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THIRD EDITION

FOOD CHEMICALS CODEX

COMMITTEE ON CODEX SPECIFICATIONS

Food and Nutrition Board
Division of Biological Sciences
Assembly of Life Sciences
National Research Council

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*The listing of individuals herein, in connection with specific meetings and programs, does not indicate that their contributions were limited solely to the activities cited.
†Explanation of abbreviations: ACS, American Chemical Society; EPA, U.S. Environmental Protection Agency; FDA, U.S. Food and Drug Administration; NF, National Formulary (of the American Pharmaceutical Association); USP, United States Pharmacopeia.

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(November 29, 1977)

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CONFERENCE ON NONPROPRIETARY NOMENCLATURE FOR FOOD, DRUG, AND COSMETIC INGREDIENTS

(Organized by the United States Adopted Names Council, with the cooperation of the Food Chemicals Codex, October 20, 1978)

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James M. Akerson
John Andrako
Durward F. Dodgen
Heinz J. Eierman
Charles Kumkumian
Howard Pippin

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William A. Ziegler (FCC)

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Daniel Banes (USP)
John V. Bergen (NF)
Durward F. Dodgen (FCC)
Harold C. Garber, Jr. (*Analyst*, Drug Standards Laboratory, NF)
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Sy-rong Sun (USP)
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JOINT FCC/USP TASK FORCE ON POLYETHYLENE GLYCOLS AND OTHER ETHOXYLATES

1—September 23, 1976; *2*—April 4, 1977; *3*—March 2, 1978; *4*—November 6, 1978; *5*—June 19, 1979; *6*—November 5, 1980

Edward Aman *3*
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Sy-rong Sun (USP) *5, 6*
Edgar E. Theimer (USP) *3, 5, 6*
Dorothy K. Wyatt (USP) *5, 6*

JOINT FCC/EPA/FDA/USP AD HOC MEETING ON ACTIVATED CARBON (June 2, 1978)

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J. A. T. de Muynk
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Richard M. Herman
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Gerald E. Stobby
Ralph Treat
Carl J. Turska
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EVALUATION OF AMINO ACIDS, SELENIUM LIMIT TEST, AND FCC REFERENCE STANDARDS (RS) BY DRUG STANDARDS LABORATORY (1972–1973) AND BY DRUG RESEARCH AND TESTING LABORATORY (1975–1980)

DRUG STANDARDS LABORATORY

1—Amino Acids; *2*—Selenium Limit Test; *3*—RS 2-*tert*-Butyl-4-hydroxyanisole; *4*—RS 3-*tert*-Butyl-4-hydroxyanisole; *5*—RS Disodium Guanylate; *6*—RS Disodium Inosinate; *7*—RS Ethyl Maltol; *8*—RS Gibberellic Acid; *9*—RS Maltol.

DRUG RESEARCH AND TESTING LABORATORY

10—RS Diketopiperazine; *11*—RS Mono-*tert*-Butyl-*p*-benzoquinone

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Preface to the Third Edition

The historical events and developmental work leading to the publication of the first edition of the *Food Chemicals Codex* in October 1966, under the direction of Dr. Justin L. Powers, are summarized in the *Preface to the First Edition*, reprinted herein on pages xxix–xxxi.

ORGANIZATION

From its inception in 1961 until 1979, the *Food Chemicals Codex* project was under the administrative supervision of the Committee on Food Protection of the Food and Nutrition Board. An advisory panel was formed in 1961, during the first year of the project, to set general policy and to develop guidelines to be followed in the preparation of the first edition, and a Committee on Specifications of the Advisory Panel was organized as the working group to develop the monographs and general test procedures. Both of these groups continued to operate until 1970, when the Advisory Panel, having served its function in guiding the project through the first edition, was released. After publication of the second edition in 1972, the name of the working group was changed to Subcommittee on Codex Specifications, to indicate more appropriately its relationship to the parent committee. This arrangement continued until July 1979, when the Subcommittee was made a full committee of the Food and Nutrition Board. During the period 1973–1976, advice was sought from the Committee on Food Protection and its Subcommittees on Food Toxicology and Nonnutritive Sweeteners on several matters related to purity criteria (see page vi).

SCOPE

The scope of the second edition was broadened slightly over that of the first edition, and that of the third edition has continued to expand. Substances included in the first edition were limited largely to chemicals added directly to foods to perform some desired function, whereas for the second edition many substances not added directly to foods but that come into contact with foods, such as food processing aids (e.g., extraction solvents, filter media), were included. Furthermore, a number of substances that are not considered to be "chemical additives" in the conventional sense were

included in the second edition; examples are the modified food starches, the masticatory substances used in chewing gum base, and pectin. For the third edition, specifications have been prepared for such ingredients as dextrose and fructose, which are more generally regarded as foods than as additives. Thus the word *Chemicals* in the title of the book—though retained for reasons of historical continuity—is in some measure inappropriate.

With few exceptions, all of the 639 monographs from the second edition, plus the 28 monographs added via the three supplements to that edition, have been incorporated in the third edition. The exceptions are as follows: *Aluminum Sulfate Solution* was deleted because it appears to be no longer used in foods and is not commercially available for such use; and *Papain* and *Pepsin* were subsumed by the monograph on *Enzyme Preparations*, which was published initially in the second supplement to the second edition. In addition, the monograph on *Sodium Metaphosphate* was in effect deleted, because it was expanded to three separate monographs (*Sodium Metaphosphate, Insoluble*; *Sodium Polyphosphates, Glassy*; and *Sodium Trimetaphosphate*). The addition of 113 completely new monographs not previously published brings to 776 the total number of monographs in the third edition. The number of substances for which specifications are provided exceeds 800, however, because several monographs provide specifications for a number of individual substances belonging to a homologous series, e.g., *Enzyme Preparations* covers 27 different enzymes; *Modified Food Starches*, 21 starches; *Natural Masticatory Substances*, 19 substances; and *Spice Oleoresins*, 6 different resins. (NOTE: Although the second edition monographs on flavor aromatic chemicals and isolates have been transferred to a separate tabular section for this edition, as discussed below, each set of specifications is regarded as an individual "monograph.")

DESIGN, FORMAT, AND MONOGRAPH CONTENTS

The design and format of the third edition are substantially different from those used for the first two. Whereas the larger page size and two-column format may be the most obvious physical changes, the Committee on Codex Specifications also reorganized the contents of the monographs for various technical reasons. The monograph section previously entitled *Specifications* was changed to *Requirements*, and the section on *Identification*, which was previously an independent section (not part of the *Specifications* section), was made part of the new *Requirements* section. Any tests that served for identification purposes but that were previously part of the *Description* sections were also transferred to the *Requirements* section as part of *Identification*. Thus, it was the Committee's intention to show that all previous tests for identification, whether occurring under an *Identification* section (which the Committee regarded as *Requirements*) or under a *Description* section (which, by definition in the *General Provisions* of the second edition, were not *Requirements*), should be part of the new *Requirements*.

Specifications for the flavor aromatic chemicals and isolates have been transferred from the general monographs section of the book to a separate tabular section, where the various physical constants and other *Requirements* may be readily compared. The tabular format also lends itself to easy accommodation of additional specifications on

such ingredients, many of which are expected to be added to this edition via supplemental revisions.

In reaffirming its policy with regard to tests for identification, the Committee on Codex Specifications has encouraged the use of infrared spectra, especially for those substances for which specific tests for identification may be lacking. This edition contains approximately 400 infrared spectra, most of which are for the essential oils and the flavor aromatic chemicals and isolates.

In implementing another new policy, the Committee has made an effort to provide assay methods, or quantitative tests to serve in lieu of assays, for as many substances as possible.

The Committee's policy on *Added Substances* (see page 5) was changed for this edition. The effect of this change is that the third edition specifications apply to the primary substance identified by the title of the individual monograph and not to mixtures of the primary substance with "added substances" (e.g., anticaking agents, antioxidants, emulsifiers), unless such additions are specifically provided for in the individual monograph. Compliance with *Food Chemicals Codex* specifications, however, can be declared for the components of such mixtures, provided that the product is properly labeled (see *Labeling* below).

LABELING

When a *Food Chemicals Codex* substance is available commercially in solution form or as a component of a mixture, and there is no provision in the Codex for such solution or mixture, the vendor may indicate on the label that the product contains substances meeting *Food Chemicals Codex* specifications by use of the initials "FCC" after the name of the component(s) that meets the FCC requirements.

REVISION AND DEVELOPMENT OF SPECIFICATIONS

In addition to the general revisions in design and format described above, the specifications and test procedures in many of the individual monographs from the second edition have been revised. The revisions were initiated by the manufacturers or suppliers, by users of the ingredients, or by the Committee itself. To the extent possible, and where the affected parties could be identified, proposed revisions were sent to the manufacturers and suppliers for review and comment. Before final adoption, all of the proposed revisions and comments related to them were studied and approved by the Committee.

Specifications for new monographs being included in this edition for the first time were requested from the manufacturers or suppliers, or they were submitted by them voluntarily. A number of new specifications, as well as many proposals for revision of existing specifications, were obtained as a result of notices published in the *Federal Register*, at the request of the Committee on Codex Specifications, in April 1978 and November 1979.

It should be emphasized that Codex specifications are under continuing scrutiny and that many revisions that could not be implemented in time for publication in this edition will be made via supplemental revisions.

For further information regarding the development and revision of Codex specifications, see *Operating Procedures of the Food Chemicals Codex*, page 571.

SYMPOSIUM ON SPECIFICATIONS

A symposium on specifications for food chemicals was organized by and held under the sponsorship of the Committee on Codex Specifications on March 14, 1975, in Washington, D.C. Financial support was provided by the Food and Drug Administration; the Nutrition Foundation, Inc.; the Grocery Manufacturers of America, Inc.; and the Chemical Manufacturers Association (formerly the Manufacturing Chemists Association). The aims of the symposium were to focus attention on the need for a careful reexamination of the procedures and criteria used in the development, application, and regulation of food additive specifications; to bring together in a public forum the latest scientific views concerning purity criteria for chemicals used in foods; to make manufacturers and users of food additives more aware of the need for ensuring that the chemicals used in foods are of suitable purity; and to stimulate interest and foster cooperation within industry in connection with the *Food Chemicals Codex* project.

Participants in the symposium (see page vii) discussed specifications as they relate to toxicology, manufacturing processes, expiration dating, analytical methods, and the safety evaluation of food chemicals. These issues were examined from the points of view of the regulator, the supplier, the user, and the consumer. The Codex project was reviewed in terms of its history and regulatory status, specifications development and revision, analytical methodology, and interaction with industry. Efforts to promote international agreement on specifications for food additives were discussed in a presentation by the chairman of the Joint FAO/WHO Codex Alimentarius Committee on Food Additives.

LIMITS OF IMPURITIES

With the exception of its application to flavoring agents, the policy regarding limits of impurities that was developed originally for the first edition, and subsequently used for the second edition, has been observed in preparing the third edition. The policy followed in developing specifications for the first edition is quoted below:

> It will be the policy of the *Food Chemicals Codex* to set maximum limits for trace impurities wherever they are deemed to be important for a particular chemical, and they shall be set at levels consistent with safety and good manufacturing practice. The maximum limits for heavy metals shall be 40 parts per million, for lead 10 parts per million, and for arsenic 3 parts per million, except in instances where higher levels cannot be avoided [under conditions of good manufacturing practice]. Where a heavy metals limit of 10 parts per million can be established, a separate limit for lead need not be specified.
>
> Flavoring agents used in foods at levels of 0.01 percent or less require only a heavy metals limit of 40 parts per million, and separate arsenic and lead tolerances may be safely omitted from [specifications for] these substances.
>
> Maximum limits for other inorganic trace impurities [e.g., fluoride, mercury, selenium] will be included in any monographs where safety or manufacturing experience indicates their desirability.

The policy in connection with limits of impurities in flavoring agents was modified for the second edition. Long experience had shown that the heavy metals test

is always negative when a vast class of flavoring agents is tested, specifically those agents that (a) are organic liquids, (b) are purified by distillation, (c) are immiscible with water, and (d) do not dissolve inorganic substances. In such cases, the heavy metals limits do not contribute to safety or to good manufacturing practice, especially when the flavoring agents are used in foods at levels of 0.01% or lower. Consequently, it was decided that limits for arsenic, heavy metals, and lead need not be included in the specifications for flavoring agents used in foods at levels of 0.01% or lower, provided that they meet the above criteria.

The Committee on Codex Specifications further revised the policy with regard to flavoring agents for the third edition. Again, the decision was made on the basis of reported experience in the manufacture and use of these ingredients. The revised policy is as follows: (a) For volatile oils prepared by distillation, the only requirement needed is a simple test for heavy metals using a 1:1 acidified mixture of the oil and water, through which hydrogen sulfide is passed. The oil passes the test, which is sensitive to 10 ppm Pb, if there is no darkening in color in either the oil or the water. (b) For cold-pressed oils (i.e., those not purified by distillation), the limits for arsenic, heavy metals, and lead as specified in the second edition will be retained for the third edition. (c) For flavor aromatic chemicals that are liquids at or near room temperature and that are prepared and/or purified by distillation, no limits for arsenic, heavy metals, or lead are required, but for flavor aromatic chemicals that are crystalline materials, or for other solids not prepared by distillation, the limits for arsenic, heavy metals, and lead as specified in the second edition will be retained for the third edition.

The Subcommittee on Toxicology of the Food and Nutrition Board's Committee on Food Protection provided advice on several occasions regarding limits of certain impurities (see listing on page vii). The Subcommittee's advice was sought most frequently when manufacturers petitioned the Committee on Codex Specifications to increase the fluoride limits for certain substances, principally the phosphates and compounds containing calcium or magnesium. Such petitions were usually accompanied by extensive toxicological reports from the literature, in addition to analyses of production lots over a period of months and data on the estimated increase in the fluoride load in the total dietary.

The decisions of the Subcommittee to recommend granting or denying these requests were based on the toxicological risk involved, upon the principles of good manufacturing practice, and upon the availability from other sources of substances meeting the *Food Chemicals Codex* limits current at the time.

With regard to such requests for increasing fluoride limits that may arise in the future, the Subcommittee on Toxicology established the following guidelines, which were accepted by the Committee on Codex Specifications: (a) In no case, except under the most unusual circumstances, should the current highest limit of 50 ppm be exceeded. (b) Restraint will be exercised in advancing other limits (up to a possible maximum of 50 ppm), and requests to increase such limits will not be granted unless the increases are judged to be essential. (c) All requests to increase fluoride limits will be considered in light of available information concerning the amounts of fluoride contributed to the total dietary by public water supplies, by foods containing fluorides, and by special food products (e.g., fish protein concentrate), as well as by processed foods to which fluoride-containing substances are added intentionally.

In developing these guidelines, it was recognized that the uncontrolled addition of fluoride to the diet via food additives is inadvisable, especially in view of the widely variable consumption of fluoride-containing food additives and of the highly irregular dietary intake of fluoride from sources other than processed foods.

GOOD MANUFACTURING PRACTICE

Although the specifications of the *Food Chemicals Codex* have been traditionally based on safety and good manufacturing practice (GMP), the Committee had not, until this edition, attempted to define what it considered GMP to be. Under the direction of committee member Samuel M. Tuthill, a set of GMP guidelines was developed for publication in this edition (see page 573 in *Section 8*). It should be emphasized that these guidelines are presented for information only and are not intended to be mandatory in any sense as regards compliance with *Food Chemicals Codex* specifications; legal and regulatory requirements concerning the manufacture of food ingredients are established and enforced by the Food and Drug Administration, not by the Committee on Codex Specifications.

NOMENCLATURE

The titles of a number of monographs were changed from the first to the second edition, and others have been changed for this edition. A listing of former and current titles is provided in *Section 8* on page 574.

The Committee on Codex Specifications has recognized the desirability of using monograph titles that are consistent with the names of substances cited in Title 21, Chapter I, Subchapter B of the *Code of Federal Regulations*. In a few cases, however, the Committee has used titles that are different from those in the regulations. *Sodium Ferrocyanide* (instead of *Yellow Prussiate of Soda*) is an example. One title, *Poloxamer*, which is applied to two substances belonging to a series of polyols, was coined specifically for use in the second edition and has been retained for this edition, although the name does not appear in the regulations. The name *Poloxamer* was developed with the cooperation and assistance of the U.S. Adopted Names Council. Additional nonproprietary names to be used as titles of Codex monographs may be developed in this manner for other substances hitherto known only by their trade names or by long, unwieldy chemical names.

Problems related to the development and use of nonproprietary names for food, drug, and cosmetic ingredients were addressed during a conference held at the Academy in October 1978 (see page x).

TEST PROCEDURES

The *General Test* procedures employed in the first and second editions have been retained for use in the third edition. Many of the older procedures have undergone substantial revision, and several new procedures have been added. With few exceptions, the methods employed in this Codex are considered to be adequate *for their intended use* in determining compliance of the substances with the requirements.

The limitations of the conventional heavy metals test procedure, however, are

well known, and an extensive two-year collaborative study was conducted prior to publication of the second edition in 1972. The study was an attempt to improve the accuracy and precision of the method. Although inconclusive in other respects, the results indicated that the reproducibility of the method is approximately 7 ppm at the 95% confidence level for substances containing 10 ppm of heavy metals. Whereas the test procedure seldom gives an accurate indication of the actual heavy metals content, it does demonstrate that the test substance is not grossly contaminated with heavy metals. In this manner, the test serves a useful function in partially defining the purity of food-grade chemicals.

Nevertheless, a continuing effort has been made to improve the test and the methods of sample preparation to the greatest extent practicable. *Method III* (see page 513), which first appeared as *Method IV* in the *First Supplement* to the second edition, was developed through a collaborative study sponsored jointly by the Committee on Codex Specifications and the Committee on Analytical Reagents of the American Chemical Society. The major difference between *Method III* and *Method II* (page 513) is the manner in which the sample is prepared for the test. Further efforts to improve the heavy metals test were made in a joint study sponsored by the Codex Committee, the American Chemical Society, the National Formulary, and the United States Pharmacopeia (see page x).

In those cases wherein the conventional heavy metals test cannot be improved to give a reasonably accurate indication of the heavy metals content, or in those cases where, for other reasons, the test is not appropriate, consideration will be given to the use of other procedures (e.g, atomic absorption spectroscopy) that will determine individual elements for which limits will be specified in lieu of a general heavy metals limit.

LEGAL STATUS

The first edition of the *Food Chemicals Codex* was given quasi-legal recognition by means of a letter of endorsement from the Commissioner of Food and Drugs, which was reprinted in the book. At that time (April 1966), the Commissioner stated that "the FDA will regard the specifications in the Food Chemicals Codex as defining an 'appropriate food grade' within the meaning of Sec. 121.101(b)(3) [now §182.1(b)(3)] and Sec. 121.1000(a)(2) [now §172.5(a)(2)] of the food additive regulations," although such endorsement could not be construed to exempt substances from compliance with requirements of acts of Congress or with regulations and rulings issued by the Food and Drug Administration (FDA) under authority of such acts.

Later, the *Food Chemicals Codex* was officially recognized by FDA when the definitions and procedural and interpretive regulations under §170.30, relating to eligibility of substances for classification as *generally recognized as safe* (GRAS), were revised and published in the *Federal Register* of June 25, 1971 (36 FR 12093).

Food Chemicals Codex specifications have also been adopted, under certain conditions, by the National Health and Medical Research Council of Australia; the Health Protection Branch of the Department of National Health and Welfare of Canada; the Ministries of Agriculture, Fisheries, and Food of Great Britain; and the Department of Health (Food and Nutrition Branch) of New Zealand. In addition, the *Food Chemicals Codex* has served as the source of many specifications developed by

the Joint FAO/WHO Expert Committee on Food Additives, and by the International Union of Pure and Applied Chemistry.

COMMITTEE TASK FORCES AND SPECIAL ASSIGNMENTS

The Chairman of the Committee on Codex Specifications established several task forces, drawn from the committee membership, to investigate certain matters or prepare material for consideration by the Committee as a whole. In addition, individual committee members were given special assignments to represent the Committee on outside study panels or projects involving other organizations and to conduct studies of a particular nature. Two of the more active task forces were those on *Hydrochloric Acid*, involving Mr. Bryant (Chairman), Dr. Campbell, Mr. Fletcher, Dr. Howard, Mr. Morecombe, Mr. Schmitz, Mr. Stobby, and Dr. Tuthill; and on *Good Manufacturing Practice*, involving Dr. Tuthill (Chairman), Mr. Bryant, Dr. Campbell, Mr. Morecombe, and Mr. Schmitz.

Mr. Boyd was given primary responsibility for the review of the essential oils specifications, for liaison with the Essential Oil Association and the American Spice Trade Association, and for providing infrared spectra for the essential oils and other substances. Mr. Broderick was responsible for review of the flavor aromatic chemicals and isolates and the incorporation of their specifications in a tabular format, for liaison with the Technical Committee of the Flavor and Extract Manufacturers Association, and for providing infrared spectra for many of the flavor aromatic chemicals and isolates. Mr. Fletcher served as primary committee liaison with the United States Pharmacopeia in a Joint Task Force on Polyethylene Glycols, with participation also by Mr. Morecombe and Mr. Stobby. Dr. Haenni provided special assistance to the project staff officer, by accepting a larger than usual assignment of proofreading material, and to the Committee on particular problems involving analytical methodology. Dr. Howard was instrumental in organizing the Workshop on Microbiological Criteria (see page ix) and in leading the Committee toward its revision of the policy on *Added Substances* (see page 5). Most of the committee members participated in the review and revision of the *General Tests* chapters (see *Section 6*); Dr. Medwick accepted responsibility for undertaking a complete revision of the chapters on *Chromatography* and *Spectrophotometry*, and for preparing a new chapter on *Nuclear Magnetic Resonance*. Mr. Morecombe served as Chairman of the Joint FCC/ACS/NF-USP Task Force on Heavy Metals, with participation also by Mr. Bryant, Mr. Schmitz, and Dr. Tuthill. Mr. Read and Mr. Fletcher conducted surveys on the possibility of nitrosamine contamination of FCC-grade substances; Mr. Read also kept the Committee informed on numerous matters concerning Canadian specifications, particularly with respect to *Caramel* and *Saccharin*. Mr. Schmitz conducted an extensive survey of the *Food Chemicals Codex* specifications, with emphasis on the need for identification tests, assays, and adequacy of methodology; he also served as liaison with the Ad Hoc Enzyme Technical Committee. Dr. Tuthill (with the assistance of John A. Caughlan) was responsible for checking all molecular weights in the third edition and for revising them to conform with the *1979 Revision of the International Atomic Weights*. Dr. Kirschman advised on all problems dealing with toxicology and suggested ways in which the Committee might give its fullest consideration to matters of toxicological significance.

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Portions of many of the monographs and most of the general tests included in the first edition were adapted from, and used with permission granted by the parent organizations of, the following publications: *United States Pharmacopeia*, Sixteenth Revision; *National Formulary*, Eleventh Edition; *Reagent Chemicals—A.C.S. Specifications 1960*; *Official and Tentative Methods of the American Oil Chemists Society*; *Essential Oil Association of USA Specifications, Infrared Spectra, and Revisions*; and *Specifications for Flavoring Materials, Flavoring and Extract Manufacturers Association*. In addition, the following ASTM methods (many of which were included in the first and second editions) have been adapted for use in the third edition, with permission, from the *Annual Book of ASTM Standards* (copyright © American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103): B 214-66, D 721-56T, D 938-62, D 1078-58, D 1347-56, D 1394-63(70), D 1416, D 1417, D 1439, D 1493-58T, D 1824, D 20008-62, D 2701-73, D 2920-70, E 1, E 28-67, E 77, and EL 62. The American Oil Chemists Society has granted permission for use of the following AOCS methods: Ca 5-40, Ca 6a-40, Ca 14-56, Cd 3-25, Cd 3a-63, Cd 4-40, Cd 11-57, Cd 13-60, Te 1a-64, and T1 1a-64.

The following organizations are among those participating in the development and review of specifications, or in other activities, pertaining to this edition:

Ad Hoc Enzyme Technical Committee
American Chemical Society (Committee on Analytical Reagents)
American Gelatin Importers Association
American Spice Trade Association (Extractors Committee)
Annatto Industry Committee
Association of Official Analytical Chemists
Calorie Control Council
Chemical Manufacturers Association (formerly Manufacturing Chemists Association)
Chlorine Institute
Compressed Gas Association
Corn Refiners Association
Cosmetic, Toiletry and Fragrance Association
Council on White Mineral Oil
Drug Research and Testing Laboratory (formerly Drug Standards Laboratory)
Environmental Defense Fund
Essential Oil Association (Scientific and Instrumental Committees)
Fatty Acid Producers Council
Federation of American Societies for Experimental Biology (Life Sciences Research Office)
Flavor and Extract Manufacturers Association (Technical Committee)
Food Safety Council

Gelatin Manufacturers Institute of America
Glycerin Producers' Association
Grocery Manufacturers of America, Inc.
Institute of Shortening and Vegetable Oils
International Food Additives Council
International Life Sciences Institute
International Pectin Producers Association
International Technical Caramel Association
Marinalg International
National Association of Chewing Gum Manufacturers
National Food Processors Association (formerly National Canners Association)
National Formulary
Nutrition Foundation
Perlite Institute
Salt Institute
United States Adopted Names Council
United States Pharmacopeia
Water Soluble Gum Association
Whey Products Institute

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Many persons associated with food processing companies, chemical manufacturers, and other organizations have contributed to this project by supplying information related to specifications and analytical test procedures, and by participating in laboratory studies and in other activities and programs of the Committee. The names of these individuals are listed on pages xiv–xvi, and their assistance is gratefully acknowledged.

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FUTURE REVISIONS

In line with previous practices, the third edition will be kept up-to-date by the issuance of supplements, which will be sent to all holders of the book at no charge. It

is expected that a fourth edition of the Codex will be published in about five or six years.

Constructive criticisms and suggestions regarding the specifications and analytical procedures incorporated in this Codex are entirely welcome. Notations as to errors, suggestions for revisions, and any other comments should be addressed to Food Chemicals Codex, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

November 1980 D.F.D.

Preface to the First Edition

The need for a compilation of standards for food-grade chemicals has been recognized for quite some time, but it was not until 1958, soon after the enactment of the Food Additives Amendment, that any positive action was taken to compile such a compendium. Although the federal Food and Drug Administration (FDA) had by regulations and informal statements defined in general terms quality requirements for food chemicals generally recognized as safe (GRAS), these requirements were not designed to be sufficiently specific to serve as release, procurement, and acceptance specifications by primary chemical manufacturers and food processors. Since complete specifications and quality-control procedures required by the FDA in food-additive petitions for chemicals not included in the GRAS lists were not published in the official regulations, their use for general guidance was restricted. It was therefore incumbent upon food processors to provide detailed procurement specifications when ordering food-additive chemicals from primary manufacturers or distributors. This system may have functioned satisfactorily in most instances, but it was generally believed that the availability of a book of standards designed especially for food-additive chemicals would be more convenient and would promote greater uniformity of quality and thus provide added assurance of safety.

For these and other reasons, the Food Protection Committee of the National Academy of Sciences–National Research Council received requests in 1958 from its Industry Liaison Panel and other sources to undertake a project designed to produce a Food Chemicals Codex comparable in many respects to the United States Pharmacopeia (U.S.P.) and the National Formulary (N.F.).

In response to these requests, advice was sought from special committees composed of representatives of industry, government agencies, and others experienced in the operation of the U.S.P. and the N.F. It was the consensus of these groups that there was a definite need for a Food Chemicals Codex and that the Food Protection Committee was a suitable agency to assume responsibility for the project.

This first edition of the Food Chemicals Codex, parts of which were published in loose-leaf form between 1963 and 1966, is the result of an effort by the Food Protection Committee started in 1961 to provide objective quality standards for food-

grade chemicals. The aim of the Codex is to define a substantial number of food-grade chemicals in terms of minimum identity and purity specifications based on the elements of safety and good manufacturing practice. It is believed that this objective has been achieved. As indicated in a letter written by Dr. James L. Goddard, Commissioner of Food and Drugs, the Food Chemicals Codex specifications have received endorsement by the federal Food and Drug Administration as constituting adequate minimum requirements of purity for chemicals permitted for intentional and purposeful use in food for man. With this official endorsement, it is expected that the Codex standards will be utilized by food processors as procurement and acceptance specifications and by primary manufacturers of food-grade chemicals as release specifications.

SCOPE

The scope of this first edition of the Codex is limited to substances amenable to chemical characterization or biological standardization which are added directly to food to perform some desired function. Such substances were selected from food additives generally recognized as safe, those approved by prior sanctions, and those for which special use tolerances have been established by FDA regulations.

SOURCES OF SPECIFICATIONS

Specifications and analytical procedures required for the Codex have been adapted from compendia devoted to standards for chemicals, from original scientific literature sources, and from data supplied by chemical manufacturers and food processors. In some instances where procedures required laboratory study, the facilities of commercial consulting laboratories have been utilized, but often the necessary work has been done in industry laboratories as a service to the Codex project.

DESIGN

Specifications and procedures for their determination are presented in the form of monographs, which constitute the major portion of the Codex. Other sections cover subjects such as general provisions designed to interpret the relative significance that should be attached to the different types of specifications, and general tests and solutions frequently referred to in the monographs.

MECHANISM OF COMPILATION

In general, provisional specifications, based on information obtained from reliable sources, were prepared in the office of the director of the project and then circulated for review to selected members of committees and panels associated with the Food Protection Committee and the Codex, and to all manufacturers who submitted data on their products. Suggestions and recommendations for revisions received from these sources resulted in revisions prior to the publication between 1963 and 1966 of a loose-leaf edition of the Codex in ten parts, which was made generally available upon a subscription basis. Finally, the loose-leaf edition was further revised, the pages collated in appropriate sequence, and the material published in its present form.

FUTURE REVISIONS

If the Food Chemicals Codex is to function effectively as an authoritative book of standards for food-grade chemicals, provision for its continuous revision under appropriate sponsorship and supervision is highly essential. It is a source of gratification to those who have made the publication of this first edition possible that such provision has been made. The Governing Board of the National Academy of Sciences has approved a plan for continuing the sponsorship of the Codex for a second five-year period under the administrative supervision of the Food Protection Committee of the Food and Nutrition Board. The approved plan provides for the issuance and distribution of interim revision supplements whenever necessary and the publication of a second, completely revised edition of the Codex in 1971.

ASSISTANCE AND SUPPORT

During the course of the development and compilation of the Food Chemicals Codex, cooperation was received from many sources.

The many constructive suggestions offered by the members of the Food Protection Committee, its subcommittees, and its Liaison Panel have been most helpful.

In devising suitable specifications for flavoring agents, the assistance of the Scientific Section of the Essential Oil Association of the USA and Scientific Research Committee of the Flavoring Extract Manufacturers' Association has been particularly notable, and appreciation is expressed to these two groups for their valuable contributions.

Many individuals associated with food processors and primary manufacturers of chemicals have contributed greatly to the project by furnishing information and advice relating to specifications and analytical procedures for food-grade chemicals and by reviewing provisional specifications prior to their publication.

The Food Protection Committee and those directly responsible for the compilation of the Food Chemicals Codex wish to express appreciation for encouragement and support by the Public Health Service whose Research Grant No. EF-00222 from the *Division of Environmental Engineering and Food Protection* has made possible the compilation and publication of the Food Chemicals Codex. Comparable appreciation should also be recorded for the contribution of supplementary grants in support of the Codex project by industry, and by associations and foundations.

June 1966 J.L.P.

1 / *General Provisions Applying to Specifications, Tests, and Assays of the* Food Chemicals Codex

TITLE OF BOOK

The title of this book, including supplements thereto issued separately, is the *Food Chemicals Codex*, Third Edition. It may be abbreviated to FCC III.

When manufacturers of FCC substances wish to indicate on their labels that the substances conform to FCC specifications, the designation "Food Chemicals Codex Grade," or "FCC Grade," or simply "FCC" (implying the concurrent edition of the FCC) may be used.

Where the term "Codex" is used without further qualification in the text of this book, it applies to the *Food Chemicals Codex*, Third Edition.

CODEX SPECIFICATIONS

Food Chemicals Codex specifications, comprising the *Description*, *Requirements*, and *Tests*, are presented in monograph form (*Section 2*) or tabular form (*Section 3*) for each individual substance or group of substances and are designed to serve for ingredients of a quality level sufficiently high to ensure their safety under usual conditions of intentional use in foods or in food processing. Thus, Codex specifications generally represent acceptable levels of quality and purity of food-grade ingredients available in the United States (or in other countries in which FCC specifications are recognized).

The titles of Codex monographs are in most instances the common or usual names. The FCC specifications apply equally to substances bearing the main titles, or synonyms listed under the main titles, or names derived by transposition of definitive words in main titles.

The assays and tests described constitute methods upon which the specifications of the *Food Chemicals Codex* depend. The analyst is not prevented, however, from applying alternative methods if he is satisfied that the procedures he uses will produce results of equal accuracy. In the event of doubt or disagreement concerning a substance purported to comply with the requirements of this Codex, only the methods described herein are applicable and authoritative.

ATOMIC WEIGHTS AND CHEMICAL FORMULAS

Computation of molecular weights and volumetric and gravimetric factors stated in tests and assays are based upon the *1979 Revision of the International Atomic Weights*.

Molecular and structural formulas and molecular weights immediately following titles are included for the purpose of information and are not to be considered an indication of the purity of the substance. Molecular formulas given in specifications, tests, and assays, however, denote the pure chemical entity.

ASSAYS AND TESTS

Analytical Samples In the description of assays and tests, the approximate quantity of the analytical sample to be used is usually indicated. The quantity actually used, however, should not deviate by more than 10% from that stated.

Some substances are directed to be dried before a sample is taken for an assay or test. When a *Loss on Drying* or *Water* test is specified, the undried substance may be used and the results calculated on the dried basis, provided that any moisture or other volatile matter in the undried sample does not interfere with the specified assay and test procedures.

The word "accurately," used in connection with gravimetric or volumetric measurements, means that the operation should

be carried out within the limits of error prescribed under *Volumetric Apparatus*, page 551, or under *Weights and Balances*, page 554. The same significance also applies to the term "exactly" or expressions such as "100.0 ml" or "50.0 mg."

The word "transfer," when used in describing assays and tests, means that the procedure should be carried out quantitatively.

Apparatus With the exception of volumetric flasks and other exact measuring or weighing devices, directions to use a definite size or type container or other laboratory apparatus are intended only as recommendations, unless otherwise specified.

Where an instrument for physical measurement, such as a thermometer, spectrophotometer, gas chromatograph, etc., is designated by its distinctive name or tradename in a test or assay, a similar instrument of equivalent or greater sensitivity or accuracy may be employed.

Where low-actinic or light-resistant containers are specified, clear glass containers that have been rendered opaque by application of a suitable coating or wrapping may be used.

Blank Tests Where a blank determination is specified in a test or assay, it is to be conducted by using the same quantities of the same reagents and by the same procedure repeated in every detail except that the substance being tested is omitted.

A *residual blank titration* may be stipulated in assays and tests involving a back titration in which a volume of a volumetric solution larger than is required to react with the sample is added, and the excess of this solution is then titrated with a second volumetric solution. Where a residual blank titration is specified or where the procedure involves such a titration, a blank is run as directed in the preceding paragraph. The volume of the titrant consumed in the back titration is then subtracted from the volume required for the blank. The difference between the two, equivalent to the actual volume consumed by the sample, is the corrected volume of the volumetric solution to be used in calculating the quantity of the substance being determined.

Constant Weight A direction that a substance is to be "dried to constant weight" means that the drying should be continued until two consecutive weighings differ by not more than 0.5 mg per g of sample taken, the second weighing to follow an additional hour of drying.

The direction "ignite to constant weight" means that the ignition should be continued at 800° ± 25°, unless otherwise specified, until two consecutive weighings do not differ by more than 0.5 mg per g of sample taken, the second weighing to follow an additional 15-min ignition period.

Desiccators and Desiccants The expression "in a desiccator" means using a tightly closed container of appropriate design in which a low moisture content can be maintained by means of a suitable desiccant. Preferred desiccants include anhydrous calcium chloride, magnesium perchlorate, phosphorus pentoxide, and silica gel.

Identification The tests described under this heading in monographs are designed for application to substances taken from labeled containers and are provided only as an aid to substantiate identification. These tests, regardless of their specificity, are not necessarily sufficient to establish proof of identity, but failure of a substance taken from a labeled container to meet the requirements of a prescribed identification test means that it does not conform to the requirements of the monograph.

Indicators The quantity of an indicator solution used should be 0.2 ml (approximately 3 drops) unless otherwise directed in an assay or test.

Loss on Drying and Water In general, a limit test, to be determined by the *Karl Fischer Titrimetric Method*, is provided under the heading *Water* for compounds containing water of crystallization or adsorbed water.

Limit tests under the heading *Loss on Drying*, determined by other methods, are designed for compounds in which the loss on drying may not necessarily be attributable to water.

Microbiological Attributes The supplier and/or user of FCC substances should apply microbiological criteria as necessary to ensure that the substance is not contaminated with pathogenic or other objectionable organisms and that the substance is otherwise suitable for its intended use. Where the Codex recognizes a specific need for microbiological criteria for an individual substance, such requirements are included in the specifications.

Negligible The term "negligible," as used in some *Residue on Ignition* specifications, indicates a quantity not exceeding 0.5 mg.

Odorless This term, when used in describing a substance, applies to the examination, after exposure to air for 15 min, of about 25 g of the substance that has been transferred quickly from the original container to an open evaporating dish of about 100-ml capacity. If the package contains 25 g or less, the entire contents should be examined.

Reagents Specifications for reagents are not included in the *Food Chemicals Codex*. Unless otherwise specified, reagents required in tests and assays should conform to the specifications of the current editions of the *United States Pharmacopeia* or *Reagent Chemicals—American Chemical Society Specifications*. Reagents not covered by any of these specifications should be of a grade suitable to the proper performance of the method of assay or test involved.

NOTE: It is recognized that certain chemical reagents specified in FCC test procedures may be considered to be hazardous or toxic by the Occupational Safety and Health Administration, by the Environmental Protection Agency (under provisions of the Toxic Substances Control Act), or by health authorities in other countries in which the *Food Chemicals Codex* is recognized. In preparing this edition, the Committee on Codex Specifications has attempted to specify use of different reagents where suitable substitutes are known. For some procedures, however, the original

chemicals have been retained, due to the lack of information on suitable substitutes. In such cases, the analyst is encouraged to investigate the use of suitable substitute reagents as appropriate, and to inform the Committee of the results so obtained.

Reference Standards Some instrumental and chromatographic tests and assays specify the use of a reference standard. Where a reference standard is designated as "USP," it may be obtained from the United States Pharmacopeia, 12601 Twinbrook Parkway, Rockville, Md. 20852. Reference standards bearing the abbreviation FCC are supplied by the *Food Chemicals Codex*, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

Significant Figures Where tolerance limits are expressed numerically, the values are considered to be significant to the number of digits indicated. Values should be rounded off to the nearest indicated digit according to the commonly used practice of rejecting or increasing numbers less than or greater than 5. For example, a requirement of not less than 96.0% would be met by a result of 95.96% but not by a result of 95.94%. When the digit to be dropped is exactly 5, the value should be rounded off to the closest even digit. Thus, both 1.4755 and 1.4765 would be rounded off to 1.476. When a range is stated, the upper and lower limits are inclusive so that the range consists of the two values themselves, properly rounded off, and all intermediate values between them.

Solutions All solutions, unless otherwise specified, are to be prepared with distilled or deionized water conforming to the USP requirements for *Purified Water*.

Such expressions as "1 in 10" or "10%" mean that *1 part by volume* of a liquid or *1 part by weight* of a solid is to be dissolved in a volume of the diluent or solvent sufficient to make the finished solution 10 parts by volume. Directions for the preparation of colorimetric solutions (CS), test solutions (TS), and volumetric solutions (VS), are provided on pages 557, 558, and 564, respectively.

A volumetric solution should be prepared to have a normality (molarity) within 10% of the stated value and should be standardized to four significant figures. When volumetric equivalence factors are provided in tests and assays, the term 0.X *N* (*M*) is understood to mean a VS having a normality (molarity) of exactly 0.X000 *N* (*M*). If the normality (molarity) of the VS employed in a particular procedure differs from 0.X000, an appropriate correction factor must be applied.

Specific Gravity Numerical values for specific gravity, unless otherwise noted, refer to the ratio of the weight of a substance in air at 25° to that of an equal volume of water at the same temperature. Specific gravity may be determined by any reliable method, unless otherwise specified.

Time Limits Unless otherwise specified, 5 min is to be allowed for a reaction to take place in conducting limit tests for trace impurities such as chloride, iron, etc.

Expressions such as "exactly 5 min" mean that the stated period should be accurately timed.

Temperatures Unless otherwise specified, temperatures are expressed in centigrade (Celsius) degrees, and all measurements are to be made at 25° unless otherwise directed.

Test Solutions See *Solutions*.

Tolerances The minimum purity tolerances specified for *Food Chemicals Codex* items have been established with the expectation that the substances to which they apply will be used as food additives, ingredients, or food-processing aids. These tolerance limits should neither bar the use of lots of articles that more nearly approach 100% purity nor should they constitute a basis for a claim that such lots exceed the quality prescribed by the *Food Chemicals Codex*.

When a maximum assay tolerance is not given, the assay should show the equivalent of not more than 100.5%.

Trace Impurities Tests for inherent trace impurities are provided to limit such substances to levels consistent with good manufacturing practice (see page 573) and that are safe and otherwise unobjectionable under conditions in which the food additive or ingredient is customarily employed.

It is obviously impossible to provide limits and tests in each monograph for the detection of all possible unusual or unexpected impurities, the presence of which would be inconsistent with good manufacturing practice. The limits and tests provided are those considered to be necessary according to currently recognized methods of manufacture and are based on information available to or provided to the Committee on Codex Specifications. If other methods of manufacture or other than the usual raw materials are used, or if other possible impurities may be present, additional tests may be required and should be applied, as necessary, by the vendor or user to demonstrate that the substance is suitable for its intended application in foods or in food processing.

In instances where both a heavy metals and a lead limit are specified in a monograph and the former is found to be 10 ppm or less, the lead content need not be determined.

Vacuum The unqualified use of the term "in vacuum" means a pressure at least as low as that obtainable by an efficient aspirating water pump (not higher than 20 mm of Hg).

Weights and Measures, Symbols and Abbreviations The metric system of weights and measures is used in most specifications, assays, and tests in this *Food Chemicals Codex*. The metric units and other abbreviations commonly employed are:

kg	= kilogram
g	= gram
mg	= milligram
μg	= microgram
ng	= nanogram
L	= liter
ml	= milliliter
μl	= microliter
m	= meter
cm	= centimeter

dm = decimeter
mm = millimeter
μm = micrometer
nm = nanometer
A = ampere
V = volt
dc = direct current
ft = foot
in. = inch
$in.^3$ = cubic inch
gal. = gallon
lb = pound
oz = ounce
ppm = parts per million (10^6) parts
ppb = parts per billion (10^9) parts
psi = pounds per square inch
sp. gr. = specific gravity
b.p. = boiling point
m.p. = melting point
id = inside diameter
od = outside diameter
h = hour
min = minute
s = second
% = percent
N = normality
M = molarity

GENERAL SPECIFICATIONS

Certain specifications in the monographs of the *Food Chemicals Codex* are not amenable to precise description and accurate determination within narrow limiting ranges. Because of the subjective or general nature of these specifications, good judgment, based upon experience, must be used in interpreting and attaching significance to them. Specifications that are most likely to cause doubt are discussed in the subsequent paragraphs.

Description The material given under this heading in monographs is provided for general information only and is not intended to be interpreted as rigidly as measurable characteristics described under tests and assays should be. It includes a description of physical characteristics such as color, odor, taste, form, etc., and information on stability under certain conditions of exposure to air and light. Statements in this section may also cover approximate indications of properties such as solubility (see below) in various solvents, pH, melting point, and boiling point, with numerical values modified by "about," "approximately," "usually," and other comparable nonspecific terms.

Solubility Statements included in the *Requirements* section in monographs under headings such as *Solubility in Alcohol* express exact requirements and constitute quality specifications.

Statements relating to solubility given under the heading *Description*, however, are intended as information regarding approximate solubilities only and are not to be considered as Codex quality requirements. Such statements are considered to be of minor significance as a means of identification or determination of purity. For those purposes, dependence must be placed upon other specifications.

Approximate solubilities are indicated by the following descriptive terms:

Descriptive Term	Parts of Solvent Required for 1 Part of Solute
Very Soluble	less than 1
Freely Soluble	from 1 to 10
Soluble	from 10 to 30
Sparingly Soluble	from 30 to 100
Slightly Soluble	from 100 to 1000
Very Slightly Soluble	from 1000 to 10,000
Practically Insoluble or Insoluble	more than 10,000

Soluble substances, when brought into solution, may show slight physical impurities, such as fragments of filter paper, fibers, and dust particles, unless excluded by definite tests or other requirements; however, significant amounts of black specks, metallic chips, glass fragments, or other insoluble matter are not permitted.

Functional Use in Foods A statement of functional classification is provided in each monograph as useful information to indicate the principal applications or technical effect of the substance in foods or in food processing. The statement is not intended to limit in any way the choice or use of the substance or to indicate that it has no other utility.

Packaging and Storage Statements in monographs relating to packaging are advisory in character and are intended only as general information to emphasize instances where deterioration may be accelerated under adverse packaging and storage conditions, such as exposure to air, light, or extremes of temperature, or where safety hazards are involved.

Containers The container is the device that holds the substance and that is or may be in direct contact with it. The immediate container is in direct contact with the substance at all times. The closure is a part of the container.

The container should not interact physically or chemically with the material that it holds so as to alter its strength, quality, or purity, and the food (additive) contact surface of the container should comply with the food additive regulations promulgated under the Food, Drug and Cosmetic Act (or with applicable laws and regulations in other countries in which FCC specifications are recognized).

Light-Resistant Container A light-resistant container is designed to prevent deterioration of the contents beyond the prescribed limits of strength, quality, or purity under the ordinary or customary conditions of handling, shipment, storage, and sale. A colorless container may be made light-resistant by enclosing it in an opaque carton or wrapper (see also *Apparatus*, page 2).

Well-Closed Container A well-closed container protects the contents from extraneous solids and from loss of the chemical under the ordinary or customary conditions of handling, shipment, storage, and sale.

Tight Container A tight container protects the contents from contamination by extraneous liquids, solids, or vapors, from loss of the chemical, and from efflorescence, deliquescence, or evaporation under the ordinary or customary conditions of handling, shipment, storage, and sale, and is capable of tight reclosure.

ADDED SUBSTANCES

FCC specifications are intended for application to individual substances (single entities) and not to proprietary blends or other mixtures. Some specifications, however, provide for "added substance" (i.e., functional secondary ingredients such as anticaking agents, antioxidants, diluents, emulsifiers, and preservatives) intentionally added when necessary to ensure the integrity, stability, utility, or functionality of the primary substance in commercial use.

When an FCC monograph provides for such additions, the added substance(s) must meet the following requirements: (a) it is approved for use in foods by the U.S. Food and Drug Administration, or by the responsible government agency in other countries in which FCC specifications are recognized; (b) it is of appropriate food-grade quality and meets the requirements of the *Food Chemicals Codex*, if listed therein; (c) it is used in an amount not to exceed the minimum required to impart its intended technical effect or function in the primary substance; (d) its use will not result in concentrations exceeding permitted levels in any food as a consequence of the subsequent use in foods of the FCC primary substance(s) to which it has been added; and (e) it does not interfere with the assays and tests prescribed for determining compliance with the FCC requirements for the primary substance, unless the monograph for the primary substance has provided for such interferences.

An FCC substance to which are added substances not specifically provided for and mentioned by name or function in its monograph should not be designated as an FCC substance. Such a combination is a mixture to be described by disclosure of its ingredients.

2 / *Monographs*

Acacia

Gum Arabic

DESCRIPTION

A dried gummy exudation obtained from the stems and branches of *Acacia senegal* (L.) Willd. or of related species of *Acacia* (Fam. *Leguminosae*). Unground acacia occurs as white or yellowish white spheroidal tears of varying size or in angular fragments. It is also available commercially in the form of white to yellowish white flakes, granules, or powder. One g dissolves in 2 ml of water, forming a solution that flows readily and is acid to litmus. It is insoluble in alcohol. A 1 in 10 solution is slightly levorotatory.

REQUIREMENTS

Identification

To 10 ml of a cold 1 in 50 solution of acacia add 0.2 ml of diluted lead subacetate TS. A flocculent, or curdy, white precipitate is formed immediately.

Arsenic (as As) Not more than 3 ppm.
Ash (Acid-Insoluble) Not more than 0.5%.
Ash (Total) Not more than 4%.
Heavy Metals (as Pb) Not more than 0.004%.
Insoluble Matter Not more than 1%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 15%.
Starch or Dextrin Passes test.
Tannin-Bearing Gums Passes test.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Acid-Insoluble) Determine as directed in the general method, page 466.

Ash (Total) Determine as directed in the general method, page 466.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Matter Dissolve a 5-g sample in about 100 ml of water contained in a 250-ml Erlenmeyer flask, add 10 ml of diluted hydrochloric acid TS, and boil gently for 15 min. Filter the hot solution by suction through a tared filtering crucible, wash thoroughly with hot water, dry at 105° for 2 h, and weigh.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 5 h. Unground samples should be powdered to pass through a No. 40 sieve and mixed well before weighing.

Starch or Dextrin Boil a 1 in 50 solution, cool, and add a few drops of iodine TS. No bluish or reddish color is produced.

Tannin-Bearing Gums To 10 ml of a 1 in 50 solution add about 0.1 ml of ferric chloride TS. No blackish coloration or blackish precipitate is formed.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Acetic Acid, Glacial

CH_3COOH

$C_2H_4O_2$ Mol wt 60.05

DESCRIPTION

A clear, colorless liquid having a pungent, characteristic odor and, when well diluted with water, an acid taste. It boils at about 118° and has a specific gravity of about 1.049. It is miscible with water, with alcohol, and with glycerin.

REQUIREMENTS

Identification

A 1 in 3 solution gives positive tests for *Acetate*, page 515.

Assay Not less than 99.5%, by weight, of $C_2H_4O_2$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Nonvolatile Residue Not more than 0.005%.
Readily Oxidizable Substances Passes test.
Solidification Point Not lower than 15.6°.

TESTS

Assay Measure about 2 ml into a tared, glass-stoppered flask, and weigh accurately. Add 40 ml of water, then add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 60.05 mg of $C_2H_4O_2$.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals To the residue obtained in the test for *Nonvolatile Residue* add 8 ml of 0.1 *N* hydrochloric acid, warm gently until solution is complete, and dilute to 100 ml with water. A 10-ml portion of this solution diluted to 25 ml meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Evaporate 19 ml (20 g), accurately measured, in a tared dish on a steam bath, and dry at 105° for 1 h.

Readily Oxidizable Substances Dilute 2 ml in a glass-stoppered container with 10 ml of water, and add 0.1 ml of 0.1 *N* potassium permanganate. The pink color is not changed to brown within 2 h.

Solidification Point Determine as directed in the general procedure, page 538.

Packaging and Storage Store in tight containers.
Functional Use in Foods Acidifier; flavoring agent.

Acetone

2-Propanone; Dimethyl Ketone

CH_3COCH_3

C_3H_6O Mol wt 58.08

DESCRIPTION

A clear, colorless, volatile liquid having a characteristic odor. It is miscible with water, with alcohol, with ether, with chloroform, and with most volatile oils. Its refractive index is about 1.356.

Caution: Acetone is highly flammable.

REQUIREMENTS

Identification

Mix 0.1 ml of the sample with 10 ml of water, add 5 ml of sodium hydroxide TS, warm, and add 5 ml of iodine TS. A yellow precipitate of iodoform is produced.

Assay Not less than 99.5% of C_3H_6O, by weight.
Acidity (as acetic acid) Not more than 0.002%.
Aldehydes (as formaldehyde) Not more than 0.002%.
Alkalinity (as NH_3) Not more than 10 ppm.
Distillation Range Within a range of 1°, including 56.1°.
Heavy Metals (as Pb) Not more than 1 ppm.
Methanol Not more than 0.05%.
Nonvolatile Residue Not more than 10 ppm.
Phenols Passes test.
Solubility in Water Passes test.
Substances Reducing Permanganate Passes test.
Water Not more than 0.5%.

TESTS

Assay Its specific gravity, determined by any reliable method (see page 3), is not greater than 0.7880 at 25°/25° (equivalent to 0.7930 at 20°/20°).

Acidity Mix 38 ml (about 30 g) of the sample with an equal volume of carbon dioxide–free water, add 0.1 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Not more than 0.1 ml is required to produce a pink color.

Aldehydes Dilute 2.5 ml (about 2 g) of the sample with 7.5 ml of water. Prepare a standard solution containing 40 µg of formaldehyde in 10 ml of water. To each solution add 0.15 ml of a 5% solution of 5,5-dimethyl-1,3-cyclohexanedione in alcohol, and evaporate on a steam bath until the acetone is volatilized. Dilute to 10 ml with water, and cool quickly in an ice bath while stirring vigorously. Any turbidity in the sample solution does not exceed that produced in the standard.

Alkalinity Add 1 drop of methyl red TS to 25 ml of water, add 0.1 *N* sulfuric acid until a red color just appears, then add 23 ml (about 18 g) of the sample, and mix. Not more than 0.1 ml of 0.1 *N* sulfuric acid is required to restore the red color.

Distillation Range Proceed as directed in the general method, page 478.

Heavy Metals Evaporate 25 ml (about 20 g) of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Methanol Dilute 10 ml of the sample to 100 ml with water. Prepare a standard solution in water containing 40 µg of methanol in each ml. To 1 ml of each solution add 0.2 ml of 10% phosphoric acid and 0.25 ml of potassium permanganate solution (1 in 20). Allow to stand for 15 min, then add 0.3 ml of sodium bisulfite solution (1 in 10), and shake until colorless. Slowly add 5 ml of ice-cold 80% sulfuric acid, keeping the mixture cold during the addition. Add 0.1 ml of chromotropic acid solution (1 in 100), mix, and digest on a steam bath for 20 min. Any violet color produced in the sample solution does not exceed that produced in the standard.

Nonvolatile Residue Evaporate 125 ml (about 100 g) of the sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Phenols Evaporate 3 ml of the sample to dryness at 60°. To the residue add 3 drops of a solution of 100 mg of sodium nitrite in 5 ml of sulfuric acid, allow to stand for about 3 min, and then carefully add 3 ml of 2 *N* sodium hydroxide. No color is produced.

Solubility in Water Mix 38 ml (about 30 g) of the sample with an equal volume of carbon dioxide–free water. The solution remains clear for at least 30 min.

Substances Reducing Permanganate Transfer 10 ml of the sample into a glass-stoppered cylinder, add 0.05 ml of 0.1 *N* potassium permanganate, mix, and allow to stand for 15 min. The pink color is not entirely discharged.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552, using freshly distilled pyridine instead of methanol as the solvent.

Packaging and Storage Store in tight containers, remote from fire.

Functional Use in Foods Extraction solvent.

Acetone Peroxides

DESCRIPTION

A mixture of monomeric and linear dimeric acetone peroxides (mainly 2,2-hydroperoxypropane), with minor proportions of higher polymers, usually mixed with an edible carrier such as cornstarch. The cornstarch mixture is a fine, white, free-flowing powder having a sharp, acrid odor similar to that of hydrogen peroxide when the container is first opened.

Caution: Acetone peroxides are strong oxidizing agents. Exposure to the skin and eyes should be avoided.

REQUIREMENTS

Identification

Dissolve about 20 mg of the sample in 5 ml of dilute sulfuric acid (1 in 10), allow to stand for a few min, and add a drop of potassium permanganate TS. The pink color is discharged.

Assay It yields an amount of hydrogen peroxide equivalent to not less than 16.0% of acetone peroxides.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Transfer about 200 mg, accurately weighed, into a 250-ml beaker, add 50 ml of dilute sulfuric acid (1 in 10), allow to stand for at least 3 min, stirring occasionally, and titrate with 0.1 *N* potassium permanganate to a light pink color that persists for at least 20 s. Calculate the total peroxides, *P*, as g of hydrogen peroxide equivalents per 100 g of the sample, by the formula

$$V \times N \times 0.017 \times 100/W,$$

in which *V* and *N* are the volume and exact normality, respectively, of the potassium permanganate, 0.017 is the milliequivalent weight of H_2O_2, and *W* is the weight, in g, of the sample taken. Multiply the value *P* so obtained by 1.6 to convert to percentage of acetone peroxides.

Sample Solution for the Determination of Arsenic and Heavy Metals Mix 10 g with 100 ml of dilute sulfuric acid (1 in 10), allow to stand for 5 min, stirring occasionally, and filter. Heat the filtrate on a steam bath for 15 min, then boil for 1 min, cool, and dilute to 100 ml with water.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dilute 20 ml of the *Sample Solution* to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in a cool, dry place, preferably below 24°.

Functional Use in Foods Bleaching agent; maturing agent; dough conditioner.

Acetylated Monoglycerides

Acetylated Mono- and Diglycerides

DESCRIPTION

Acetylated monoglycerides consist of partial or complete esters of glycerin with a mixture of acetic acid and edible fat-forming fatty acids. They may be manufactured by the interesterification of edible fats with triacetin and glycerin in the presence of catalytic agents, followed by molecular distillation, or by the direct acetylation of edible monoglycerides with acetic anhydride without the use of catalyst or molecular distillation. They vary in consistency from clear, thin liquids to solids, and are from white to pale yellow in color. They may have an acetic acid odor, but are practically bland in taste. They are insoluble in water, but are soluble in alcohol, in acetone, and in other organic solvents, the extent of solubility depending upon the degree of esterification and the melting range.

REQUIREMENTS

Acid Value Not more than 6.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Reichert-Meissl Value Between 75 and 150.

The following specifications should conform to the representations of the vendor: **Free Glycerin, Iodine Value,** and **Saponification Value.**

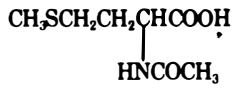
TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Free Glycerin Determine as directed in the general method, page 504.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Reichert-Meissl Value Determine as directed in the general method, page 508.
Saponification Value Determine as directed in the general method, page 509.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Emulsifier; coating agent; texture-modifying agent; solvent; lubricant.

N-Acetyl-L-Methionine

N-Acetyl-L-2-amino-4-(methylthio)butyric Acid



$C_7H_{13}NO_3S$ Mol wt 191.24

DESCRIPTION

A colorless or lustrous white crystalline solid, or a white powder. It is odorless or practically odorless. It is soluble in water, in alcohol, in alkali solutions, and in dilute mineral acids, but is practically insoluble in ether.

REQUIREMENTS

Identification

Dissolve 250 mg of the sample in 2.5 ml of isopropyl alcohol, dilute to 25 ml with water, and then dilute 10 ml of this solution to 100 ml with water. Spot 0.5, 30, and 50 μl of the solution 2 cm from the bottom of a suitable thin-layer chromatographic plate coated with silica gel (e.g., 20- × 20-cm Brinkman Silica Gel 60, 250 μm, or equivalent), and allow the plate to develop for a distance of 10 cm in a sealed equilibrated thin-layer chromatographic chamber, using a solvent composed of 75 volumes of *n*-butanol, 20 volumes of acetic acid, and 20 volumes of water. Dry the plate overnight, and spray with iodoplatinate solution prepared fresh before use by mixing 3 ml of 10% hexachloroplatinic (IV) acid with 97 ml of water and 100 ml of 6% potassium iodide solution. The sample forms a single colorless spot having an R_f value of 0.67 ± 0.1.

Assay Not less than 99.0% of $C_7H_{13}NO_3S$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20}$: Between −19.0° and −23.0°.

TESTS

Assay Transfer about 250 mg of the sample, accurately weighed, into a glass-stoppered flask, and add 100 ml of water, 5 g of dibasic potassium phosphate, 2 g of monobasic potassium phosphate, and 2 g of potassium iodide. Mix well to dissolve, add 50.0 ml of 0.1 *N* iodine, stopper the flask, and mix. Allow to stand for 30 min, and then titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a residual blank titration (see page 2). Each ml of 0.1 *N* iodine is equivalent to 9.562 mg of $C_7H_{13}NO_3S$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying Dry at 105° for 2 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution containing 5 g in sufficient 2 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Adipic Acid

Hexanedioic Acid; 1,4-Butanedicarboxylic Acid

$$HOOC(CH_2)_4COOH$$

$C_6H_{10}O_4$ Mol wt 146.14

DESCRIPTION

White crystals or crystalline powder. It is soluble in acetone, freely soluble in alcohol, and slightly soluble in water. It is not hygroscopic.

REQUIREMENTS

Assay Not less than 99.6% and not more than the equivalent of 101.0% of $C_6H_{10}O_4$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Melting Range Between 151.5° and 154°.

Residue on Ignition Not more than 0.002%.

Water Not more than 0.2%.

TESTS

Assay Mix about 1.5 g, accurately weighed, with 75 ml of recently boiled and cooled water in a 250-ml glass-stoppered Erlenmeyer flask, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a faint pink endpoint that persists for at least 30 s, shaking the flask as the endpoint is approached. Each ml of 0.5 *N* sodium hydroxide is equivalent to 36.54 mg of $C_6H_{10}O_4$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition Transfer 100.0 g to a tared 125-ml platinum dish that has been previously cleaned by fusing with 5 g of potassium pyrosulfate or bisulfate, followed by boiling in diluted sulfuric acid TS and rinsing with water. Melt the sample completely over a gas burner, then ignite the melt with the burner. After ignition starts, lower or remove the flame in order to prevent the sample from boiling and to keep it burning slowly until it is completely carbonized. Ignite at 850° in a muffle furnace for 30 min or until the carbon is completely removed, cool, and weigh.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Buffer; neutralizing agent.

Agar

DESCRIPTION

A dried hydrophilic, colloidal polygalactoside extracted from *Gelidium cartilagineum* (L.) Gaillon (Fam. *Gelidiaceae*), *Gracilaria confervoides* (L.) Greville (Fam. *Sphaerococcaceae*), and related red algae (Class *Rhodophyceae*). It is commercially available in bundles consisting of thin, membranous agglutinated strips, or in cut, flaked, granulated, or powdered forms. It is white to pale yellow in color, is either odorless or has a slight characteristic odor, and has a mucilaginous taste. Agar is insoluble in cold water, but is soluble in boiling water.

REQUIREMENTS

Identification

A. Place a few fragments of unground agar or a small amount of the powder on a slide, add a few drops of water, and examine microscopically. The agar appears granular and somewhat filamentous. A few fragments of the spicules of sponges and a few frustules of diatoms may be present.

B. Boil 1 g with 65 ml of water for 10 min with continuous stirring, and adjust to a concentration of 1.5%, by weight, with hot water. A clear liquid is obtained that congeals between 32° and 39° to form a firm, resilient gel that does not liquefy below 85°.

Arsenic (as As) Not more than 3 ppm.

Ash (Acid-Insoluble) Not more than 0.5% on the dried basis.

Ash (Total) Not more than 6.5% on the dried basis.

Gelatin Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.
Insoluble Matter Not more than 1%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 20%.
Starch Passes test.
Water Absorption Passes test.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Acid-Insoluble) Determine as directed in the general method, page 466.

Ash (Total) Determine as directed in the general method, page 466.

Gelatin Dissolve about 1 g in 100 ml of boiling water, and allow to cool to about 50°. To 5 ml of the solution add 5 ml of trinitrophenol TS. No turbidity appears within 10 min.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Insoluble Matter To 7.5 g add sufficient water to make 500 g, boil for 15 min, and readjust to the original weight. To 100 g of the mixture add hot water to make 200 ml, heat almost to boiling, filter while hot through a tared filtering crucible, rinse the container with several portions of hot water, and pass the rinsings through the crucible. Dry the crucible and its contents at 105° to constant weight, cool, and weigh. The weight of the residue does not exceed 15 mg.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 5 h. Cut unground agar into pieces from 2 to 5 mm square before drying.

Starch Boil 100 mg in 100 ml of water, cool, and add a few drops of iodine TS. No blue color is produced.

Water Absorption Place 5 g in a 100-ml graduated cylinder, fill to the mark with water, mix, and allow to stand at about 25° for 24 h. Pour the contents of the cylinder through moistened glass wool, allowing the water to drain into another 100-ml graduated cylinder. Not more than 75 ml of water is obtained.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; emulsifier; thickener.

DL-Alanine

DL-2-Aminopropanoic Acid

$CH_3CH(NH_2)COOH$

$C_3H_7NO_2$ Mol wt 89.09

DESCRIPTION

A white, odorless, crystalline powder having a sweetish taste. It is freely soluble in water, but sparingly soluble in alcohol. It is optically inactive. The pH of a 1 in 20 solution is between 5.5 and 7.0. It melts with decomposition at about 198°.

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS for 3 min. A violet color is produced.
B. Dissolve 200 mg in 10 ml of water, add 100 mg of potassium permanganate, and heat to boiling. The odor of acetaldehyde is detected.

Assay Not less than 98.5% and not more than the equivalent of 102.0% of $C_3H_7NO_2$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Dissolve about 200 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 8.909 mg of $C_3H_7NO_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

L-Alanine

L-2-Aminopropanoic Acid

$CH_3CH(NH_2)COOH$

$C_3H_7NO_2$ Mol wt 89.09

DESCRIPTION

A white, odorless, crystalline powder having a sweetish taste. It is freely soluble in water, sparingly soluble in alcohol, and insoluble in ether. The pH of a 1 in 20 solution is between 5.5 and 7.0.

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS for 3 min. A violet color is produced.
B. Dissolve 200 mg in 10 ml of water, add 100 mg of potassium permanganate, and heat to boiling. The odor of acetaldehyde is detected.

Assay Not less than 98.5% and not more than the equivalent of 102.0% of $C_3H_7NO_2$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.2%.
Specific Rotation $[\alpha]_D^{20}$: Between +13.5° and +15.5°, on the dried basis.

TESTS

Assay Dissolve about 200 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 8.909 mg of $C_3H_7NO_2$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 10 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Alginic Acid

$(C_6H_3O_6)_n$ Equiv wt, *Calculated*, 176.13
Equiv wt, *Actual* (Avg), 200.00

DESCRIPTION

Alginic acid is a hydrophilic colloidal carbohydrate extracted by the use of dilute alkali from various species of brown seaweeds (*Phaeophyceae*). It may be described chemically as a linear glycuronoglycan consisting mainly of β-(1 → 4) linked D-mannuronic and L-guluronic acid units in the pyranose ring form. It occurs as a white to yellowish white, fibrous powder. It is odorless and tasteless. Alginic acid is insoluble in water, readily soluble in alkaline solutions, and insoluble in organic solvents. The pH of a 3 in 100 suspension in water is between 2.0 and 3.4.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 150 solution in 0.1 *N* sodium hydroxide add 1 ml of calcium chloride TS. A voluminous gelatinous precipitate is formed.
B. To 5 ml of the solution prepared for *Identification Test A* add 1 ml of diluted sulfuric acid TS. A heavy gelatinous precipitate is formed.
C. To about 5 mg, contained in a test tube, add 5 ml of water, 1 ml of a freshly prepared 1 in 100 solution of naphtholresorcinol in ethanol, and 5 ml of hydrochloric acid. Heat the mixture to boiling, boil gently for about 3 min, and then cool to about 15°. Transfer the contents of the test tube to a 30-ml separator with the aid of 5 ml of water and extract with 15 ml of isopropyl ether. Perform a blank test (see page 2). The isopropyl ether extract from the sample exhibits a deeper purplish hue than that from the blank.

Assay It yields not less than 20% and not more than 23% of carbon dioxide (CO_2), corresponding to between 91% and 104.5% of alginic acid (Equiv wt 200.00), calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Ash Not more than 4% after drying.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 15%.

TESTS

Assay Proceed as directed under *Alginates Assay*, page 463. Each ml of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 25 mg of alginic acid (equiv wt 200.00).

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash Weigh accurately about 3 g in a tared crucible, and incinerate at about 650° until free from carbon. Cool the crucible and its contents in a desiccator, weigh, and determine the weight of the ash.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, but use nitric acid instead of sulfuric acid to wet the sample prior to ignition, and cautiously ignite in a platinum crucible. Any color does not exceed that produced in a control (*Solution A*) containing 20 μg of lead ion (Pb).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Stabilizer; thickener; emulsifier.

Almond Oil, Bitter, FFPA

Bitter Almond Oil Free from Prussic Acid

DESCRIPTION

A volatile oil obtained from *Prunus amygdalus* Batsch var. *amara* (De Candolle) Focke (Fam. *Rosaceae*), apricot kernel (*Prunus armeniaca* L.), and other fruit kernels containing amygdalin. It is prepared by steam distillation of a water-macerated, powdered, and pressed cake that has been specially treated and redistilled to remove hydrocyanic acid. It is a colorless to slightly yellow liquid having a strong almondlike aroma and a slightly astringent, mild taste. It is soluble in most fixed oils and in propylene glycol, and it is slightly soluble in mineral oil. It is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 585, using the same test conditions as specified therein.

Assay Not less than 95.0% of aldehydes, calculated as benzaldehyde (C_7H_6O).

Acid Value Not more than 8.0.

Angular Rotation Optically inactive, or not more than ±0.15°.

Chlorinated Compounds Passes test.

Heavy Metals (as Pb) Passes test.

Hydrocyanic Acid Passes test (about 0.15%).

Refractive Index Between 1.541 and 1.546 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 1.040 and 1.050.

TESTS

Assay Weigh accurately about 1 ml, and proceed as directed under *Aldehydes*, page 499, using 53.05 as the equivalence factor (*e*) in the calculation.

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Chlorinated Compounds Proceed as directed in the general method, page 500.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Hydrocyanic Acid To a 1-ml sample in a test tube, add 1 ml of water, 5 drops of a 1 in 10 sodium hydroxide solution, and 5 drops of a 1 in 10 ferrous sulfate solution. Shake thoroughly and acidify with 0.5 *N* hydrochloric acid. No blue precipitate or color forms.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves to form a clear solution in 2 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Aluminum Ammonium Sulfate

Ammonium Alum

$AlNH_4(SO_4)_2{\cdot}12H_2O$ Mol wt 453.32

DESCRIPTION

Large, colorless crystals, white granules, or a powder. It is odorless and has a sweetish, strongly astringent taste. One g dissolves in 7 ml of water at 25° and in about 0.3 ml of boiling water. It is insoluble in alcohol, and is freely, but slowly, soluble in glycerin. Its solutions are acid to litmus.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Aluminum*, page 515, for *Ammonium*, page 515, and for *Sulfate*, page 517.

Assay Not less than 99.5% of $AlNH_4(SO_4)_2 \cdot 12H_2O$.
Alkalies and Alkaline Earths Passes test.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 1 g, dissolve it in 50 ml of water, add 50.0 ml of 0.05 *M* disodium EDTA, and boil gently for 5 min. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone TS. Titrate with 0.05 *M* zinc sulfate to a bright rose pink color, and perform a blank determination (see page 2). Each ml of 0.05 *M* disodium EDTA is equivalent to 22.67 mg of $AlNH_4(SO_4)_2 \cdot 12H_2O$.

Alkalies and Alkaline Earths Completely precipitate the aluminum from a boiling solution of 1 g of the sample in 100 ml of water by the addition of enough ammonia TS to render the solution distinctly alkaline to methyl red TS, and filter. Evaporate the filtrate to dryness, and ignite. The weight of the residue does not exceed 5 mg.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride

Lime Suspension Carefully slake about 56 g of low-fluorine calcium oxide (about 2 ppm F) with 250 ml of water, and add 250 ml of 60% perchloric acid slowly and with stirring. Add a few glass beads, and boil to copious fumes of perchloric acid, then cool, add 200 ml of water, and boil again. Repeat the dilution and boiling once more, cool, dilute considerably, and filter through a fritted-glass filter if precipitated silicon dioxide appears. Pour the clear solution, with stirring, into 1000 ml of sodium hydroxide solution (1 in 10), allow the precipitate to settle, and siphon off the supernatant liquid. Remove the sodium salts from the precipitate by washing five times in large centrifuge bottles, shaking the mass thoroughly each time. Finally, shake the precipitate into a suspension and dilute to 2000 ml. Store in paraffin-lined bottles and shake well before use. (NOTE: 100 ml of this suspension should give no appreciable fluoride blank when evaporated, distilled, and titrated as directed in the *Fluoride Limit Test*, page 510.)

Procedure Assemble the distilling apparatus as described in the *Fluoride Limit Test*, page 510, and add to the distilling flask 1.67 g of the sample, accurately weighed, and 25 ml of dilute sulfuric acid (1 in 2). Distil until the temperature reaches 160°, then maintain at 160° to 165° by adding water from the funnel, collecting 300 ml of distillate. Oxidize the distillate by the cautious addition of 2 or 3 ml of fluorine-free 30% hydrogen peroxide (to remove sulfates), allow to stand for a few min, and evaporate in a platinum dish with an excess of *Lime Suspension*. Ignite briefly at 600°, then cool and wet the ash with about 10 ml of water. Cover the dish with a watch glass, and cautiously introduce under cover just sufficient 60% perchloric acid to dissolve the ash. Add the contents of the dish through the dropping funnel of a freshly prepared distilling apparatus (the distilling flask should contain a few glass beads), using a total of 20 ml of the perchloric acid for dissolving the ash and transferring the solution. Add 10 ml of water and a few drops of silver perchlorate solution (1 in 2) through the dropping funnel, and continue as directed in the *Fluoride Limit Test*, page 510, beginning with "Distil until the temperature reaches 135°. . . . "

Heavy Metals Dissolve 1 g in 20 ml of water, add a few drops of diluted hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Buffer; neutralizing agent.

Aluminum Potassium Sulfate

Potassium Alum

$AlK(SO_4)_2 \cdot 12H_2O$ Mol wt 474.38

DESCRIPTION

Large, transparent crystals or crystalline fragments, or a white crystalline powder. It is odorless and has a sweetish, astringent taste. One g dissolves in 7.5 ml of water at 25° and in about 0.3 ml of boiling water. It is insoluble in alcohol, but is freely soluble in glycerin. Its solutions are acid to litmus.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Aluminum*, page 515, for *Potassium*, page 517, and for *Sulfate*, page 517.

Assay Not less than 99.5% of $AlK(SO_4)_2.12H_2O$.
Ammonium Salts Passes test.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 1 g, dissolve it in 50 ml of water, add 50.0 ml of 0.05 *M* disodium EDTA, and boil gently for 5 min. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone TS. Titrate with 0.05 *M* zinc sulfate to a bright rose pink color, and perform a blank determination (see page 2). Each ml of 0.05 *M* disodium EDTA is equivalent to 23.72 mg of $AlK(SO_4)_2.12H_2O$.

Ammonium Salts Heat 1 g with 10 ml of sodium hydroxide TS on a steam bath for 1 min. The odor of ammonia is not perceptible.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in the test for *Fluoride* under *Aluminum Ammonium Sulfate*, page 15.

Heavy Metals Dissolve 1 g in 20 ml of water, add a few drops of diluted hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Buffer; neutralizing agent; firming agent.

Aluminum Sodium Sulfate

Soda Alum; Sodium Alum

$AlNa(SO_4)_2$ Mol wt 242.09

DESCRIPTION

Aluminum sodium sulfate is anhydrous or may contain up to 12 molecules of water of hydration. It occurs as colorless crystals, white granules, or a powder. It is odorless and has a saline, astringent taste. The anhydrous form is slowly soluble in water. The dodecahydrate is freely soluble in water, and it effloresces in air. Both forms are insoluble in alcohol.

REQUIREMENTS

Identification

It responds to the flame test for *Sodium*, page 517, and gives positive tests for *Aluminum*, page 515, and for *Sulfate*, page 517.

Assay *Anhydrous form:* Not less than 96.5% of $AlNa(SO_4)_2$ after drying; *dodecahydrate:* not less than 99.5% of $AlNa(SO_4)_2$ after drying.
Ammonium Salts Passes test.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying *Anhydrous form:* Not more than 10%; *dodecahydrate:* not more than 47.2%.
Neutralizing Value *Anhydrous form:* Between 103 and 107.
Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 500 mg of a sample previously dried as directed in the test for *Loss on Drying*, moisten with 1 ml of acetic acid, and dissolve it in 50 ml of water, warming gently on a steam bath until solution is complete. Cool, neutralize with ammonia TS, add 50.0 ml of 0.05 *M* disodium EDTA, and boil gently for 5 min. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone TS. Titrate with 0.05 *M* zinc sulfate to a bright rose pink color, and perform a blank determination (see page 2). Each ml of 0.05 *M* disodium EDTA is equivalent to 12.10 mg of $AlNa(SO_4)_2$.

Ammonium Salts Heat 1 g with 10 ml of sodium hydroxide TS on a steam bath for 1 min. The odor of ammonia is not perceptible.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in the test for *Fluoride* under *Aluminum Ammonium Sulfate*, p. 15.

Heavy Metals Dissolve 1 g in 20 ml of water, add a few drops of diluted hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 *Anhydrous form:* dry at 200° for 16

h; *dodecahydrate:* dry first at 50° to 55° for 1 h, then at 200° for 16 h.

Neutralizing Value Weigh accurately 500 mg of the anhydrous form into a 200-ml Erlenmeyer flask, add 30 ml of water and 4 drops of phenolphthalein TS, and boil until the sample dissolves. Add 13.0 ml of 0.5 *N* sodium hydroxide, boil for a few seconds, and titrate with 0.5 *N* hydrochloric acid to the disappearance of the pink color, adding the acid dropwise and agitating vigorously after each addition. Calculate the neutralizing value, as parts of $NaHCO_3$ equivalent to 100 parts of the sample, by the formula $8.4V$, in which V is the volume, in ml, of 0.5 *N* sodium hydroxide consumed by the sample.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; neutralizing agent; firming agent.

Aluminum Sulfate

$Al_2(SO_4)_3 \cdot xH_2O$ Mol wt (anhydrous) 342.14

DESCRIPTION

Aluminum sulfate is anhydrous or contains 18 molecules of water of crystallization. Due to efflorescence, the hydrate may have a composition approximating the formula $Al_2(SO_4)_3 \cdot 14H_2O$. It occurs as a white powder, as shining plates, or as crystalline fragments. It is odorless and has a sweet taste, becoming mildly astringent. One g of the hydrate dissolves in about 2 ml of water. The anhydrous product approaches the same solubility, but the rate of solution is so slow that it initially appears to be relatively insoluble. The pH of a 1 in 20 solution is 2.9 or above.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Aluminum*, page 515, and for *Sulfate*, page 517.

Assay $Al_2(SO_4)_3$ (anhydrous): not less than 99.5% of $Al_2(SO_4)_3$, calculated on the ignited basis; $Al_2(SO_4)_3 \cdot 18H_2O$ (hydrate): not less than 99.5% and not more than the equivalent of 114.0% of $Al_2(SO_4)_3 \cdot 18H_2O$, corresponding to not more than approximately 101.7% of $Al_2(SO_4)_3 \cdot 14H_2O$.

Alkalies and Alkaline Earths Passes test (about 0.4%).

Ammonium Salts Passes test.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.003%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Ignition $Al_2(SO_4)_3$ (anhydrous): not more than 5%. [NOTE: This specification does not apply to $Al_2(SO_4)_3 \cdot 18H_2O$.]

Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately an amount of sample equivalent to about 4 g of $Al_2(SO_4)_3$, transfer into a 250-ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 250-ml beaker, add 25.0 ml of 0.05 *M* disodium EDTA, and boil gently for 5 min. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone TS. Titrate with 0.05 *M* zinc sulfate until the color changes from green violet to rose pink, and perform a blank determination (see page 2), substituting 10 ml of water for the sample. Each ml of 0.05 *M* disodium EDTA is equivalent to 8.554 mg of $Al_2(SO_4)_3$ or to 16.66 mg of $Al_2(SO_4)_3 \cdot 18H_2O$.

Alkalies and Alkaline Earths To a boiling solution of 2 g in 150 ml of water add a few drops of methyl red TS, and then add ammonia TS until the color of the solution just changes to a distinct yellow. Add hot water to restore the original volume, and filter while hot. Evaporate 75 ml of the filtrate to dryness, and ignite to constant weight. Not more than 4 mg of residue remains.

Ammonium Salts Heat 1 g with 10 ml of sodium hydroxide TS on a steam bath for 1 min. The odor of ammonia is not perceptible.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in the test for *Fluoride* under *Aluminum Ammonium Sulfate*, page 15.

Heavy Metals Dissolve 500 mg in 20 ml of water, add a few drops of diluted hydrochloric acid TS, and evaporate to dryness in a porcelain dish. Treat the residue with 20 ml of water, and add 50 mg of hydroxylamine hydrochloride. Heat on a steam bath for 10 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 50 mg of hydroxylamine hydrochloride in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Ignition Weigh accurately about 2 g of $Al_2(SO_4)_3$ (anhydrous), and ignite, preferably in a muffle furnace, at about 500° for 3 h. [NOTE: This test does not apply to $Al_2(SO_4)_3 \cdot 18H_2O$.]

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Firming agent.

Ambrette Seed Oil

Ambrette Seed Liquid

DESCRIPTION

The volatile oil obtained by steam distillation from the partially dried and crushed seeds of the plant *Abelmoschus moschatus* Moench, syn. *Hibiscus Abelmoschus* L. (Fam. *Malvaceae*). It is refined by solvent extraction to remove fatty acids, or precipitation of the fatty acid salts. It is a clear yellow to amber liquid having the strong musky odor of ambrettolide. It is soluble in most fixed oils and in mineral oil, often with cloudiness. It is relatively insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 608, using the same test conditions as specified therein.

Acid Value Not more than 3.0.
Angular Rotation Between $-2.5°$ and $+3°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.468 and 1.485 at 20°.
Saponification Value Between 140 and 200.
Specific Gravity Between 0.898 and 0.920.

TESTS

Acid Value Determine as directed in the general method, page 499.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using about 1 g, accurately weighed.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Ammonium Alginate

Algin

$(C_6H_7O_6NH_4)_n$ Equiv wt, *Calculated*, 193.16
Equiv wt, *Actual* (Avg), 217.00

DESCRIPTION

The ammonium salt of alginic acid (see *Alginic Acid*, page 13) occurs as a white to yellowish, fibrous or granular powder. It dissolves in water to form a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than about 30% by weight. It is insoluble in chloroform, in ether, and in acids having a pH lower than about 3.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 100 solution add 1 ml of calcium chloride TS. A voluminous, gelatinous precipitate is formed.
B. To 10 ml of a 1 in 100 solution add 1 ml of diluted sulfuric acid TS. A heavy gelatinous precipitate is formed.
C. Ammonium alginate meets the requirements of *Identification Test C* under *Alginic Acid*, page 13.
D. To about 1 g of ammonium alginate contained in a test tube add 5 ml of sodium hydroxide TS, and shake the mixture briefly. The odor of ammonia is evolved.

Assay It yields not less than 18% and not more than 21% of carbon dioxide (CO_2), corresponding to between 88.7% and 103.6% of ammonium alginate (equiv wt 217.00), calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Ash Not more than 4% after drying.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 15%.

TESTS

Assay Proceed as directed under *Alginates Assay*, page 14. Each ml of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.12 mg of ammonium alginate (equiv wt 217.00).
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Ash Determine as directed under *Ash* in the monograph on *Alginic Acid*, page 14.
Heavy Metals Determine as directed in the test for *Heavy Metals* under *Alginic Acid*, page 14.
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Ammonium Bicarbonate

NH_4HCO_3 Mol wt 79.06

DESCRIPTION

White crystals or a crystalline powder having a slight odor of ammonia. At a temperature of 60° or above it volatilizes rapidly, dissociating into ammonia, carbon dioxide, and water, but at room temperature it is quite stable. One g dissolves in about 6 ml of water. It is insoluble in alcohol.

REQUIREMENTS

Identification

It gives positive tests for *Ammonium*, page 515, and for *Bicarbonate*, page 516.

Assay Not less than 99.0% of NH_4HCO_3.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 10 ppm.
Nonvolatile Residue Not more than 0.05% (0.55% for products containing a suitable anticaking agent).
Sulfur Compounds Not more than 0.007%.

TESTS

Assay Weigh accurately about 3 g, dissolve it in 40 ml of water, add methyl orange TS, and titrate with 1 *N* sulfuric acid. Each ml of 1 *N* sulfuric acid is equivalent to 79.06 mg of NH_4HCO_3.
Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.
Chloride, page 471 Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 15 µg of chloride ion (Cl).
Heavy Metals Dissolve the residue from the test for *Nonvolatile Residue* in 1 ml of diluted hydrochloric acid TS, evaporate to dryness, and dissolve the residue in 50 ml of water. A 25-ml portion of this solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Nonvolatile Residue Transfer 4 g into a tared dish, add 10 ml of water, evaporate on a steam bath, and then dry at 105°. The weight of the residue does not exceed 2 mg. Retain the residue for the *Heavy Metals Test*.
Sulfur Compounds Dissolve 4 g in 40 ml of water, add about 10 mg of sodium carbonate and 1 ml of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed in the *Sulfate Limit Test*, page 471. Any turbidity produced does not exceed that shown by 280 µg of sulfate ion (SO_4).

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Alkali; leavening agent.

Ammonium Carbonate

DESCRIPTION

Ammonium carbonate consists of ammonium bicarbonate (NH_4HCO_3) and ammonium carbamate (NH_2COONH_4) in varying proportions. It occurs as a white powder or as hard, white or translucent masses. Its solutions are alkaline to litmus. On exposure to air it becomes opaque and is finally converted into porous lumps or a white powder of ammonium bicarbonate due to the loss of ammonia and carbon dioxide. One g dissolves slowly in about 4 ml of water.

REQUIREMENTS

Identification

When heated, it volatilizes without charring and the vapor is alkaline to moistened litmus paper. A 1 in 20 solution effervesces upon the addition of an acid.

Assay Not less than 30.0% and not more than 34.0% of NH_3.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 10 ppm.
Nonvolatile Residue Not more than 0.05%.
Sulfur Compounds Not more than 0.005%.

TESTS

Assay Place about 10 ml of water in a weighing bottle, tare the bottle and its contents, add about 2 g of ammonium carbonate, and weigh accurately. Transfer the contents of the bottle to a 250-ml flask, and slowly add, with mixing, 50.0 ml of 1 *N* sulfuric acid, allowing for the release of carbon dioxide. When solution has been effected, wash down the sides of the flask with a few ml of water, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each ml of 1 *N* sulfuric acid is equivalent to 17.03 mg of NH_3.
Arsenic A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.
Chloride Dissolve 500 mg in 10 ml of hot water, add about 5 mg of sodium carbonate, and evaporate to dryness on a steam bath. Treat the residue as directed in the *Chloride Limit Test*, page 471. Any turbidity produced does not exceed that shown in a control containing 15 µg of chloride ion (Cl).
Heavy Metals Dissolve the residue from the test for *Nonvolatile Residue* in 1 ml of diluted hydrochloric acid TS, and

evaporate to dryness. Dissolve the residue in water to make 50 ml. A 25-ml portion of this solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Transfer 4 g into a tared dish, add 10 ml of water, evaporate on a steam bath, and then dry for 1 h at 105°. The weight of the residue does not exceed 2 mg. Retain the residue for the *Heavy Metals Test*.

Sulfur Compounds Dissolve 4 g in 40 ml of water, add about 10 mg of sodium carbonate and 1 ml of 30% hydrogen peroxide, and evaporate the solution to dryness on a steam bath. Treat the residue as directed in the *Sulfate Limit Test*, page 471. Any turbidity produced does not exceed that shown in a control containing 200 µg of sulfate (SO_4).

Packaging and Storage Store in tight, light-resistant containers, preferably at a temperature not exceeding 30°.

Functional Use in Foods Miscellaneous and general purpose; buffer; neutralizing agent.

Ammonium Chloride

NH_4Cl Mol wt 53.49

DESCRIPTION

Colorless crystals, or a white, fine or coarse crystalline powder. It has a cool, saline taste and is somewhat hygroscopic. One g dissolves in 2.6 ml of water at 25°, in 1.4 ml of boiling water, in about 100 ml of alcohol, and in about 8 ml of glycerin. The pH of a 1 in 20 solution is between 4.5 and 6.0.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Ammonium*, page 515, and for *Chloride*, page 516.

Assay Not less than 99.0% of NH_4Cl after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

TESTS

Assay Dry about 200 mg over silica gel for 4 h, weigh accurately, and dissolve it in about 40 ml of water in a glass-stoppered flask. Add, while agitating, 3 ml of nitric acid, 5 ml of nitrobenzene, 50.0 ml of 0.1 *N* silver nitrate, shake vigorously, then add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each ml of 0.1 *N* silver nitrate is equivalent to 5.349 mg of NH_4Cl.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry over silica gel for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Yeast food; dough conditioner.

Ammonium Hydroxide

Strong Ammonia Solution; Stronger Ammonia Water

NH_4OH Mol wt 35.05

DESCRIPTION

A clear, colorless solution of NH_3 having an exceedingly pungent, characteristic odor. Upon exposure to air it loses ammonia rapidly. Its specific gravity is about 0.90.

REQUIREMENTS

Identification

Dense, white fumes are produced when a glass rod wet with hydrochloric acid is held near the surface of the liquid.

Assay Not less than 27.0% and not more than 30.0%, by weight, of NH_3.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 5 ppm.

Nonvolatile Residue Not more than 0.02%.

Readily Oxidizable Substances Passes test.

TESTS

Assay Tare accurately a 125-ml glass-stoppered Erlenmeyer flask containing 35.0 ml of 1 *N* sulfuric acid. Partially fill a 10-ml graduated pipet from near the bottom of a sample, previously cooled in the original sample bottle to 10° or lower. (Do not use vacuum for drawing up the sample.) Wipe off any liquid adhering to the outside of the pipet, and discard the first ml. Hold the pipet just above the surface of the acid, and transfer 2 ml into the flask, leaving at least 1 ml in the pipet. Stopper the flask, mix, and weigh again to obtain the weight of the sample. Add methyl red TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each ml of 1 *N* sulfuric acid is equivalent to 17.03 mg of NH_3.

Arsenic Evaporate 11 ml (10-g sample) to about 2 ml on a steam bath, dilute to 50 ml with water, and mix. A 5-ml portion of this solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Transfer 22 ml (20-g sample) to a beaker, add about 5 mg of sodium chloride, evaporate to dryness on a steam bath, and dissolve the residue in 2 ml of diluted acetic acid TS and sufficient water to make 50 ml. A 10-ml portion

of this solution, diluted to 25 ml with water, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Evaporate 11 ml (10-g sample) in a tared platinum or porcelain dish to dryness, dry at 105° for 1 h, cool, and weigh.

Readily Oxidizable Substances Dilute 4 ml with 6 ml of water, and add a slight excess of diluted sulfuric acid TS and 0.1 ml of 0.1 *N* potassium permanganate. The pink color does not completely disappear within 10 min.

Packaging and Storage Store in tight containers, preferably at a temperature not exceeding 25°.

Functional Use in Foods Alkali.

Ammonium Phosphate, Dibasic

Diammonium Phosphate

$(NH_4)_2HPO_4$ Mol wt 132.06

DESCRIPTION

White, odorless crystals, crystalline powder, or granules having a cooling, saline taste. It is freely soluble in water. The pH of a 1 in 100 solution is between 7.6 and 8.2.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Ammonium*, page 515, and for *Phosphate*, page 517.

Assay Not less than 96.0% and not more than 102.0% of $(NH_4)_2HPO_4$.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Dissolve about 600 mg, accurately weighed, in 40 ml of water, and titrate to a pH of 4.6 with 0.1 *N* sulfuric acid. Each ml of 0.1 *N* sulfuric acid is equivalent to 13.21 mg of $(NH_4)_2HPO_4$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*). (NOTE: Use glacial acetic acid in making the pH adjustment.)

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; dough conditioner; leavening agent; yeast food.

Ammonium Phosphate, Monobasic

Monoammonium Phosphate

$NH_4H_2PO_4$ Mol wt 115.02

DESCRIPTION

White, odorless crystals, crystalline powder, or granules. It is freely soluble in water. The pH of a 1 in 100 solution is between 4.3 and 5.0.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Ammonium*, page 515, and for *Phosphate*, page 517.

Assay Not less than 96.0% and not more than 102.0% of $NH_4H_2PO_4$.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Dissolve about 500 mg, accurately weighed, in 50 ml of water, and titrate to a pH of 8.0 with 0.1 *N* sodium hydroxide. Each ml of 0.1 *N* sodium hydroxide is equivalent to 11.50 mg of $NH_4H_2PO_4$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.1 ml of *Fluoride Standard Solution*.

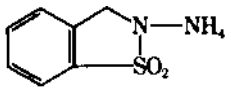
Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; dough conditioner; leavening agent; yeast food.

Ammonium Saccharin

1,2-Benzisothiazolin-3-one 1,1-Dioxide Ammonium Salt



$C_7H_8N_2O_3S$ Mol wt 200.21

DESCRIPTION

White crystals or a white crystalline powder. It is freely soluble in water. The pH of a 1 in 3 solution is between 5 and 6. It is intensely sweet.

REQUIREMENTS

Identification

A. Dissolve about 100 mg in 5 ml of sodium hydroxide solution (1 in 20), evaporate to dryness, and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with diluted hydrochloric acid TS, and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet color.

B. Mix 20 mg with 40 mg of resorcinol, cautiously add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid results.

C. A 1 in 10 solution gives positive tests for *Ammonium*, page 515.

D. To 10 ml of a 1 in 10 solution add 1 ml of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water and dry at 105° for 2 h. It melts between 226° and 230° (*Class Ia*, page 519).

Assay Not less than 98.0% and not more than the equivalent of 101.0% of $C_7H_8N_2O_3S$.
Arsenic (as As) Not more than 3 ppm.
Benzoate and Salicylate Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Readily Carbonizable Substances Passes test.
Selenium Not more than 0.003%.
Toluenesulfonamides Not more than 25 ppm.
Water Not more than 0.3%.

TESTS

Assay Weigh accurately about 500 mg, and transfer it quantitatively to a separator with the aid of 10 ml of water. Add 2 ml of diluted hydrochloric acid TS, and extract the precipitated saccharin first with 30 ml, then with five 20-ml portions of a solvent composed of 9 volumes of chloroform and 1 volume of alcohol. Filter each extract through a small filter paper moistened with the solvent mixture, and evaporate the combined filtrates on a steam bath to dryness with the aid of a current of air. Dissolve the residue in 75 ml of hot water, cool, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination, and make any necessary correction (see page 2). Each ml of 0.1 *N* sodium hydroxide is equivalent to 20.02 mg of $C_7H_8N_2O_3S$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Benzoate and Salicylate To 10 ml of a 1 in 20 solution previously acidified with 5 drops of acetic acid, add 3 drops of ferric chloride TS. No precipitate or violet color appears.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Readily Carbonizable Substances, page 532 Dissolve 200 mg in 5 ml of sulfuric acid TS, and keep at a temperature of 48° to 50° for 10 min. The color is no darker than *Matching Fluid A*.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Toluenesulfonamides Determine as directed under *Sodium Saccharin*, page 298.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nonnutritive sweetener.

Ammonium Sulfate

$(NH_4)_2SO_4$ Mol wt 132.13

DESCRIPTION

Colorless or white, odorless crystals or granules that decompose at temperatures above 280°. One g is soluble in about 1.5 ml of water, and is insoluble in alcohol. The pH of a 0.1 *M* solution is about 4.5 to 6.0.

REQUIREMENTS

Identification

It gives positive tests for *Ammonium*, page 515, and for *Sulfate*, page 517.

Assay Not less than 99.0% of $(NH_4)_2SO_4$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Residue on Ignition Not more than 0.25%.
Selenium Not more than 0.003%.

TESTS

Assay Transfer about 2 g, accurately weighed, into a 250-ml flask and dissolve it in 100 ml of water. To the solution add

40 ml of a mixture of equal volumes of formaldehyde and water, previously neutralized to phenolphthalein TS with 1 *N* sodium hydroxide. Mix, allow to stand for 30 min, and titrate the mixture with 1 *N* sodium hydroxide to a pink endpoint that persists for 5 min. Each ml of 1 *N* sodium hydroxide is equivalent to 66.06 mg of $(NH_4)_2SO_4$.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Miscellaneous and general purpose; dough conditioner; yeast food.

Amyris Oil, West Indian Type

Sandalwood Oil, West Indian Type

DESCRIPTION

The volatile oil obtained by steam distillation from the wood of *Amyris balsamifera* L. (Fam. *Rutaceae*). It is a clear, pale yellow, viscous liquid having a distinct odor suggestive of sandalwood. It is soluble in most fixed oils and usually in mineral oil. It is soluble in an equal volume of propylene glycol, the solution often becoming opalescent on further dilution. It is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 585, using the same test conditions as specified therein.

Acid Value Not more than 3.0.

Angular Rotation Between +10° and +53°.

Ester Value Not more than 7.

Ester Value after Acetylation Between 115 and 165.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.503 and 1.512 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.943 and 0.976.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Ester Value after Acetylation Proceed as directed under *Total Alcohols*, page 499, using about 2 g of the dried acetylated oil, accurately weighed. Reflux for a period of 2 h. Calculate the *Ester Value after Acetylation* by the formula $A \times 28.05/B$, in which A is the number of ml of 0.5 *N* alcoholic potassium hydroxide consumed in the saponification, and B is the weight, in g, of the acetylated oil used in the test.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 80% alcohol, often with opalescence.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably aluminum, glass, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Angelica Root Oil

DESCRIPTION

Angelica root oil is obtained by steam distillation of the dried slender rootlets of *Angelica archangelica* L. It is a pale yellow to deep amber liquid having a warm pungent odor and bitter-sweet taste. It is soluble in most fixed oils, slightly soluble in mineral oil, but relatively insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 585, using the same test conditions as specified therein.

Acid Value Not more than 7.0.

Angular Rotation Optically inactive, or not more than +46.0°.
Ester Value Between 10 and 65.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.473 and 1.487 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.850 and 0.880.

TESTS

Acid Value Determine as directed in the general method, page 499.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Ester Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 90% alcohol, often with turbidity, and remains in solution on further addition of alcohol to a total of 10 ml.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably dark glass bottles, or aluminum or tin-lined containers, in a cool place protected from light. The oils increase in specific gravity and viscosity on storage.
Functional Use in Foods Flavoring agent.

Angelica Seed Oil

DESCRIPTION

Angelica seed oil is obtained by steam distillation of the fresh seeds of *Angelica archangelica* L. It is a light yellow liquid having a sweeter and more delicate aroma than the root oil. It is soluble in most fixed oils, slightly soluble in mineral oil, but relatively insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 586, using the same test conditions as specified therein.

Acid Value Not more than 3.0.
Angular Rotation Between +4° and +16°.
Ester Value Between 14.0 and 32.0.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.480 and 1.488 at 20°C.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.853 and 0.876.

TESTS

Acid Value Determine as directed in the general method, page 499.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Ester Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 90% alcohol, often with considerable turbidity, and remains in solution on further addition of alcohol to a total of 10 ml.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably dark glass bottles, or aluminum or tin-lined containers, in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Anise Oil

DESCRIPTION

Anise oil is obtained by steam distillation of the dried ripe fruit of *Pimpinella anisum* L. (Fam. *Umbelliferae*) or *Illicium verum* Hooker filius (Fam. *Magnoliaceae*). It is a colorless to pale yellow, strongly refractive liquid having the characteristic odor and taste of anise.

NOTE: If solid material has separated, carefully warm the anise oil until it is completely liquefied, and mix before using it.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths

(or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 586, using the same test conditions as specified therein.

Angular Rotation Between $-2°$ and $+1°$.
Heavy Metals (as Pb) Passes test.
Phenols Passes test.
Refractive Index Between 1.553 and 1.560 at 20°.
Solidification Point Not lower than 15°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.978 and 0.988.

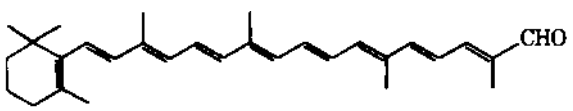
TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Phenols Prepare a 1 in 3 solution of recently distilled anise oil in 90% alcohol. It is neutral to moistened litmus paper, and the mixture develops no blue or brownish color upon the addition of 1 drop of ferric chloride TS to 5 ml of the solution.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solidification Point Determine as directed in the general method, page 538.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 90% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.
Functional Use in Foods Flavoring agent.

β-Apo-8′-Carotenal

Apocarotenal; APO



$C_{30}H_{40}O$ Mol wt 416.65

DESCRIPTION

A fine crystalline powder with a dark metallic sheen. It is freely soluble in chloroform and sparingly soluble in acetone, but is insoluble in water.

REQUIREMENTS

Identification

A. Determine the absorbance of *Sample Solution B*, prepared as directed in the *Assay*, at 488 nm and at 460 nm. The ratio A_{488}/A_{460} is between 0.77 and 0.85.
B. Determine the absorbance of *Sample Solution B* at 460 nm, and that of *Sample Solution A*, prepared as directed in the *Assay*, at 332 nm. The ratio $A_{332}/(10 \times A_{460})$ is between 0.063 and 0.075.

Assay Not less than 96.0% and not more than 101.0% of $C_{30}H_{40}O$.
Arsenic (as As) Not more than 1 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Melting Range Between 136° and 142°, with decomposition.
Residue on Ignition Not more than 0.2%.

TESTS

Assay

NOTE: Carry out all work in low-actinic glassware and in subdued light.

Sample Solution A Transfer about 40 mg of the sample, accurately weighed, into a 100-ml volumetric flask, dissolve in 10 ml of acid-free chloroform, dilute to volume with cyclohexane, and mix. Pipet 2 ml of this solution into a 50-ml volumetric flask, dilute to volume with cyclohexane, and mix.

Sample Solution B Pipet 5 ml of *Sample Solution A* into a 50-ml volumetric flask, dilute to volume with cyclohexane, and mix.

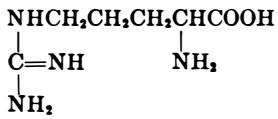
Procedure Determine the absorbance of *Sample Solution B* in a 1-cm cell at the wavelength of maximum absorption at about 460 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in mg, of $C_{30}H_{40}O$ in the sample taken by the formula $25{,}000A/264$, in which A is the absorbance of the solution and 264 is the absorptivity of pure β-apo-8′-carotenal.

Arsenic A *Sample Solution* prepared from a 3-g sample as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Melting Range Determine as directed in the general procedure, page 519.
Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Packaging and Storage Store in tight light-resistant containers under inert gas.
Functional Use in Foods Color.

L-Arginine

L-1-Amino-4-guanidovaleric Acid



$C_6H_{14}N_4O_2$ Mol wt 174.20

DESCRIPTION

White crystals or a white crystalline powder. It is soluble in water, but insoluble in ether and sparingly soluble in alcohol. It is strongly alkaline, and its water solutions absorb carbon dioxide from the air.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A reddish purple color appears.

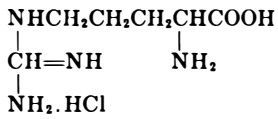
Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_6H_{14}N_4O_2$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 1%.
Residue on Ignition Not more than 0.2%.
Specific Rotation $[\alpha]_D^{20}$: Between +25.0° and +27.9°, on the dried basis.

TESTS

Assay Dissolve about 200 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 8.710 mg of $C_6H_{14}N_4O_2$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 80° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.
Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

L-Arginine Monohydrochloride

L-1-Amino-4-guanidovaleric Acid Monohydrochloride



$C_6H_{14}N_4O_2.HCl$ Mol wt 210.66

DESCRIPTION

A white or nearly white, practically odorless crystalline powder. It is soluble in water, slightly soluble in hot alcohol, and insoluble in ether. It melts with decomposition at about 235°.

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS. A reddish purple color is produced.
B. A 1 in 1000 solution gives positive tests for *Chloride*, page 516.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_6H_{14}N_4O_2.HCl$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20}$: Between +20.5° and +23.0°, on the dried basis.

TESTS

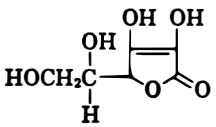
Assay Transfer about 200 mg, accurately weighed, into a 250-ml flask, and dissolve in 3 ml of formic acid and 50 ml of glacial acetic acid. Add 10 ml of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure green color or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 10.53 mg of $C_6H_{14}N_4O_2.HCl$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Ascorbic Acid

Vitamin C; L-Ascorbic Acid



$C_6H_8O_6$ Mol wt 176.13

DESCRIPTION

White or slightly yellow crystals or powder, melting at about 190°. It gradually darkens on exposure to light, is reasonably stable in air when dry, but rapidly deteriorates in solution in the presence of air. One g is soluble in about 3 ml of water and in about 30 ml of alcohol. It is insoluble in chloroform, in ether, and in benzene.

REQUIREMENTS

Identification

A. A 1 in 50 solution slowly reduces alkaline cupric tartrate TS at 25°, but more readily upon heating.
B. To 2 ml of a 1 in 50 solution of the sample add 4 drops of methylene blue TS, and warm to 40°. The deep blue color is practically discharged within 3 min.
C. Dissolve 15 mg of the sample in 15 ml of a 1 in 20 solution of trichloroacetic acid, add about 200 mg of activated charcoal, shake vigorously for 1 min, and filter through a small fluted filter, returning the filtrate, if necessary, until clear. To 5 ml of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, and then heat in a water bath at 50°. A blue color develops.

Assay Not less than 99.0% of $C_6H_8O_6$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{25}$: Between +20.5° and +21.5°.

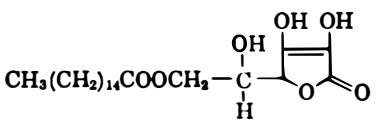
TESTS

Assay Dissolve about 400 mg, accurately weighed, in a mixture of 100 ml of water, recently boiled and cooled, and 25 ml of diluted sulfuric acid TS. Titrate the solution immediately with 0.1 *N* iodine, adding starch TS near the endpoint. Each ml of 0.1 *N* iodine is equivalent to 8.806 mg of $C_6H_8O_6$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 1 g in 10 ml of water.

Packaging and Storage Store in tight, light-resistant containers.
Functional Use in Foods Preservative; antioxidant; nutrient; dietary supplement.

Ascorbyl Palmitate

Palmitoyl L-Ascorbic Acid



$C_{22}H_{38}O_7$ Mol wt 414.54

DESCRIPTION

A white or yellowish white powder having a slight odor. It is very slightly soluble in water and in vegetable oils. One g dissolves in about 4.5 ml of alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution in alcohol decolorizes dichlorophenolindophenol TS.

Assay Not less than 95.0% of $C_{22}H_{38}O_7$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 2%.
Melting Range Between 107° and 117°.

Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{25°}$: Between +21° and +24°, calculated on the dried basis.

TESTS

Assay Dissolve about 300 mg, accurately weighed, in 50 ml of alcohol in a 250-ml Erlenmeyer flask, add 30 ml of water, and immediately titrate with 0.1 *N* iodine to a yellow color that persists for at least 30 s. Each ml of 0.1 *N* iodine is equivalent to 20.73 mg of $C_{22}H_{38}O_7$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry in a vacuum oven at 56° to 60° for 1 h.
Melting Range Determine as directed in *Procedure for Class Ia*, page 519.
Residue on Ignition Ignite 2 g as directed in the general method, page 533.
Specific Rotation, page 530 Determine in a solution containing 1 g in 10 ml of methanol.

Packaging and Storage Store in tight containers, preferably in a cool, dry place.
Functional Use in Foods Antioxidant.

L-Asparagine

L-α-Aminosuccinamic Acid

$$H_2NOCCH_2CH(NH_2)COOH$$

$C_4H_8N_2O_3.H_2O$ Mol wt 150.13

DESCRIPTION

White crystals or crystalline powder having a slightly sweet taste. It is soluble in water and practically insoluble in alcohol and in ether. Its solutions are acid to litmus. It melts at about 234°.

REQUIREMENTS

Identification

To 100 mg of the sample add 5 ml of sodium hydroxide TS, heat on a water bath for 1 h, adjust the pH to 5.0 with diluted hydrochloric acid TS, and add 100 mg of triketohydrindene hydrate. The vapor evolved changes to blue the color of acetaldehyde test paper.

Assay Not less than 98.0% and not more than 101.0% of $C_4H_8N_2O_3$ after drying.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Between 11.5% and 12.5%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20°}$: Between +33.0° and +36.5° after drying.

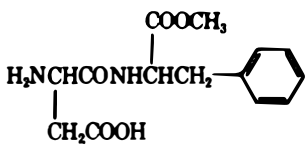
TESTS

Assay Dissolve about 130 mg of the sample, previously dried at 105° for 4 h and accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 13.21 mg of $C_4H_8N_2O_3$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 105° for 4 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 10 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Aspartame

N-L-α-Aspartyl-L-phenylalanine 1-Methyl Ester; APM



$C_{14}H_{18}N_2O_5$ Mol wt 294.31

DESCRIPTION

A white, odorless, crystalline powder having a sweet taste. It is sparingly soluble in water and slightly soluble in alcohol. The pH of an 0.8% solution is between about 4 and 6.5.

REQUIREMENTS

Identification

A. Dissolve 2 g of triketohydrindene in 75 ml of dimethylsulfoxide, add 62 mg of hydrindantin, dilute to 100 ml with 4

M lithium acetate buffer solution (pH 9), and filter. Transfer about 10 mg of the sample to a test tube, add 2 ml of the reagent solution, and heat. A dark purple color forms.

B. Dissolve about 20 mg of the sample in 1 ml of methanol, add 0.5 ml of methanol saturated with hydroxylamine hydrochloride, mix, and then add 0.3 ml of 5 *N* potassium hydroxide in methanol. Heat the mixture to boiling, then cool, adjust the pH to between 1 and 1.5 with hydrochloric acid TS, and add 0.1 ml of ferric chloride TS. A burgundy color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{14}H_{18}N_2O_5$ after drying.

Arsenic (as As) Not more than 3 ppm.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Not more than 1.5%.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 4.5%.

Residue on Ignition Not more than 0.2%.

Specific Rotation $[\alpha]_D^{20}$: Between +12.5° and +17.5°, calculated on the dried basis.

Transmittance Passes test.

TESTS

Assay (*Caution:* Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminum foil while dissolving the aspartame sample and during the titration.) Transfer about 150 mg of the sample, previously dried at 105° for 4 h and accurately weighed, into a 150-ml beaker, add 35 ml of dimethylformamide, and stir until the sample is completely dissolved. Add 5 drops of thymol blue TS, and titrate to a dark blue endpoint with 0.1 *M* lithium methoxide. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *M* lithium methoxide is equivalent to 29.43 mg of $C_{14}H_{18}N_2O_5$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

5-Benzyl-3,6-dioxo-2-piperazineacetic Acid

Apparatus Use a suitable gas chromatograph (see page 475) equipped with a hydrogen flame ionization detector and designed for handling glass columns with on-column injection (Micro-Tek 220 or equivalent), containing a 1.83-m (6-ft) × 4-mm (id) glass column packed with 3% OV-1 on 80/100-mesh Supelcoport (Supelco, Inc., or equivalent). Condition the column overnight at 250° before readjustment and equilibration to the operating conditions. To preclude buildup of silicon oxide, clean the detector with acetone frequently.

Operating Conditions The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: *column temperature*, 200°; *inlet temperature*, 200°; *detector temperature*, 275°; *carrier gas*, helium, flowing at a rate of 75 ml per min; *hydrogen and air flow to burner*, optimized to give maximum sensitivity; *recorder*, 1 mv full scale. (NOTE: For the Micro-Tek, the attenuation is 16 × 10.)

Silylation Reagent Just before use, dilute 3 parts, by volume, of *N,O*-bis(trimethylsilyl)acetamide with 2 parts of dimethylformamide.

Standard Preparation Transfer about 25 mg of FCC 5-Benzyl-3,6-dioxo-2-piperazineacetic Acid Reference Standard, accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Pipet 10.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with methanol, and mix. Pipet 3.0 ml of the second solution into a 2-dram vial with Teflon-lined cap, and evaporate to dryness on a steam bath. Add 1.0 ml of the *Silylation Reagent* to the residue, cap the vial tightly, shake, and heat in an oven at 80° for 30 min. Remove the vial from the oven, shake it for 15 s, and cool to room temperature.

Sample Preparation Transfer about 10 mg of the aspartame sample, accurately weighed, into a 2-dram vial with Teflon-lined cap, add 1.0 ml of the *Silylation Reagent*, cap tightly, shake, and heat in an oven at 80° for 30 min. Remove the vial from the oven, shake it for 15 s, and cool to room temperature.

Procedure Inject a 3-µl portion of the *Standard Preparation* into the gas chromatograph, obtain the chromatogram, measure the height of the peak produced by the 5-benzyl-3,6-dioxo-2-piperazineacetic acid, and record it as *P*. Under the stated conditions, the elution time is about 7 to 9 min. Similarly, inject a 3-µl portion of the *Sample Preparation*, obtain the chromatogram, measure the height of the peak produced by any 5-benzyl-3,6-dioxo-2-piperazineacetic acid contained in the sample, and record it as *p*. Calculate the percentage of 5-benzyl-3,6-dioxo-2-piperazineacetic acid in the sample by the formula

$$(W \times p \times 100)/(w \times P \times 167),$$

in which *W* is the exact weight of the Reference Standard taken, in mg, *w* is the exact weight of the aspartame sample taken, in mg, and 167 is the dilution factor for the Reference Standard.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 4 h.

Residue on Ignition Ignite a 1-g sample as directed in the general method, page 533.

Specific Rotation, page 530 Determine in a solution containing 4 g of sample in sufficient 15 *N* formic acid to make 100 ml. Make the determination within 30 min of preparation of the sample solution.

Transmittance The transmittance (*T*, page 540) of a 1% solution, in 2 *N* hydrochloric acid, determined in a 1-cm cell at 430 nm with a suitable spectrophotometer, using 2 *N* hydrochloric acid as the blank, is not less than 0.95, equivalent to an absorbance (*A*, page 539) of not more than approximately 0.022.

Packaging and Storage Store in well-closed containers in a cool, dry place.

Functional Use in Foods Sweetener; sugar substitute; flavor enhancer.

DL-Aspartic Acid

DL-Aminosuccinic Acid

$HOOCCH_2CH(NH_2)COOH$

$C_4H_7NO_4$ Mol wt 133.10

DESCRIPTION

Colorless or white, odorless crystals having an acid taste. It is slightly soluble in water, but insoluble in alcohol and in ether. It is optically inactive and melts with decomposition at about 280°.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A bluish purple color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_4H_7NO_4$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 100 ml of recently boiled and cooled water, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to the first appearance of a faint pink color that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 13.31 mg of $C_4H_7NO_4$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

L-Aspartic Acid

L-Aminosuccinic Acid

$HOOCCH_2CH(NH_2)COOH$

$C_4H_7NO_4$ Mol wt 133.10

DESCRIPTION

White, odorless crystals or crystalline powder having a slightly acid taste. It is slightly soluble in water but insoluble in alcohol and in ether. It melts at about 270°.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A bluish purple color appears.

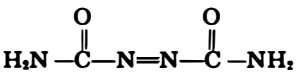
Assay Not less than 98.5% of $C_4H_7NO_4$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.25%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20}$: Between +24.5° and +26.0° after drying.

TESTS

Assay Dissolve about 250 mg, previously dried at 105° for 3 h and accurately weighed, in 100 ml of recently boiled and cooled water, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to the first appearance of a faint pink color that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 13.31 mg of $C_4H_7NO_4$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Azodicarbonamide



$C_2H_4N_4O_2$ Mol wt 116.08

DESCRIPTION

A yellow to orange red, odorless, crystalline powder. It is practically insoluble in water and in most organic solvents. It is slightly soluble in dimethyl sulfoxide. It melts above 180° with decomposition.

REQUIREMENTS

Identification

A solution of 35 mg of the sample in 1000 ml of water exhibits an ultraviolet absorption maximum at about 245 nm.

Assay Not less than 98.6% of $C_2H_4N_4O_2$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.003%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Nitrogen Between 47.2% and 48.7%.
pH of a 2% Suspension Not less than 5.0.
Residue on Ignition Not more than 0.15%.

TESTS

Assay Transfer about 225 mg of the sample, previously dried in a vacuum oven at 50° for 2 h and accurately weighed, into a 250-ml glass-stoppered iodine flask. Add about 23 ml of dimethyl sulfoxide to the flask, washing any adhered sample down with the solvent, then stopper the flask, and place about 2 ml of the solvent in the cup or lip of the flask. Swirl occasionally until complete solution of the sample is effected, and then loosen the stopper to drain the remainder of solvent into the flask and to rinse down any dissolved sample into the solution. Add 5.0 g of potassium iodide followed by 15 ml of water, then immediately pipet 10 ml of 0.5 *N* hydrochloric acid into the flask, and stopper rapidly. Swirl until the potassium iodide dissolves, and allow to stand for 20 to 25 min protected from light. Titrate the liberated iodine with 0.1 *N* sodium thiosulfate to the disappearance of the yellow color. Titrate with additional thiosulfate if any yellow color appears within 15 min. Perform a blank determination on a solution consisting of 25 ml of dimethyl sulfoxide, 5.0 g of potassium iodide, 15 ml of water, and 5 ml of 0.5 *N* hydrochloric acid, and make any necessary correction. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 5.804 mg of $C_2H_4N_4O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 670-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry in a vacuum oven at 50° for 2 h.

Nitrogen Transfer about 50 mg into a 100-ml Kjeldahl flask, add 3 ml of concentrated hydriodic acid solution (57% freshly assayed), and digest the mixture with gentle heating for 1.25 h, adding sufficient water, when necessary, to maintain the original volume. Increase the heat at the end of the digestion period, and continue heating until the volume is reduced by about one-half. Cool to room temperature, add 1.5 g of potassium sulfate, 3 ml of water, and 4.5 ml of sulfuric acid, and heat until iodine fumes are no longer evolved. Allow the mixture to cool, wash down the sides of the flask with water, heat until charring occurs, and again cool to room temperature. To the charred material add 40 mg of mercuric oxide, heat until the color of the solution is pale yellow, then cool, wash down the sides of the flask with a few ml of water, and digest the mixture for 3 additional h. Cool the digest, add 20 ml of ammonia-free water, 16 ml of a 50% sodium hydroxide solution, and 5 ml of a 44% sodium thiosulfate solution. Immediately connect the flask to a distillation apparatus as directed under *Nitrogen Determination*, page 521, and distil, collecting the distillate in 10 ml of a 4% boric acid solution. Add a few drops of methyl red–methylene blue TS to the distillate and titrate with 0.05 *N* sulfuric acid. Perform a blank determination (see page 2). Each ml of 0.05 *N* sulfuric acid is equivalent to 0.7004 mg of N.

pH of a 2% Suspension Add 2 g to 100 ml of water, agitate the mixture with a power stirrer for 5 min, and determine the pH of the resulting suspension potentiometrically (page 531).

Residue on Ignition Ignite 1.5 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Maturing agent for flour.

Balsam Peru Oil

DESCRIPTION

The oil obtained by extraction or distillation from Peruvian Balsam obtained from *Myroxylon pereirae* Royle Klotzsche (Fam. *Leguminosae*). It is a yellow to pale brown, slightly viscous liquid having a sweet balsamic odor. Occasionally, crystals may separate from the liquid. It is soluble in most fixed oils, and is soluble, with turbidity, in mineral oil. It is partly soluble in propylene glycol, but it is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 586, using the same test conditions as specified therein.

Acid Value Between 30 and 60.
Angular Rotation Between $-1°$ and $+2°$.
Ester Value Between 200 and 225.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.567 and 1.579 at 20°C.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.095 and 1.110.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Proceed as directed in the general method, page 501, using about 1 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 90% alcohol and remains in solution upon dilution to 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Basil Oil, Comoros Type

Basil Oil Exotic; Basil Oil, Réunion Type

DESCRIPTION

Basil oil, Comoros type, is obtained by steam distillation of the flowering tops or the entire plant of *Ocimum basilicum* L. It may be distinguished from other types, such as European basil oil, by its camphoraceous odor and physicochemical constants. It is a light yellow liquid with a spicy odor. It is soluble in most fixed oils and, with turbidity, in mineral oil. One ml is soluble in 20 ml of propylene glycol with slight haziness, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 586, using the same test conditions as specified therein.

Acid Value Not more than 1.0.
Angular Rotation Between $-2°$ and $+2°$.
Ester Value after Acetylation Between 25 and 45.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.512 and 1.520 at 20°C.
Saponification Value Between 4 and 10.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.952 and 0.973.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value after Acetylation Proceed as directed under *Linalool Determination*, page 501, using 2.5 g of the dry acetylated oil, accurately weighed, for the saponification. Calculate the *Ester Value after Acetylation* by the formula $a \times 28.05/b$, in which a is the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and b is the weight of the acetylated oil, in g, used in the test.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 5 g of sample, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 80% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Basil Oil, European Type

Basil Oil, Italian Type; Sweet Basil Oil

DESCRIPTION

Basil oil, European type, is obtained by steam distillation of the flowering tops or the entire plant of *Ocimum basilicum* L. It may be distinguished from other types, such as basil oil, Comoros type, or basil oil, Réunion type, by its more floral odor and its physicochemical constants. It is a pale yellow to yellow liquid with a floral-spicy odor. It is soluble in most fixed oils and, with turbidity, in mineral oil. One ml is soluble in 20 ml of propylene glycol with slight haziness, but it is insoluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 609, using the same test conditions as specified therein.

Acid Value Not more than 2.5.
Angular Rotation Between −5° and −15°.
Ester Value after Acetylation Between 140 and 180.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.483 and 1.493 at 20°C.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.900 and 0.920.

TESTS

Acid Value Determine as directed in the general method, page 499.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Ester Value after Acetylation Proceed as directed under *Linalool Determination*, page 501, using 2.5 g of the dry acetylated oil, accurately weighed, for the saponification. Calculate the *Ester Value after Acetylation* by the formula $a \times 28.05/b$, in which a is the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and b is the weight of the acetylated oil, in g, used in the test.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 80% alcohol.
Specific Gravity Determine by any reliable method (see page 3).
Packaging and Storage Store in full, tight, preferably glass or aluminum containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Bay Oil

Myrcia Oil

DESCRIPTION

The volatile oil distilled from the leaves of *Pimenta acris* Kostel. It occurs as a yellow or brownish yellow liquid with a pleasant aromatic odor and a pungent, spicy taste. It is soluble in alcohol and in glacial acetic acid. Its solutions in alcohol are acid to litmus.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 587, using the same test conditions as specified therein.
B. Shake 1 ml with 20 ml of hot water and filter. The filtrate gives not more than a slight acid reaction with litmus, and on the addition of 1 drop of ferric chloride TS yields only a transient grayish green, not a blue or purple color.

Assay Not less than 50% and not more than 65%, by volume, of phenols.
Angular Rotation Levorotatory, but not more than −3°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.507 and 1.516 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.950 and 0.990.

TESTS

Assay Proceed as directed under *Phenols*, page 502.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 95% alcohol to form a clear or only slightly turbid solution.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Beeswax, White

White Wax

DESCRIPTION

The bleached, purified wax from the honeycomb of the bee *Apis mellifera* L. (Fam. *Apidae*). It is a yellowish white solid, somewhat translucent in thin layers, having a faint characteristic odor, free from rancidity. Its specific gravity is about 0.95. White beeswax is insoluble in water and sparingly soluble in cold alcohol. Boiling alcohol dissolves cerotic acid and part of the myricin, which are constituents of the wax. It is completely soluble in chloroform, in ether, and in fixed and volatile oils. It is partly soluble in cold benzene and in cold carbon disulfide, but is completely soluble in these liquids at temperatures of 30° or above.

REQUIREMENTS

Acid Value Between 17 and 24.

Arsenic (as As) Not more than 3 ppm.

Carnauba Wax Passes test.

Ester Value Between 72 and 79.

Fats, Japan Wax, Rosin, and Soap Passes test.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Melting Range Between 62° and 65°.

Saponification Cloud Test Passes test.

TESTS

Acid Value, page 503 Warm about 3 g, accurately weighed, in a 200-ml flask with 25 ml of absolute alcohol, previously neutralized to phenolphthalein with potassium hydroxide, until the sample is melted. Shake the mixture, add 1 ml of phenolphthalein TS, and titrate the warm solution with 0.5 *N* alcoholic potassium hydroxide to a permanent, faint pink color.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Carnauba Wax Place 100 mg in a test tube, and add 20 ml of *n*-butanol. Immerse the test tube in boiling water, and shake the mixture gently until solution is complete. Transfer the test tube into a beaker of water at 60°, and allow it to cool to room temperature. A loose mass of fine, needlelike crystals separate from a clear mother liquor. Under the microscope the crystals appear as loose needles or stellate clusters, and no amorphous masses are observed (*absence of carnauba wax*).

Ester Value To the solution resulting from the determination of *Acid Value* add 25.0 ml of 0.5 *N* alcoholic potassium hydroxide and 50 ml of alcohol, heat the mixture under a reflux condenser for 4 h, and titrate the excess alkali with 0.5 *N* hydrochloric acid. Perform a residual blank titration, and calculate the *Ester Value* as the number of mg of potassium hydroxide required for each g of the sample taken for the test.

Fats, Japan Wax, Rosin, and Soap Boil 1 g for 30 min with 35 ml of a 1 in 7 solution of sodium hydroxide, maintaining the volume by the occasional addition of water, and cool the mixture. The wax separates and the liquid remains clear. Filter the cold mixture and acidify the filtrate with hydrochloric acid. No precipitate is formed.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Melting Range Determine as directed for *Class II* substances in the general procedure, page 520.

Saponification Cloud Test

Saponifying Solution Dissolve 40 g of potassium hydroxide in about 900 ml of aldehyde-free alcohol maintained at a temperature of 15° until solution is complete, then warm to room temperature, and add sufficient aldehyde-free alcohol to make 1000 ml.

Procedure Transfer 3.00 g into a round-bottom, 100-ml boiling flask provided with a ground-glass joint, add 30 ml of the *Saponifying Solution*, attach a reflux condenser to the flask, and heat the mixture gently on a steam bath for 2 h. At the end of this period, remove the reflux condenser, insert a thermometer into the solution, and place the flask in a water bath at a temperature of 80°. Rotate the flask while both the bath and the solution cool to 65°. The solution shows no cloudiness or globule formation before this temperature is reached.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Candy glaze and polish; miscellaneous and general purpose; flavoring agent.

Beeswax, Yellow

Yellow Wax

DESCRIPTION

The purified wax from the honeycomb of the bee *Apis mellifera* L. (Fam. *Apidae*). It is a yellowish to grayish brown solid having an agreeable, honeylike odor. It is somewhat brittle when cold, and presents a dull, granular, noncrystalline fracture when broken. It becomes pliable at a temperature of about 35°. Its specific gravity is about 0.95. Yellow beeswax is insoluble in water and sparingly soluble in cold alcohol. Boiling alcohol dissolves cerotic acid and part of the myricin, which are constituents of the wax. It is completely soluble in chloroform, in ether, and in fixed and volatile oils. It is partly soluble in cold

benzene and in cold carbon disulfide, but is completely soluble in these solvents at temperatures of 30° or above.

REQUIREMENTS

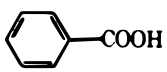
Acid Value Between 18 and 24.
Arsenic (as As) Not more than 3 ppm.
Carnauba Wax Passes test.
Ester Value Between 72 and 77.
Fats, Japan Wax, Rosin, and Soap Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Melting Range Between 62° and 65°.
Saponification Cloud Test Passes test.

TESTS

For the determination of *Acid Value*; *Arsenic*; *Carnauba Wax*; *Ester Value*; *Fats, Japan Wax, Rosin, and Soap*; *Heavy Metals*; *Lead*; *Melting Range*; and *Saponification Cloud Test*, proceed as directed in the monograph on *White Beeswax*, page 34.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Candy glaze and polish; miscellaneous and general purpose; flavoring agent.

Benzoic Acid



$C_7H_6O_2$ Mol wt 122.12

DESCRIPTION

White crystals, scales, or needles. It is odorless or has a slightly benzoinlike or benzaldehydelike odor. It begins to sublime at about 100° and is volatile with steam. One g is soluble in 275 ml of water at 25°, in 20 ml of boiling water, in 3 ml of alcohol, in 5 ml of chloroform, and in 3 ml of ether. It is soluble in fixed and in volatile oils, and is sparingly soluble in solvent hexane.

REQUIREMENTS

Identification

Dissolve 1 g in a mixture of 20 ml of water and 1 ml of sodium hydroxide TS, filter the solution, and add about 1 ml of ferric chloride TS. A buff-colored precipitate is formed.

Assay Not less than 99.5% and not more than 100.5% of $C_7H_6O_2$, calculated on the anhydrous basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Readily Carbonizable Substances Passes test.
Readily Oxidizable Substances Passes test.
Residue on Ignition Not more than 0.05%.
Solidification Point Between 121° and 123°.
Water Not more than 0.7%.

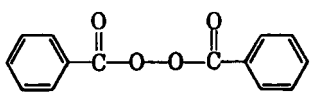
TESTS

Assay Dissolve about 500 mg of the sample, accurately weighed, in 25 ml of 50% alcohol previously neutralized with 0.1 *N* sodium hydroxide, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Each ml of 0.1 *N* sodium hydroxide is equivalent to 12.21 mg of $C_7H_6O_2$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Volatilize 2 g over a low flame. To the residue add 2 ml of nitric acid and about 10 mg of sodium carbonate, and evaporate to dryness on a steam bath. Dissolve the residue in a mixture of 1 ml of diluted acetic acid TS and 24 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Readily Carbonizable Substances, page 532 Dissolve 500 mg in 5 ml of sulfuric acid TS. The color is no darker than *Matching Fluid Q*.
Readily Oxidizable Substances To a mixture of 100 ml of water and 1.5 ml of sulfuric acid heated to 100° add dropwise 0.1 *N* potassium permanganate until a pink color persists for 30 s. Dissolve 1.0 g of the benzoic acid in the hot solution, and titrate with 0.1 *N* potassium permanganate to a pink color that persists for 15 s. The volume of 0.1 *N* potassium permanganate consumed does not exceed 0.5 ml.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.
Solidification Point Determine as directed in the general method, page 538.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552, using methanol in pyridine (1 in 2) as the solvent.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Preservative; antimicrobial agent.

Benzoyl Peroxide

Benzoyl Superoxide



$C_{14}H_{10}O_4$ Mol wt 242.23

DESCRIPTION

A colorless, crystalline solid having a faint odor of benzaldehyde. It is insoluble in water, slightly soluble in alcohol, and soluble in benzene, chloroform, and ether. One g dissolves in 40

ml of carbon disulfide. It melts between 103° and 106° with decomposition.

Caution: Benzoyl peroxide, especially in the dry form, is a dangerous, highly reactive, oxidizing material and has been known to explode spontaneously. Observe safety precautions printed on the label of the container.

REQUIREMENTS

Identification

To 500 mg of the sample add 50 ml of 0.5 *N* alcoholic potassium hydroxide, heat gradually to boiling, and continue boiling for 15 min. Cool, dilute to 200 ml with water, and make the solution strongly acid with 0.5 *N* hydrochloric acid. Extract with ether, dry the extract with anhydrous sodium sulfate, and then evaporate to dryness on a steam bath. The residue of benzoic acid so obtained melts between 121° and 123° (see page 519).

Assay Not less than 96.0% of $C_{14}H_{10}O_4$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 15 ml of acetone in a 100-ml glass-stoppered bottle, and add 3 ml of potassium iodide solution (1 in 2). Swirl for 1 min, then immediately titrate with 0.1 *N* sodium thiosulfate (without the addition of starch TS). Each ml of 0.1 *N* sodium thiosulfate is equivalent to 12.11 mg of $C_{14}H_{10}O_4$.

Arsenic Mix 1 g with 10 ml of sodium hydroxide TS, slowly evaporate to dryness on a steam bath, and cool. A *Sample Solution*, prepared as directed for organic compounds from the residue obtained, meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Mix 500 mg with 5 ml of sodium hydroxide TS, slowly evaporate to dryness on a steam bath, cool, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 5 ml of sodium hydroxide TS in the control (*Solution A*).

Lead Mix 1 g with 10 ml of sodium hydroxide TS, slowly evaporate to dryness on a steam bath, and cool. A *Sample Solution*, prepared as directed for organic compounds from the residue so obtained, meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in the original container and observe the safety precautions printed on the label.
Functional Use in Foods Bleaching agent.

Bergamot Oil, Coldpressed

DESCRIPTION

A volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of *Citrus bergamia* Risso et Poiteau (Fam. *Rutaceae*). It is a green to yellowish green or yellowish brown liquid having a fragrant, sweet-fruity odor. It is miscible with alcohol and with glacial acetic acid. It is soluble in most fixed oils, but is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 587, using the same test conditions as specified therein.

Assay Not less than 36.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between +8° and +24°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.465 and 1.468 at 20°.
Residue on Evaporation Not more than 6.0%.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.875 and 0.880.
Ultraviolet Absorbance Not less than 0.32.

TESTS

Assay Weigh accurately about 2 g, and proceed as directed under *Ester Determination*, page 500, but heat the mixture for 30 min on the steam bath. Use 98.15 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Residue on Evaporation Proceed as directed in the general method, page 502, heating for 5 h.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 90% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

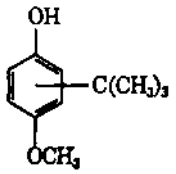
Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 50 mg of sample, accurately weighed. The absorbance maximum occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

BHA

Butylated Hydroxyanisole



$C_{11}H_{16}O_2$ Mol wt 180.25

DESCRIPTION

BHA is predominately 3-*tert*-butyl-4-hydroxyanisole (3-BHA), with varying amounts of 2-*tert*-butyl-4-hydroxyanisole (2-BHA). It occurs as a white or slightly yellow, waxy solid having a faint characteristic odor. It is insoluble in water, but is freely soluble in alcohol and in propylene glycol. It melts between 48° and 63°.

REQUIREMENTS

Identification

To 5 ml of a 1 in 10,000 solution of the sample in 72% alcohol add 2 ml of sodium borate TS and 1 ml of a 1 in 10,000 solution of 2,6-dichloroquinonechlorimide in absolute alcohol, and mix. A blue color develops.

Assay Not less than 98.5% of $C_{11}H_{16}O_2$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Residue on Ignition Not more than 0.05%.

TESTS

Assay

Standard Reference Curve Weigh accurately 900.0 mg, 950.0 mg, and 1.0000 g of FCC 3-*tert*-Butyl-4-hydroxyanisole Reference Standard, representing 90.0%, 95.0%, and 100.0% of $C_{11}H_{16}O_2$, respectively, into separate 10-ml volumetric flasks. Dissolve each standard in carbon disulfide, dilute to volume with carbon disulfide, and mix. Measure the infrared absorption spectrum of each solution from 10.5 to 12.5 μm with a suitable double-beam infrared spectrophotometer, using 0.15-mm sample cells, a 1.3-cm rock salt plate in the reference beam, 2× slits, and normal scanning speed. Draw a background line on the spectrogram from 11.2 to 12.0 μm, and determine the net absorbance of each solution at 11.42 μm by subtracting the background absorbance at this wavelength from the total absorption of each solution. Plot the calculated net absorbances against the percentage of $C_{11}H_{16}O_2$ in each solution.

Assay Preparation Transfer 1.0000 g of the sample, accurately weighed, into a 10-ml volumetric flask, dissolve it in carbon disulfide, dilute to volume with carbon disulfide, and mix. Measure the infrared absorption spectrum of this solution using the same conditions described above, determine the net absorbance of the sample at 11.42 μm, and obtain the apparent percentage of $C_{11}H_{16}O_2$ by means of the *Standard Reference Curve*.

Calculation Calculate the true, total percentage of $C_{11}H_{16}O_2$ in the sample of butylated hydroxyanisole taken by the formula

$$(\text{Apparent \% BHA}) + 0.16(100 - \text{\% 3-BHA}),$$

in which Apparent % BHA is the percentage found by the procedure described under *Assay Preparation*, 0.16 is a factor to correct for the decreased absorbance of the 2-isomer (which absorbs only 84% as strongly as does the 3-isomer at 11.42 μm), and % 3-BHA is the percentage of 3-*tert*-butyl-4-hydroxyanisole in the sample obtained by the procedure described below.

Percent 3-BHA Accurately weigh 1.0000 g of the sample, previously melted in a water bath and thoroughly mixed, transfer it into a 10-ml volumetric flask, dilute to volume with carbon disulfide, and mix. Measure the infrared absorption spectrum of this solution from 10 to 12 μm, using a 0.4-mm sample cell and a 1.3-cm rock salt plate in the reference beam. Subtract the background absorbance at 10.40 μm from the absorbance at 10.75 μm and at 10.95 μm. Using these net absorbance values, calculate the absorbance ratio $A_{10.75}/A_{10.95}$ by dividing the net absorbance found at 10.75 μm by that found at 10.95 μm. (The exact position of these absorption bands may vary, depending upon the instrument. If a recording instrument is used, the position of maximum absorbance on the spectrogram should be taken. With a nonrecording instrument, the exact wavelength and slit settings should be determined.) Determine the percentage of 3-*tert*-butyl-4-hydroxyanisole in the sample taken by means of a calibration curve obtained as follows: Prepare three 10.0-ml solutions in carbon disulfide containing, respectively, the following quantities of the specified FCC Reference Standards: (a) 1.0000 g of 3-*tert*-butyl-4-hydroxyanisole, (b) 900.0 mg of 3-*tert*-butyl-4-hydroxyanisole and 100.0 mg of 2-*tert*-butyl-4-hydroxyanisole, and (c) 800.0 mg of 3-*tert*-butyl-4-hydroxyanisole and 200.0 mg of 2-*tert*-butyl-4-hydroxyanisole. Measure the infrared absorption spectrum of each solution under the same conditions employed for the sample, and plot the calculated absorbance ratios against the corresponding percentages of 3-*tert*-butyl-4-hydroxyanisole.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

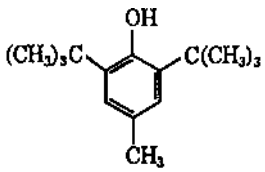
Residue on Ignition Ignite 10 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Antioxidant.

BHT

Butylated Hydroxytoluene; 2,6-Di-*tert*-butyl-*p*-cresol



$C_{15}H_{24}O$ Mol wt 220.35

DESCRIPTION

A white crystalline solid having a faint characteristic odor. It is insoluble in water and in propylene glycol, but is freely soluble in alcohol.

REQUIREMENTS

Identification

To 10 ml of a 1 in 100,000 solution of the sample in methanol add 10 ml of water, 2 ml of sodium nitrite solution (3 in 1000), and 5 ml of dianisidine solution (200 mg of 3,3′-dimethoxybenzidine dihydrochloride dissolved in a mixture of 40 ml of methanol and 60 ml of 1 *N* hydrochloric acid). An orange red color develops within 3 min. Add 5 ml of chloroform, and shake. The chloroform layer exhibits a purple or magenta color that fades when exposed to light.

Assay Not less than 99.0 weight % of $C_{15}H_{24}O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Residue on Ignition Not more than 0.002%.

TESTS

Assay Its solidification point (see page 538) is not lower than 69.2°, indicating a purity of not less than 99.0% of $C_{15}H_{24}O$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

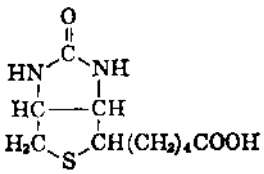
Residue on Ignition Transfer a 50-g sample into a tared crucible, ignite until thoroughly charred, and cool. Moisten the ash with 1 ml of sulfuric acid, and complete the ignition by heating for 15-min periods at 800° ± 25° to constant weight.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Antioxidant.

Biotin

cis-Hexahydro-2-oxo-1H-thieno[3,4]imidazole-4-valeric Acid; *d*-Biotin



$C_{10}H_{16}N_2O_3S$ Mol wt 244.31

DESCRIPTION

A practically white, crystalline powder. It is stable to air and heat. One g dissolves in about 5000 ml of water at 25° and in about 1300 ml of alcohol; it is more soluble in hot water and in dilute alkali, and is insoluble in other common organic solvents.

REQUIREMENTS

Identification

A saturated solution in warm water decolorizes bromine TS, added dropwise.

Assay Not less than 97.5% of $C_{10}H_{16}N_2O_3S$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Melting Range Between 229° and 232° with decomposition.

Specific Rotation $[\alpha]_D^{25}$: Between +89° and +93°.

TESTS

Assay Mix about 500 mg, accurately weighed, with 100 ml of water, add phenolphthalein TS, and titrate the suspension slowly, while heating and stirring continuously, with 0.1 *N* sodium hydroxide to a pink color. Each ml of 0.1 *N* sodium hydroxide is equivalent to 24.43 mg of $C_{10}H_{16}N_2O_3S$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Melting Range Determine as directed in the general procedure, page 519.
Specific Rotation, page 530 Determine in a solution in 0.1 *N* sodium hydroxide containing 500 mg in each 25 ml.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Birch Tar Oil, Rectified

DESCRIPTION

The pyroligneous oil obtained by dry distillation of the bark and the wood of *Betula pendula* Roth and related species of *Betula* (Fam. *Betulaceae*) and rectified by steam distillation. It is a clear, dark brown liquid having a strong leatherlike odor. It is soluble in most fixed oils, but it is insoluble in glycerin, in mineral oil, and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 587, using the same test conditions as specified therein.

Heavy Metals (as Pb) Passes test.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.886 and 0.950.

TESTS

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of absolute alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Black Pepper Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried, unripened fruit of the plant *Piper nigrum* L. (Fam. *Piperaceae*). It is an almost colorless to slightly greenish liquid having the characteristic odor of pepper and a relatively mild taste. It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is sparingly soluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 587, using the same test conditions as specified therein.

Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.479 and 1.488 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.864 and 0.884.

TESTS

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 95% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Bois de Rose Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the chipped wood of *Aniba rosaeodora* var. *amazonica* Ducke, (Fam. *Lauraceae*). The oils from the coastal region of Brazil and the Amazon valley tend to differ in odor and in linalool content from that produced in the Loreto province of Peru. The oil is a colorless to pale yellow liquid having a slightly camphoraceous,

pleasant floral odor. It is soluble in most fixed oils and in propylene glycol. It is soluble in mineral oil, occasionally with turbidity, but only slightly soluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 588, using the same test conditions as specified therein.

Assay Not less than 82.0% and not more than 92.0% of total alcohols, calculated as linalool ($C_{10}H_{18}O$).
Angular Rotation Between $-4°$ and $+6°$.
Distillation Range Not less than 70% distils between 195° and 205°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.462 and 1.470 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.868 and 0.889.

TESTS

Assay Proceed as directed under *Linalool Determination*, page 501, using about 1.2 g of the acetylated oil, accurately weighed.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Distillation Range Proceed as directed in the general method, page 478, using 50 ml of the sample, previously dried over anhydrous sodium sulfate, and employing a 125-ml flask.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 6 ml of 60% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Brominated Vegetable Oil

DESCRIPTION

Brominated vegetable oil is a bromine addition product of vegetable oil or oils. It is a pale yellow to dark brown, viscous, oily liquid having a bland or fruity odor and a bland taste. It is insoluble in water, but is soluble in alcohol, in chloroform, in ether, in hexane, and in fixed oils.

REQUIREMENTS

Identification

Mix about 0.2 ml of the sample with 1 g of anhydrous sodium carbonate in a suitable crucible, cover the mixture with an additional 1 g of sodium carbonate, compact the mixture by gentle tapping, and heat the crucible rapidly and strongly for 10 min. Cool the crucible and its contents, dissolve the residue in 20 ml of hot water, and filter. To the filtrate add diluted nitric acid TS until effervescence ceases, then add 1 ml of silver nitrate TS. A curdy, yellowish precipitate, which is insoluble in nitric acid but soluble in an excess of stronger ammonia water, is formed.

Arsenic (as As) Not more than 3 ppm.
Free Bromine Passes test.
Free Fatty Acids (as oleic) Not more than 2.5%.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Not more than 16.
Specific Gravity Within the range specified by the vendor.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Free Bromine Dissolve 1 g in 20 ml of acetone, add 1 g of sodium iodide, and allow to stand in a stoppered flask in the dark for 30 min, with occasional shaking. Add 25 ml of water and 1 ml of starch TS. No blue color is produced.
Free Fatty Acids Determine as directed in the general procedure, page 504, using 28.2 as the equivalence factor (*e*) in the calculation for oleic acid. Titrate with the appropriate normality of sodium hydroxide solution, shaking vigorously, to the first permanent pink color of the same intensity as that of the neutralized alcohol, or, if the color of the sample interferes, titrate to a pH of 8.5, determined with a suitable instrument.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Specific Gravity Determine as directed in the general procedure, page 3, at the temperature specified by the vendor.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Flavoring agent; beverage stabilizer.

Butadiene-Styrene 75/25 Rubber

DESCRIPTION

Butadiene-styrene 75/25 rubber is available as a liquid latex or solid rubber that is produced by the emulsion polymerization of butadiene and styrene, using fatty acid soaps (free from chick-edema factor) as emulsifiers, a persulfate catalyst, a suitable molecular weight regulator (if required), and a suitable short-stop. It is also available as a solid rubber produced by the solution-copolymerization of butadiene and styrene in a hexane solution, using butyl lithium as a catalyst.

The latex has a pH of 9.5 to 11.0 and a solids content of 26% to 42%. It is coagulated with or without other food-grade ingredients in a heated kettle. The coagulated mass is squeezed to drain off serums, then the coagulum is washed with hot water (with or without alkali), and it is rinsed with water until the batch is neutral. Finally, the coagulum is dried to remove residual volatiles. When butadiene-styrene rubber is purchased in the latex form, it must be washed by the preceding or an equivalent procedure.

In the case of the solvent-polymerized product, solvent and volatiles are removed by processing with hot water or by drum-drying. Both of the solid forms are supplied by the manufacturer either in slab form or as a uniform, free-flowing crumb and may contain a suitable food-grade antioxidant. The crumb form, in addition, may contain a suitable food-grade partitioning agent.

REQUIREMENTS

NOTE: The following REQUIREMENTS apply to the solid rubber as supplied by the manufacturer, or to the washed and dried coagulum obtained from the latex as described above.

Identification

Identify emulsion-polymerized butadiene-styrene 75/25 rubber latex and solid by comparing their infrared absorption spectra with the respective typical spectra as shown on page 714. Prepare latex samples by first drying at 105° for 4 h, then by dissolving in hot toluene and evaporating on a cesium bromide plate. Prepare solid samples by dissolving them in hot toluene and evaporating on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Bound Styrene Between 22.0% and 26.0%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Lithium Not more than 0.0075%.
Quinones Not more than 0.002%.
Residual Hexane Not more than 0.01%.
Residual Styrene Not more than 0.002%.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Bound Styrene Determine as directed in the general method, page 467.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Lithium

Atomic Absorption Spectrophotometer Use a suitable instrument, equipped with a lithium hollow cathode lamp, capable of measuring the radiation absorbed by lithium in the 6707-nm spectral band.

Standard Solution Transfer 399.3 mg of ACS reagent-grade lithium carbonate to a 1000-ml volumetric flask, dissolve in a minimum amount of 1:1 conc. hydrochloric acid–water, dilute to volume with water, and mix. Transfer 10.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with water, and mix. Finally, transfer 10.0 ml of this solution to a second 100-ml volumetric flask, add 1.0 ml of conc. hydrochloric acid, dilute to volume with water, and mix. This solution contains 75 µg of Li per 100 ml.

Sample Solution Weigh accurately 1 g of a solid rubber sample, wrap it tightly in ashless filter paper, and place in a tared platinum crucible. Heat in an oven at 100° for 15 min, and then transfer to a muffle furnace programmed to reach 500° within 1 to 3 h after introduction of the sample. Remove the crucible from the furnace 15 to 20 min after 500° has been reached, and cool in a desiccator. Quantitatively transfer the contents of the crucible to a 100-ml volumetric flask, using 1 ml of conc. hydrochloric acid and water, dilute to volume with water, and mix.

Procedure Following the manufacturer's instructions for operating the atomic absorption spectrophotometer, aspirate a suitable portion of the *Standard Solution* through the flame. In a similar manner, aspirate a suitable portion of the *Sample Solution*. Any absorbance produced by the *Sample Solution* does not exceed that produced by the *Standard Solution*.

Quinones Determine as directed in the general method, page 469.

Residual Hexane

NOTE: The isooctane (2,2,4-trimethylpentane) used in this test should be of chromatographic-grade quality.

Internal Standard Stock Solution Transfer 150 mg of *n*-

nonane, accurately weighed, to a 50-ml volumetric flask, dilute to volume with isooctane, and mix.

Dilute Internal Standard Solution Pipet 10.0 ml of *Internal Standard Stock Solution* into a 100-ml volumetric flask, dilute to volume with isooctane, and mix. Pipet 5.0 ml of this solution into a 250-ml volumetric flask, dilute to volume with isooctane, and mix. Each ml of the final solution contains 6 μg of *n*-nonane.

Hexane Standard Solution Transfer 150 mg of *n*-hexane, accurately weighed, to a 50-ml volumetric flask, dilute to volume with isooctane, and mix. Pipet 1.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with isooctane, and mix. Finally, pipet 10.0 ml of this solution and 10.0 ml of *Internal Standard Stock Solution* into a 50-ml volumetric flask, dilute to volume with isooctane, and mix.

Sample Preparation Weigh accurately 1.5 g of a solid rubber sample, transfer it into a 4-oz bottle, and pipet 25.0 ml of the *Dilute Internal Standard Solution* into the bottle. Stopper the bottle, and shake mechanically overnight to dissolve the rubber. Add 50 ml of methanol to precipitate out the polymer, and shake vigorously for 15 min. Allow the mixture to settle, and decant the liquid phase into a 250-ml separator. Wash the polymer with 25 ml of methanol, and add the wash to the separator. Add 50 to 75 ml of cold water to the separator, and shake vigorously for 1 min, venting periodically to release any pressure. Allow the phases to separate, drain off the bottom (aqueous) phase, and rewash the isooctane phase with a second 50-ml portion of cold water. Shake again, allow to separate, and drain off the bottom layer. Transfer 10 ml of the isooctane phase to a 20-ml vial for the analysis.

Procedure Use a gas chromatograph equipped with a flame-ionization detector and a column capable of separating hexane, isooctane, and *n*-nonane. Under typical conditions, the instrument contains a 10-ft × 0.125-in. stainless steel column packed with 60- to 80-mesh Chromosorb P containing 15% didecyl phthalate. The column is maintained isothermally at 120°, the injection port at 240°, and the detector at 250°. Helium is the carrier gas, flowing at a rate of 30 ml per min. A digital integrator or computer is recommended for data acquisition, although any mode (other than triangulation and planimetry) that gives accurate and reliable measurement of the peak areas is satisfactory.

Chromatograph duplicate 5-μl portions of the *Hexane Standard Solution*, and measure the areas under the hexane and nonane peaks. In a similar manner, chromatograph duplicate 5-μl portions of the *Sample Preparation*, and measure the areas under the hexane and nonane peaks. The peak area ratio of hexane to nonane (i.e., hexane divided by nonane) produced by the *Sample Preparation* does not exceed that produced by the *Hexane Standard Solution*.

Residual Styrene Determine as directed in the general method, page 469.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Butadiene-Styrene 50/50 Rubber

DESCRIPTION

A synthetic liquid latex (SBR 2000 Type) or solid rubber (SBR 1028 Type) produced by the emulsion copolymerization of butadiene and styrene, using rosin acid soaps or fatty acid soaps (free from chick-edema factor) as emulsifiers, a persulfate catalyst, a suitable molecular weight regulator (if required), and a suitable shortstop. The latex, which has a pH between 10.0 and 11.5 and a solids content of 41% to 63%, is coagulated with or without other food-grade ingredients in a heated kettle, the coagulated mass is squeezed to drain off serums, and the coagulum is washed with hot water (with or without alkali) and rinsed with water until the batch is neutral. Finally, the coagulum is dried to remove residual volatiles. When butadiene-styrene rubber is purchased in the latex form, it must be washed by the preceding or an equivalent procedure. The solid form is supplied by the manufacturer either in slab form or as a uniform, free-flowing crumb and may contain a suitable food-grade antioxidant. The crumb form, in addition, may contain a suitable food-grade partitioning agent.

REQUIREMENTS

NOTE: The following REQUIREMENTS apply to the solid rubber as supplied by the manufacturer, or to the washed and dried coagulum obtained from the latex as described above.

Identification

Identify butadiene-styrene 50/50 rubber latex and solid by comparing their infrared absorption spectra with the respective typical spectra as shown on page 715. Prepare latex samples by first drying at 105° for 4 h, then by dissolving in hot toluene and evaporating on a cesium bromide plate. Prepare solid samples by dissolving them in hot toluene and evaporating on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Bound Styrene Between 45.0% and 50.0%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Quinones Not more than 0.002%.
Residual Styrene Not more than 0.003%.

TESTS

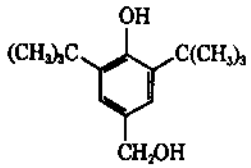
Proceed as directed under *Butadiene-Styrene 75/25 Rubber*, page 41.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Butylated Hydroxymethylphenol

4-Hydroxymethyl-2,6-di-*tert*-butylphenol



$C_{15}H_{24}O_2$ Mol wt 236.35

DESCRIPTION

A nearly white crystalline solid having a faint characteristic odor. It is insoluble in water and in propylene glycol, but is freely soluble in alcohol.

REQUIREMENTS

Identification

Butylated hydroxymethylphenol may be identified by its solidification point, as determined in the *Assay*.

Assay Not less than 98.0% of $C_{15}H_{24}O_2$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Its solidification point (see page 538) is not lower than 140°, indicating a purity of not less than 98.0%, by weight, of $C_{15}H_{24}O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Antioxidant.

1,3-Butylene Glycol

$CH_2OHCH_2CHOHCH_3$

$C_4H_{10}O_2$ Mol wt 90.12

DESCRIPTION

A clear, colorless, hygroscopic, viscous liquid having a slight, characteristic taste. It is practically odorless. It is miscible with water, with acetone, and with ether in all proportions, but it is immiscible with fixed oils. It dissolves most essential oils and synthetic flavoring substances.

REQUIREMENTS

Assay Not less than 99.0% of $C_4H_{10}O_2$.
Arsenic (as As) Not more than 3 ppm.
Distillation Range Between 200° and 215°.
Heavy Metals (as Pb) Not more than 10 ppm.
Specific Gravity Between 1.004 and 1.006 at 20°.

TESTS

Assay Prepare an acetylating reagent, within one week of use, by mixing 3.4 ml of water and 130 ml of acetic anhydride with 1000 ml of anhydrous pyridine. Pipet 20 ml of this reagent into a 250-ml iodine flask, and add about 1 g of the sample, accurately weighed. Attach a dry reflux condenser to the flask, and reflux for 1 h. Allow the flask to cool to room temperature, then rinse the condenser with 50 ml of chilled (10°) carbon dioxide–free water, allowing the water to drain into the flask. Stopper the flask, cool to below 20°, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide, swirling the contents of the flask continuously during the titration. Perform a blank determination (see page 2). Each ml of 0.5 *N* sodium hydroxide is equivalent to 2.253 mg of $C_4H_{10}O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Distillation Range Proceed as directed in the general method, page 478.

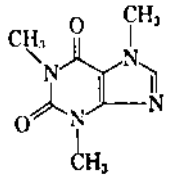
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Solvent for flavoring agents.

Caffeine

1,3,7-Trimethylxanthine



$C_8H_{10}N_4O_2$ Mol wt 194.19

DESCRIPTION

A white powder, or white, glistening needles, usually matted together. Caffeine is anhydrous or contains one molecule of water of hydration. It is odorless and has a bitter taste. Its solutions are neutral to litmus. The hydrate is efflorescent in air. One g of hydrated caffeine is soluble in about 50 ml of water, in 75 ml of alcohol, in about 6 ml of chloroform, and in 600 ml of ether.

REQUIREMENTS

Identification

A. Dissolve about 5 mg in 1 ml of hydrochloric acid in a porcelain dish, add 50 mg of potassium chlorate, and evaporate on a steam bath to dryness. Invert the dish over a vessel containing a few drops of ammonia TS. The residue acquires a purple color, which disappears upon the addition of a solution of a fixed alkali.

B. To a saturated solution of caffeine add tannic acid TS. A precipitate, which is soluble in an excess of the reagent, is formed.

C. To 5 ml of a saturated solution of caffeine add 5 drops of iodine TS. No precipitate is formed. Then add 3 drops of diluted hydrochloric acid TS. A red brown precipitate, which dissolves when a slight excess of sodium hydroxide TS is added, is formed.

Assay Not less than 98.5% and not more than the equivalent of 101.0% of $C_8H_{10}N_4O_2$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Melting Range Between 235° and 237.5°.

Other Alkaloids Passes test.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.1%.

Water *Anhydrous caffeine:* not more than 0.5%; *hydrous caffeine:* not more than 8.5%.

TESTS

Assay Dissolve about 800 mg, accurately weighed, of finely powdered caffeine, with warming, in a mixture of 80 ml of acetic anhydride and 180 ml of benzene. Cool, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 19.42 mg of $C_8H_{10}N_4O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 500 mg in 2.5 ml of hydrochloric acid and 23 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 10 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Melting Range Dry at 80° for 4 h and then determine as directed in the general procedure, page 519.

Other Alkaloids Add a few drops of mercuric-potassium iodide TS to 5 ml of a 1 in 50 solution of the sample. No precipitate forms.

Readily Carbonizable Substances, page 532 Dissolve 500 mg in 5 ml of sulfuric acid TS. The color is no darker than *Matching Fluid D*.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Water Determine the water content by drying at 80° for 4 h (page 518) or by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store hydrous caffeine in tight containers and anhydrous caffeine in well-closed containers.

Labeling Label caffeine to indicate whether it is anhydrous or hydrous.

Functional Use in Foods Flavoring agent; stimulant.

Calcium Acetate

$Ca(C_2H_3O_2)_2$ Mol wt 158.17

DESCRIPTION

A fine, white, bulky, odorless powder. It is freely soluble in water, and is slightly soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Calcium*, page 516, and for *Acetate*, page 515.

Assay Not less than 99.0% of $Ca(C_2H_3O_2)_2$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.05%.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.0025%.

Lead Not more than 10 ppm.
Sulfate Not more than 0.1%.
Water Not more than 7%.

TESTS

Assay Dissolve about 300 mg, accurately weighed, in 150 ml of water containing 2 ml of diluted hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 7.909 mg of $Ca(C_2H_3O_2)_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 40-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, page 511, except in the *Procedure* use 10 ml of 1 *N* hydrochloric acid to dissolve the sample.

Heavy Metals A solution of 1.2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 400 mg of the sample in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Sulfate, page 471 Any turbidity produced by a 200-mg sample does not exceed that shown in a control containing 200 µg of sulfate (SO_4).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Sequestrant.

Calcium Alginate

Algin

$[(C_6H_7O_6)_2Ca]_n$ Equiv wt, *Calculated*, 195.16
Equiv wt, *Actual* (Avg), 219.00

DESCRIPTION

The calcium salt of alginic acid (see *Alginic Acid*, page 13) occurs as a white to yellowish, fibrous or granular powder. It is nearly odorless and tasteless. It is insoluble in water, but is soluble in alkaline solutions or in solutions of substances that combine with the calcium. It is insoluble in organic solvents.

REQUIREMENTS

Identification

A. Calcium alginate meets the requirements of *Identification Test C* under *Alginic Acid*, page 13.
B. Extract the *Ash* from calcium alginate with diluted hydrochloric acid TS and filter. The filtrate gives positive tests for *Calcium*, page 516.

Assay It yields not less than 18% and not more than 21% of carbon dioxide (CO_2), corresponding to between 89.6% and 104.5% of calcium alginate (equiv wt 219.00), calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Ash Between 12% and 18% after drying.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 15%.

TESTS

Assay Proceed as directed under *Alginates Assay*, page 463. Each ml of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.38 mg of calcium alginate (equiv wt 219.00).

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash Determine as directed under *Ash* in the monograph on *Alginic Acid*, page 14.

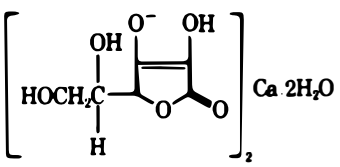
Heavy Metals Determine as directed in the test for *Heavy Metals* under *Alginic Acid*, page 14.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Calcium Ascorbate



$C_{12}H_{14}CaO_{12}\cdot 2H_2O$ Mol wt 426.35

DESCRIPTION

A white to slightly yellow, odorless, crystalline powder. It is soluble in water, slightly soluble in alcohol, and insoluble in ether. The pH of a 1 in 10 solution is between 6.8 and 7.4.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Calcium*, page 516, and it decolorizes dichlorophenol-indophenol TS.

Assay Not less than 98.0% of $C_{12}H_{14}CaO_{12}{\cdot}2H_2O$.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Oxalate Passes test.
Specific Rotation $[\alpha]_D^{25°}$: Between +95° and +97°.

TESTS

Assay Dissolve about 300 mg, accurately weighed, in 50 ml of water in a 250-ml Erlenmeyer flask, and immediately titrate with 0.1 *N* iodine to a pale yellow color that persists for at least 30 s. Each ml of 0.1 *N* iodine is equivalent to 10.66 mg of $C_{12}H_{14}CaO_{12}{\cdot}2H_2O$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Fluoride Proceed as directed in the *Fluoride Limit Test*, page 510.
Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Oxalate To a solution of 1 g in 10 ml of water add 2 drops of glacial acetic acid and 5 ml of a 1 in 10 solution of calcium acetate. The solution remains clear after standing for 5 min.
Specific Rotation, page 530 Determine in a solution containing 1 g in each 20 ml.

Packaging and Storage Store in tight containers, preferably in a cool, dry place.
Functional Use in Foods Antioxidant.

Calcium Bromate

$Ca(BrO_3)_2{\cdot}H_2O$ Mol wt 313.90

DESCRIPTION

A white crystalline powder. It is very soluble in water.

REQUIREMENTS

Identification

A. A 1 in 20 solution in diluted hydrochloric acid TS imparts a transient yellowish red color to a nonluminous flame.

B. To a 1 in 20 solution add sulfurous acid dropwise. A yellow color is produced that disappears upon the addition of an excess of sulfurous acid.

Assay Not less than 99.8% of $Ca(BrO_3)_2{\cdot}H_2O$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.

TESTS

Assay Dissolve about 900 mg, accurately weighed, in 50 ml of water in a 250-ml glass-stoppered Erlenmeyer flask. Add 3 g of potassium iodide, followed by 3 ml of hydrochloric acid. Allow the mixture to stand for 5 min, add 100 ml of cold water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination (see page 2). Each ml of 0.1 *N* sodium thiosulfate is equivalent to 26.16 mg of $Ca(BrO_3)_2{\cdot}H_2O$.
Arsenic Dissolve 1 g in a mixture of 5 ml of hydrochloric acid and 5 ml of water, and evaporate the solution until crystals appear. Cool, dissolve the residue in water, and dilute to 35 ml. This solution meets the requirements of the *Arsenic Test*, page 464.

Sample Solution for the Determination of Heavy Metals and Lead Dissolve 2 g in 10 ml of water, add 10 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 5 ml of hydrochloric acid, again evaporate to dryness, and then dissolve the residue in 40 ml of water.

Heavy Metals A 10-ml portion of the *Sample Solution*, diluted to 25 ml with water, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A 20-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Maturing agent; dough conditioner.

Calcium Carbonate

Precipitated Calcium Carbonate

$CaCO_3$ Mol wt 100.09

DESCRIPTION

A fine, white microcrystalline powder. It is colorless and tasteless, and is stable in air. It is practically insoluble in water

and in alcohol. The presence of any ammonium salt or carbon dioxide increases its solubility in water, but the presence of any alkali hydroxide reduces the solubility.

REQUIREMENTS

Identification

It dissolves with effervescence in diluted acetic acid TS, in diluted hydrochloric acid TS, and in diluted nitric acid TS, and the resulting solutions, after boiling, give positive tests for *Calcium*, page 516.

Assay Not less than 98.0% of $CaCO_3$ after drying.
Acid-Insoluble Substances Not more than 0.2%.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 0.003%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 2%.
Magnesium and Alkali Salts Not more than 1%.

TESTS

Assay Transfer about 200 mg, previously dried at 200° for 4 h and accurately weighed, into a 400-ml beaker, add 10 ml of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 ml of diluted hydrochloric acid TS from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the sample. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 ml with water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 5.004 mg of $CaCO_3$.

Acid-Insoluble Substances Suspend 5 g in 25 ml of water, cautiously add with agitation 25 ml of dilute hydrochloric acid (1 in 2), then add water to make a volume of about 200 ml. Heat the solution to boiling, cover, digest on a steam bath 1 h, cool, and filter. Wash the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it. The weight of the residue does not exceed 10 mg.

Arsenic A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, page 511.

Sample Solution for the Determination of Heavy Metals and Lead Cautiously dissolve 5 g in 25 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in about 15 ml of water, and dilute to 25 ml (1 ml = 200 mg).

Heavy Metals Neutralize 3.3 ml (667 mg) of the *Sample Solution* with sodium hydroxide TS, using phenolphthalein as the indicator, and dilute to 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 5-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 200° for 4 h.

Magnesium and Alkali Salts Mix 1 g with 40 ml of water, carefully add 5 ml of hydrochloric acid, mix, and boil for 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-ml cylinder, dilute with water to 100 ml, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Alkali; nutrient; dietary supplement; dough conditioner; firming agent; yeast food.

Calcium Chloride

$CaCl_2 2H_2O$ Mol wt 147.02

DESCRIPTION

White, hard, odorless fragments or granules. It is deliquescent. One g dissolves in 1.2 ml of water at 25°, in 0.7 ml of boiling water, in 10 ml of alcohol at 25°, and in 2 ml of boiling alcohol. The pH of a 1 in 20 solution is between 4.5 and 8.5.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Calcium*, page 516, and for *Chloride*, page 516.

Assay Not less than 99.0% and not more than the equivalent of 107.0% of $CaCl_2 2H_2O$.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.004%.
Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.
Magnesium and Alkali Salts Not more than 4%.

TESTS

Assay Transfer about 1.5 g, accurately weighed, into a 250-ml volumetric flask, dissolve it in a mixture of 100 ml of water and 5 ml of diluted hydrochloric acid TS, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a suitable container, and add 50 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 7.351 mg of $CaCl_2.2H_2O$.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, page 511.

Heavy Metals Dissolve 1 g in 2 ml of diluted acetic acid TS, and add water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Magnesium and Alkali Salts Dissolve 1 g in about 50 ml of water, add 500 mg of ammonium chloride, mix, and boil for 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-ml cylinder, dilute with water to 100 ml, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 20 mg.

Packaging and Storage Store in tight containers.
Functional Use in Foods Miscellaneous and general purpose; sequestrant; firming agent.

Calcium Chloride, Anhydrous

$CaCl_2$ Mol wt 110.99

DESCRIPTION

White, hard, odorless fragments or granules. It is deliquescent. One g dissolves in 1.5 ml of water at 25°, in 0.7 ml of boiling water, in 8 ml of alcohol at 25°, and in 1.6 ml of boiling alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Calcium*, page 516, and for *Chloride*, page 516.

Assay Not less than 93.0% of $CaCl_2$.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.004%.
Heavy Metals (as Pb) Not more than 0.002%
Lead Not more than 10 ppm.
Magnesium and Alkali Salts Not more than 5%.

TESTS

Assay Transfer about 1 g, accurately weighed, into a 250-ml volumetric flask, dissolve it in a mixture of 100 ml of water and 5 ml of diluted hydrochloric acid TS, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a suitable container, and add 50 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 5.550 mg of $CaCl_2$.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, page 511.

Heavy Metals Dissolve 1 g in 2 ml of diluted acetic acid TS, and add water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Magnesium and Alkali Salts Dissolve 1 g in about 50 ml of water, add 500 mg of ammonium chloride, mix, and boil for about 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture into a 100-ml cylinder, dilute with water to 100 ml, let it stand for 4 h or overnight, and then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg.

Packaging and Storage Store in tight containers.
Functional Use in Foods Miscellaneous and general purpose; sequestrant; firming agent.

Calcium Chloride Solution

DESCRIPTION

Calcium chloride solution occurs as a clear to slightly turbid, colorless or slightly colored liquid at room temperature. It is nominally available in a concentration range of about 35% to 45% of $CaCl_2$.

REQUIREMENTS

Identification

When diluted to a concentration of about 1 to 10 ($CaCl_2$ basis), it gives positive tests for *Calcium*, page 516, and for *Chloride*, page 516.

Assay Not less than 90.0% and not more than 110.0%, by weight, of the labeled amount of calcium chloride, expressed as $CaCl_2$.

Alkalinity [as $Ca(OH)_2$] Not more than 0.3%.

Arsenic (as As) Not more than 3 ppm, calculated on the $CaCl_2$ determined in the *Assay*.

Fluoride Not more than 0.004%, calculated on the $CaCl_2$ determined in the *Assay*.

Heavy Metals (as Pb) Not more than 0.002%, calculated on the $CaCl_2$ determined in the *Assay*.

Lead Not more than 10 ppm, calculated on the $CaCl_2$ determined in the *Assay*.

Magnesium and Alkali Salts Not more than 5%, calculated on the $CaCl_2$ determined in the *Assay*.

TESTS

Assay Transfer an accurately weighed amount of the solution, equivalent to about 1 g of $CaCl_2$, into a 250-ml volumetric flask, dissolve it in a mixture of 100 ml of water and 5 ml of diluted hydrochloric acid TS, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a suitable container, and add 50 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 5.550 mg of $CaCl_2$.

Alkalinity Dilute an accurately weighed amount of the solution, equivalent to about 5 g of $CaCl_2$, to 50 ml with water, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid. Each ml of 0.1 *N* hydrochloric acid is equivalent to 3.71 mg of $Ca(OH)_2$.

Arsenic Dilute an accurately weighed amount of the solution, equivalent to 1 g of $CaCl_2$, to 35 ml with water. The resulting solution meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in *Method III* under the *Fluoride Limit Test*, page 511, using as the sample an accurately weighed amount of the solution equivalent to 1 g of $CaCl_2$.

Heavy Metals Dilute an accurately weighed amount of the solution, equivalent to 1 g of $CaCl_2$, to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dilute an accurately weighed amount of the solution, equivalent to 1 g of $CaCl_2$, to 10 ml with water. The resulting solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Magnesium and Alkali Salts Dilute an accurately weighed amount of the solution, equivalent to 1 g of $CaCl_2$, to 50 ml with water, add 500 mg of ammonium chloride, mix, and boil for about 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture into a 100-ml cylinder, dilute with water to 100 ml, let it stand for 4 h or overnight, and then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 25 mg.

Packaging and Storage Store in tight containers.

Functional Use in Foods Miscellaneous and general purpose; sequestrant; firming agent.

Calcium Citrate

$$[CH_2(COO^-)C(OH)(COO^-)CH_2COO^-]_2Ca_3$$

$Ca_3(C_6H_5O_7)_2.4H_2O$ Mol wt 570.50

DESCRIPTION

A fine, white, odorless powder. It is very slightly soluble in water and insoluble in alcohol.

REQUIREMENTS

Identification

A. Dissolve 500 mg in 10 ml of water and 2.5 ml of diluted nitric acid TS, add 1 ml of mercuric sulfate TS, heat to boiling, and then add potassium permanganate TS. A white precipitate is formed.

B. Ignite 500 mg completely at as low a temperature as possible, cool, and dissolve the residue in 10 ml of water and 1 ml of glacial acetic acid. Filter and add 10 ml of

ammonium oxalate TS to the filtrate. A voluminous white precipitate appears that is soluble in hydrochloric acid.

Assay Not less than 97.5% of $Ca_3(C_6H_5O_7)_2$ after drying.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Between 10% and 13.3%.

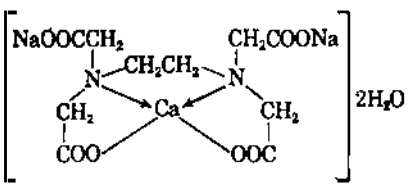
TESTS

Assay Dissolve about 350 mg, previously dried at 150° for 4 h and accurately weighed, in a mixture of 10 ml of water and 2 ml of diluted hydrochloric acid TS, and dilute to about 100 ml with water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 8.300 mg of $Ca_3(C_6H_5O_7)_2$.
Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.
Fluoride Weigh accurately 1.67 g, and proceed as directed in the *Fluoride Limit Test*, page 510.
Heavy Metals Dissolve 1 g in 20 ml of water and 2 ml of hydrochloric acid, add 1.5 ml of stronger ammonia TS, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Dissolve 1 g in 10 ml of water and 1 ml of hydrochloric acid. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry for 4 h at 150°.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Sequestrant; buffer; firming agent.

Calcium Disodium EDTA

Calcium Disodium Ethylenediaminetetraacetate;
Calcium Disodium (Ethylenedinitrilo)tetraacetate;
Calcium Disodium Edetate



$C_{10}H_{12}CaN_2Na_2O_8 \cdot 2H_2O$ Mol wt 410.30

DESCRIPTION

White, odorless crystalline granules or a white to off-white powder. It is slightly hygroscopic, has a faint saline taste, and is stable in air. It is freely soluble in water.

REQUIREMENTS

Identification

A. A 1 in 20 solution responds to the oxalate test for *Calcium*, page 516, and to the flame test for *Sodium*, page 517.
B. The infrared absorption spectrum of a liquid petrolatum dispersion of the sample exhibits maxima only at the same wavelengths as that of a similar preparation of USP Calcium Disodium Edetate Reference Standard.
C. To 5 ml of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. To the deep red solution so obtained add about 50 mg of the sample, and mix. The deep red color disappears.

Assay Not less than 97.0% and not more than the equivalent of 102.0% of $C_{10}H_{12}CaN_2Na_2O_8$, calculated on the anhydrous basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Magnesium-Chelating Substances Passes test.
pH of a 1 in 100 Solution Between 6.5 and 7.5.
Water Not more than 13%.

TESTS

Assay Transfer about 1.2 g of the sample, accurately weighed, into a 250-ml beaker, and dissolve in 75 ml of water. Add 25 ml of diluted acetic acid TS and 1.0 ml of diphenylcarbazone TS, and titrate slowly with 0.1 *M* mercuric nitrate to the first appearance of a purplish color. Each ml of 0.1 *M* mercuric nitrate is equivalent to 37.43 mg of $C_{10}H_{12}CaN_2Na_2O_8$.
Arsenic Prepare a *Sample Solution* as directed for organic compounds on page 465, but use 70% perchloric acid instead of 30% hydrogen peroxide in the decomposition of the sample. The resulting solution meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed for organic compounds on page 518, but use 70% perchloric acid instead of 30% hydrogen peroxide in the decomposition of the sample. The resulting solution meets the requirements of the *Lead Limit Test*, page 518.
Magnesium-Chelating Substances Transfer a 1-g sample, accurately weighed, to a small beaker, and dissolve it in 5 ml of water. Add 5 ml of a buffer solution prepared by dissolving 67.5 g of ammonium chloride in 200 ml of water, adding 570 ml of stronger ammonia TS, and diluting with water to 1000 ml. Then to the buffered solution add 5 drops of eriochrome black TS, and titrate with 0.1 *M* magnesium acetate to the appearance of a deep wine-red color. Not more than 2.0 ml is required.
pH of a 1 in 100 Solution Determine by the *Potentiometric Method*, page 531.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Preservative; sequestrant.

Calcium Gluconate

$[CH_2OH(CHOH)_4COO]_2Ca$

$C_{12}H_{22}CaO_{14}$ Mol wt 430.38

DESCRIPTION

White, crystalline granules or powder. It is odorless, tasteless, and stable in air. Its solutions are neutral to litmus. One g dissolves slowly in about 30 ml of water at 25° and in about 5 ml of boiling water. It is insoluble in alcohol and in many other organic solvents.

REQUIREMENTS

Identification

A. A 1 in 50 solution gives positive tests for *Calcium*, page 516.
B. Place 500 mg in a test tube and dissolve it in 5 ml of water by warming. To the warm solution add about 0.7 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, heat on a steam bath for 30 min, and allow to cool. Induce crystallization by scratching the inner surface of the tube with a glass rod. Crystals of gluconic acid phenylhydrazide form.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{12}H_{22}CaO_{14}$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 3%.
Sucrose and Reducing Sugars Passes test.

TESTS

Assay Dissolve about 800 mg, previously dried at 105° for 16 h and accurately weighed, in 100 ml of water containing 2 ml of diluted hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 21.52 mg of $C_{12}H_{22}CaO_{14}$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464, substituting nitric acid for hydrogen peroxide in the wet digestion of the sample.
Heavy Metals Mix a 1-g sample with 4 ml of 1 *N* hydrochloric acid, dilute to 25 ml with water, warm gently until dissolved, and cool. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 16 h.
Sucrose and Reducing Sugars Dissolve 500 mg in 10 ml of hot water, add 2 ml of diluted hydrochloric acid TS, boil for about 2 min, and cool. Add 5 ml of sodium carbonate TS, allow to stand for 5 min, dilute with water to 20 ml, and filter. Add 5 ml of the clear filtrate to about 2 ml of alkaline cupric tartrate TS, and boil for 1 min. No red precipitate is formed immediately.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Miscellaneous and general purpose; buffer; firming agent; sequestrant.

Calcium Glycerophosphate

$C_3H_7CaO_6P$ Mol wt 210.14

DESCRIPTION

A fine, white, odorless, almost tasteless powder. It is somewhat hygroscopic. One g dissolves in about 50 ml of water at 25°. It is more soluble in water at a lower temperature, and citric acid increases its solubility in water. It is insoluble in alcohol.

REQUIREMENTS

Identification

A. A saturated solution gives positive tests for *Calcium*, page 516.
B. Heat a mixture of 100 mg of the sample with 500 mg of potassium bisulfate. Pungent vapors of acrolein are evolved.

Assay Not less than 98.0% of $C_3H_7CaO_6P$ after drying.
Alkalinity Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 12%.

TESTS

Assay Weigh accurately about 2 g, previously dried at 150° for 4 h, and dissolve in 100 ml of water and 5 ml of diluted hydrochloric acid TS. Transfer the solution to a 250-ml volumetric flask, dilute to volume with water, and mix well. Pipet 50.0 ml of this solution into a suitable container, and add 50 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue

the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 10.51 mg of $C_3H_7CaO_6P$.

Alkalinity A solution of 1 g in 60 ml of water requires not more than 1.5 ml of 0.1 *N* sulfuric acid for neutralization, using 3 drops of phenolphthalein TS as indicator.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 500 mg in 3 ml of diluted acetic acid TS, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 150° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement.

Calcium Hydroxide

Slaked Lime

$Ca(OH)_2$ Mol wt 74.09

DESCRIPTION

A white powder, possessing an alkaline, slightly bitter taste. One g dissolves in 630 ml of water at 25°, and in 1300 ml of boiling water. It is soluble in glycerin and in a saturated solution of sucrose, but is insoluble in alcohol.

REQUIREMENTS

Identification

A. When mixed with from 3 to 4 times its weight of water, it forms a smooth magma. The clear, supernatant liquid from the magma is alkaline to litmus.

B. Mix 1 g with 20 ml of water, and add sufficient acetic acid to effect solution. The resulting solution gives positive tests for *Calcium*, page 516.

Assay Not less than 95.0% of $Ca(OH)_2$.

Acid-Insoluble Substances Not more than 0.5%.

Arsenic (as As) Not more than 3 ppm.

Carbonate Passes test.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Magnesium and Alkali Salts Not more than 4.8%.

TESTS

Assay Weigh accurately about 1.5 g, transfer to a beaker, and gradually add 30 ml of diluted hydrochloric acid TS. When solution is complete, transfer it to a 500-ml volumetric flask, rinse the beaker thoroughly, adding the rinsings to the flask, dilute with water to volume, and mix. Transfer 50.0 ml of this solution into a suitable container, and add 50 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 3.705 mg of $Ca(OH)_2$.

Acid-Insoluble Substances Dissolve 2 g in 30 ml of dilute hydrochloric acid (1 in 3), and heat to boiling. Filter the mixture, wash the residue with hot water, and ignite. The weight of the residue does not exceed 10 mg.

Arsenic A solution of 1 g in 15 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Carbonate Mix 2 g of the sample with 50 ml of water, and add an excess of diluted hydrochloric acid TS. No more than a slight effervescence is produced.

Fluoride Weigh accurately 1.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Heavy Metals Dissolve 500 mg in 10 ml of diluted hydrochloric acid TS, and evaporate to dryness on a steam bath. Dissolve the residue in 25 ml of water, and filter. The filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 15 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Magnesium and Alkali Salts Dissolve 500 mg in a mixture of 30 ml of water and 10 ml of diluted hydrochloric acid TS, and boil for 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-ml cylinder, dilute with water to 100 ml, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 12 mg.

Packaging and Storage Store in tight containers.

Functional Use in Foods Miscellaneous and general purpose; buffer; neutralizing agent; firming agent.

Calcium Iodate

$Ca(IO_3)_2.H_2O$ Mol wt 407.90

DESCRIPTION

A white powder. It is odorless or has a slight odor. It is slightly soluble in water, and is insoluble in alcohol.

REQUIREMENTS

Identification

To 5 ml of a saturated solution of the sample add 1 drop of starch TS and a few drops of 20% hypophosphorous acid. A transient blue color appears.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $Ca(IO_3)_2.H_2O$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Weigh accurately about 600 mg, dissolve it in 10 ml of 70% perchloric acid and 10 ml of water, heating gently if necessary, and dilute with water to 250.0 ml. Transfer 50.0 ml to a 250-ml glass-stoppered Erlenmeyer flask, add 1 ml of 70% perchloric acid and 5 g of potassium iodide, stopper the flask, and swirl briefly. Let stand for 5 min, then titrate with 0.1 *N* sodium thiosulfate, adding starch TS just before the endpoint is reached. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 3.398 mg of $Ca(IO_3)_2.H_2O$.

Arsenic Mix 3 ml of hydrochloric acid with a 1-g sample, evaporate to dryness on an asbestos board on a hot plate, and cool. Add 5 ml of hydrochloric acid, and again evaporate to dryness. Dissolve the residue in 15 ml of water, heat nearly to boiling, and add just enough hydrazine sulfate to discharge any yellow color. Cool, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Mix 5 ml of hydrochloric acid with a 2-g sample, evaporate to dryness on an asbestos board on a hot plate, and cool. Add 5 ml of hydrochloric acid, and again evaporate to dryness. Dissolve the residue in 15 ml of water, heat nearly to boiling, and add just enough hydrazine sulfate to discharge any yellow color. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Maturing agent; dough conditioner.

Calcium Lactate

$[CH_3CH(OH)COO]_2Ca.xH_2O$

$C_6H_{10}CaO_6.xH_2O$ Mol wt (anhydrous) 218.22

DESCRIPTION

White to cream-colored, almost odorless, crystalline powder or granules, containing up to five molecules of water of crystallization. The pentahydrate is somewhat efflorescent and at 120° becomes anhydrous. It is soluble in water and practically insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Calcium*, page 516, and for *Lactate*, page 517.

Assay Not less than 98.0% and not more than 101.0% of $C_6H_{10}CaO_6$, calculated on the anhydrous basis.
Acidity Passes test (about 0.45%, as lactic acid).
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.0015%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying *Pentahydrate*: between 22% and 27%; *trihydrate*: between 15% and 20%; *monohydrate*: between 5% and 8%; *dried form*: not more than 3%.
Magnesium and Alkali Salts Not more than 1%.
Volatile Fatty Acids Passes test.

TESTS

Assay Dissolve an accurately weighed amount of the sample, equivalent to about 350 mg of $C_6H_{10}CaO_6$, in 150 ml of water containing 2 ml of diluted hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 10.91 mg of $C_6H_{10}CaO_6$.

Acidity Dissolve 1 g in 20 ml of water, add 3 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Not more than 0.5 ml is required.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Fluoride Proceed as directed in the *Fluoride Limit Test*, page 510, using *Method I* (3.3-g sample) or *Method III* (1.0-g sample).

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dissolve 1 g in 3 ml of dilute nitric acid (1 in 2), boil for 1 min, cool, and dilute to 20 ml with water. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Distribute a sample of about 1.5 g evenly in a suitable weighing dish to a depth not exceeding 3 mm, and dry at 102° for 4 h.

Magnesium and Alkali Salts Mix 1 g with 40 ml of water, carefully add 1 ml of hydrochloric acid, boil for 1 min, and add rapidly 40 ml of oxalic acid TS. Add immediately to the warm mixture 2 drops of methyl red TS, then add ammonia TS, dropwise, from a buret until the mixture is just alkaline, and cool to room temperature. Transfer the mixture into a 100-ml graduate, dilute with water to 100 ml, mix, and allow to stand for 4 h or overnight. Decant the clear, supernatant liquid through a dry filter paper, transfer 50 ml of the clear filtrate to a tared platinum dish, and add 0.5 ml of sulfuric acid. Evaporate to a small volume on a steam bath, then carefully heat over a free flame to dryness, and continue heating to complete decomposition and volatilization of the ammonium salts. Finally, ignite the residue to constant weight. The weight of the residue does not exceed 5 mg.

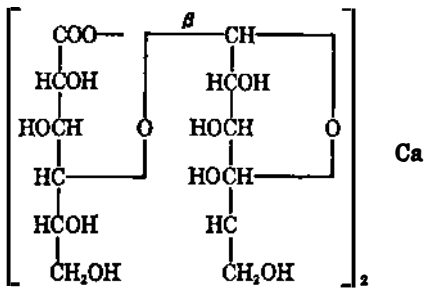
Volatile Fatty Acids Stir about 500 mg of the sample with 1 ml of sulfuric acid, and warm. The mixture does not emit an odor of volatile fatty acids.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; dough conditioner; yeast food.

Calcium Lactobionate

Calcium 4-(β,D-Galactosido)-D-gluconate



$C_{24}H_{42}CaO_{24}$ Mol wt 754.66

DESCRIPTION

A white to cream-colored, odorless, free-flowing powder. It is freely soluble in water but is insoluble in alcohol and in ether. It has a bland taste, and it readily forms double salts, such as the chloride, bromide, and gluconate. It decomposes at about 120°. The pH of a 1 in 10 solution is between 6.5 and 7.5.

REQUIREMENTS

Identification

It gives positive tests for *Calcium*, page 516.

Arsenic (as As) Not more than 3 ppm.

Bromide Passes test.

Calcium Content Not less than 5.05% and not more than 5.55% of Ca, calculated on the anhydrous basis.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 8%.

Reducing Substances Not more than 5%.

Specific Rotation $[\alpha]_D^{20}$: Between +23° and +25°.

Sulfate Not more than 0.7%.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Bromide

NOTE: Wash all glassware with diluted nitric acid TS and rinse with water to remove all traces of adsorbed bromide.

Bromide Stock Solution Dissolve 744.6 mg of anhydrous potassium bromide in a 1000-ml volumetric flask, dilute to volume with water, and mix. Each ml contains 5 µg of Br.

Bromide Working Standard Pipet 10.0 ml of the *Bromide Stock Solution* into a 1000-ml volumetric flask, dilute to volume with water, and mix. Each ml contains 5 µg of Br.

Acetate Buffer Solution Dissolve 68 g of sodium acetate trihydrate in water, add 30 ml of glacial acetic acid, dilute to 1000 ml with water, and mix. The pH of this solution is 4.6 to 4.7.

Sample Solution Place 5.0 g of the sample, accurately weighed, in a 250-ml beaker, dissolve in about 150 ml of water, transfer the solution into a 500-ml volumetric flask, dilute to volume with water, and mix.

Procedure Transfer 2.0 ml of the *Sample Solution* and 48.0 ml of water into a 125-ml Erlenmeyer flask. Into a second 125-ml Erlenmeyer flask transfer 5.0 ml of the *Bromide Working Standard* and 45.0 ml of water. To each flask add 2.0 ml of *Acetate Buffer Solution*, 2.0 ml of phenol red indicator solution (21 mg of phenolsulfonphthalein sodium salt per 100 ml of water), and 0.5 ml of a 0.5% solution of chloramine T (sodium *p*-toluenesulfonchloramide), and mix thoroughly. After exactly 20 min add 0.5 ml of 2 *M* sodium thiosulfate, with mixing, and compare the colors visually. The color of the sample mixture does not exceed that of the standard mixture.

Calcium Content Weigh accurately about 1.5 g, and dissolve it in 100 ml of water containing 2 ml of diluted hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration

to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of Ca.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 8 h.

Reducing Substances Fill a 38- × 250-mm chromatographic tube, to within 5 mm from the top, with Amberlite IR-120 ion-exchange resin or other equivalent cation-exchange resin of the sulfonated hydrocarbon type, in the hydrogen form. Dissolve 1.0 g of the sample in 35 ml of water, and pass the solution through the ion-exchange column, collecting the eluate in a 250-ml volumetric flask. Allow the solution to descend only to the level of the resin surface, then add 20 to 30 ml of water, and again let the level run down to the top of the resin. Repeat this washing procedure until 150 to 180 ml of solution has eluted into the flask. (*Caution:* The column must not be permitted to run dry during this process, as this would markedly reduce the column efficiency and also increase the eluting time.) Neutralize the solution to phenolphthalein by the addition of sodium hydroxide solution (first use sodium hydroxide TS, then approximately 0.5 *N* sodium hydroxide as the phenolphthalein endpoint is approached). Add 10 ml of copper sulfate solution (69.3 g of $CuSO_4.5H_2O$ per 1000 ml solution, filtered before use), mix well, then add 6 ml of 0.5 *N* sodium hydroxide, and mix. Dilute to volume with water, mix, and filter. Refilter through the same filter paper if necessary until a clear filtrate is obtained. Pipet a 50-ml aliquot of the clear filtrate into a 400-ml beaker, and add 50 ml of alkaline cupric tartrate TS. Cover the beaker with a watch glass, heat the mixture at such a rate that it comes to a boil in exactly 4 min, and boil for exactly 2 min longer. Remove from the heat, and filter immediately through a tared Gooch crucible. Wash with hot water until the precipitated cuprous oxide is quantitatively transferred to the crucible. (NOTE: Once the transfer has begun, it should continue without interruption to prevent the precipitate from creeping over the sides of the crucible and/or through the asbestos. If the crucible is permitted to empty more than once or twice during the filtration, serious loss of precipitate may occur.) Rinse the precipitate well with hot water and then with about 15 ml of alcohol. Finally, dry at 100° for 30 min (or equivalent conditions), cool, and weigh. The weight of the cuprous oxide does not exceed 15.2 mg.

Specific Rotation, page 530 Determine in a solution containing 500 mg, calculated on the anhydrous basis, in each 10 ml.

Sulfate Transfer about 25 g, accurately weighed, into a 600-ml beaker, dissolve it in 200 ml of water, adjust the solution to a pH between 4.5 and 6.5 with diluted hydrochloric acid TS, and filter, if necessary. Heat the filtrate or clear solution to just below the boiling point, then add 10 ml of barium chloride TS, stirring vigorously, boil gently for 5 min, and allow to stand for at least 2 h, or preferably overnight. Collect the precipitate of barium sulfate on a tared Gooch crucible, wash until free from chloride, dry, and ignite at 600° to constant weight. The weight of barium sulfate so obtained, multiplied by 0.412, represents the weight of SO_4 in the sample taken.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Firming agent in dry pudding mixes.

Calcium Oxide

Lime

CaO Mol wt 56.08

DESCRIPTION

Hard, white or grayish white masses or granules, or a white to grayish white powder. It is odorless. One g dissolves in about 840 ml of water at 25°, and in about 1740 ml of boiling water. It is soluble in glycerin, but is insoluble in alcohol.

REQUIREMENTS

Identification

Slake 1 g with 20 ml of water, and add acetic acid until the sample is dissolved. The resulting solution gives positive tests for *Calcium*, page 516.

Assay Not less than 95.0% of CaO after ignition.

Acid-Insoluble Substances Not more than 1%.

Alkalies or Magnesium Not more than 3.6%.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Ignition Not more than 10%.

TESTS

Assay Ignite about 1 g to constant weight, and dissolve the ignited sample, accurately weighed, in 20 ml of diluted hydrochloric acid TS. Cool the solution, dilute with water to 500.0 ml, and mix. Pipet 50.0 ml of this solution into a suitable container, and add 50 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.804 mg of CaO.

Acid-Insoluble Substances Slake a 5-g sample, then mix it with 100 ml of water and sufficient hydrochloric acid, added dropwise, to effect solution. Boil the solution, cool, add hydrochloric acid, if necessary, to make the solution distinctly acid, and filter through a tared crucible. Wash the residue

with water until free of chlorides, dry at 105° for 1 h, cool, and weigh.

Alkalies or Magnesium Dissolve 500 mg in 30 ml of water and 15 ml of diluted hydrochloric acid TS. Heat the solution and boil for 1 min. Add rapidly 40 ml of oxalic acid TS, and stir vigorously. Add 2 drops of methyl red TS, and neutralize the solution with ammonia TS to precipitate the calcium completely. Heat the mixture on a steam bath for 1 h, cool, dilute to 100 ml with water, mix well, and filter. To 50 ml of the filtrate add 0.5 ml of sulfuric acid, then evaporate to dryness, and ignite to constant weight in a tared platinum crucible.

Arsenic A solution of 1 g in 15 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Weigh accurately 1.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Heavy Metals Mix 2 g with 25 ml of water, cautiously add 7 ml of hydrochloric acid, followed by 3 ml of nitric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of diluted hydrochloric acid TS and 25 ml of hot water, filter, wash with a few ml of water, and dilute the filtrate to 100 ml with water. A 25-ml portion of this solution, to which has been added 1.0 ml of 10% hydroxylamine hydrochloride solution, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 15 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Ignition Ignite 1 g to constant weight in a tared platinum crucible with a blast lamp.

Packaging and Storage Store in tight containers.

Functional Use in Foods Alkali; nutrient; dietary supplement; dough conditioner; yeast food.

Calcium Pantothenate

d-Calcium Pantothenate; Dextro Calcium Pantothenate

$$[HOCH_2C(CH_3)_2CH(OH)CONH(CH_2)_2COO]_2Ca$$

$C_{18}H_{32}CaN_2O_{10}$ Mol wt 476.54

DESCRIPTION

The calcium salt of the dextrorotatory isomer of pantothenic acid occurs as a slightly hygroscopic, white powder. It is odorless and has a bitter taste. It is stable in air. One g dissolves in about 3 ml of water. It is soluble in glycerin, but is practically insoluble in alcohol, in chloroform, and in ether.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Calcium*, page 516.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 3 h, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Calcium Pantothenate Reference Standard.

C. Boil 50 mg in 5 ml of 1 *N* sodium hydroxide for 1 min, cool, and add 5 ml of 1 *N* hydrochloric acid and 2 drops of ferric chloride TS. A strong yellow color is produced.

Assay Not less than 90.0% and not more than the equivalent of 110.0% of dextrorotatory calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), calculated on the dried basis.

Alkalinity Passes test.

Alkaloids Passes test.

Calcium Content Not less than 8.2% and not more than 8.6% of Ca after drying.

Heavy Metals (as Pb) Not more than 0.002%.

Loss on Drying Not more than 5%.

Specific Rotation $[\alpha]_D^{25°}$: Between +25° and +27.5°.

TESTS

Assay Proceed as directed under *Calcium Pantothenate Assay*, page 466.

Alkalinity Dissolve 1 g in 15 ml of recently boiled and cooled water in a small flask. As soon as solution is complete, add 1.0 ml of 0.1 *N* hydrochloric acid, then add 0.05 ml of phenolphthalein TS, and mix. No pink color is produced within 5 s.

Alkaloids Dissolve 200 mg in 5 ml of water, and add 1 ml of diluted hydrochloric acid TS and 2 drops of mercuric-potassium iodide TS. No turbidity is produced in 1 min.

Calcium Content Weigh accurately about 950 mg, previously dried at 105° for 3 h, and dissolve it in 100 ml of water containing 2 ml of diluted hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of Ca.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 3 h.

Specific Rotation, page 530 Determine in a solution containing 500 mg, calculated on the dried basis, in each 10 ml.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement.

Calcium Pantothenate, Racemic

$C_{18}H_{32}CaN_2O_{10}$ Mol wt 476.54

DESCRIPTION

A mixture of the calcium salts of the dextrorotatory and levorotatory isomers of pantothenic acid. It occurs as a white, slightly hygroscopic powder. It is odorless, has a bitter taste, and is stable in air. Its solutions are neutral or alkaline to litmus. It is optically inactive. It is freely soluble in water. It is soluble in glycerin, and is practically insoluble in alcohol, in chloroform, and in ether.

NOTE: The physiological activity of racemic calcium pantothenate is approximately one-half that of the dextrorotatory isomer.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Calcium*, page 516.
B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 3 h, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Calcium Pantothenate Reference Standard.
C. Boil 50 mg in 5 ml of 1 *N* sodium hydroxide for 1 min, cool, and add 5 ml of 1 *N* hydrochloric acid and 2 drops of ferric chloride TS. A strong yellow color is produced.

Assay Not less than 42.5% of dextrorotatory calcium pantothenate ($C_{18}H_{32}CaN_2O_{10}$), calculated on the dried basis.
Alkalinity Passes test.
Alkaloids Passes test.
Calcium Content Not less than 8.2% and not more than 8.6% of Ca after drying.
Heavy Metals (as Pb) Not more than 0.002%.
Loss on Drying Not more than 5%.

TESTS

Assay Proceed as directed under *Calcium Pantothenate Assay*, page 466.
Alkalinity Dissolve 1 g in 15 ml of recently boiled and cooled water in a small flask. As soon as solution is complete, add 1.6 ml of 0.1 *N* hydrochloric acid, then add 0.05 ml of phenolphthalein TS, and mix. No pink color is produced within 5 s.
Alkaloids Dissolve 200 mg in 5 ml of water, and add 1 ml of diluted hydrochloric acid TS and 2 drops of mercuric-potassium iodide TS. No turbidity is produced in 1 min.
Calcium Content Weigh accurately about 950 mg, previously dried at 105° for 3 h, and dissolve it in 100 ml of water containing 2 ml of diluted hydrochloric acid TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of Ca.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 105° for 3 h.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Calcium Pantothenate, Calcium Chloride Double Salt

Calcium Chloride Double Salt of *dl*- or *d*-Calcium Pantothenate

$C_{18}H_{32}CaN_2O_{10}.CaCl_2$ Mol wt 587.52

DESCRIPTION

A chemical complex composed of approximately equimolecular quantities of dextrorotatory (*d*) or racemic (*dl*) calcium pantothenate and calcium chloride. It occurs as a white, odorless, free-flowing, fine powder having a bitter taste. It is freely soluble in water, but insoluble in alcohol. Its solutions in water are alkaline to litmus.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Calcium*, page 516.
B. Dissolve 50 mg in 5 ml of 1 *N* sodium hydroxide and filter. To the filtrate add 1 drop of cupric sulfate TS. A deep blue color develops.
C. Stir 1.0 g of a dried sample with 15 ml of dimethylformamide for 5 min. Centrifuge the mixture, then transfer 2.0 ml of the clear supernatant liquid to a weighing dish, evaporate it under vacuum on a steam bath, and dry the residue in an oven at 105° for 1 h. The weight of the residue, composed of uncombined calcium pantothenate and calcium chloride, in g, multiplied by 750 equals the percentage of uncomplexed material in the sample. It does not exceed 10.0% of the weight of the sample.

Assay Not less than the equivalent of 37.0% of dextro calcium pantothenate, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Calcium Content Between 12.4% and 13.6% of Ca after drying.
Chloride Between 10.5% and 12.1% of Cl after drying.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 5%.

TESTS

Assay Proceed as directed under *Calcium Pantothenate Assay*, page 466.
Arsenic A solution of 1 g in 25 ml of water meets the requirements of the *Arsenic Test*, page 464.
Calcium Content Proceed as directed for *Calcium Content* under *Calcium Pantothenate*, page 56.
Chloride Transfer about 1 g, previously dried in vacuum for 1 h and accurately weighed, into a 250-ml beaker, and add sufficient water to make 100 ml. Equip a pH meter with glass and silver electrodes, and set it on the "+ millivolt" scale. Insert the electrodes and a motor-driven glass stirring rod into the sample beaker. Add 1 to 2 drops of methyl orange TS. Stir and add, dropwise, 10% nitric acid until a pink color is obtained, then add 10 ml excess. Titrate the solution with 0.1 *N* silver nitrate to a reading of +1.0 millivolt on the pH meter. Each ml of 0.1 *N* silver nitrate is equivalent to 3.545 mg of Cl.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A solution of 1 g in 25 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry in vacuum at 100° for 1 h.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Calcium Peroxide

Calcium Dioxide; Calcium Superoxide

CaO_2 Mol wt 72.08

DESCRIPTION

A white or yellowish, odorless, almost tasteless powder or granular material. It decomposes in moist air. It is practically insoluble in water. It dissolves in acids, forming hydrogen peroxide. A 1 in 100 aqueous slurry has a pH of about 12.

REQUIREMENTS

Identification

Cautiously dissolve 250 mg in 5 ml of glacial acetic acid, and add a few drops of a saturated solution of potassium iodide. Iodine is liberated. Add 20 ml of water and sufficient sodium thiosulfate TS to remove the iodine color. The resulting solution gives positive tests for *Calcium*, page 516.

Assay Not less than 60.0% of CaO_2.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.

TESTS

Assay Transfer about 1 g of the sample, accurately weighed, into an Erlenmeyer flask, add 30 ml of water and 30 ml of 85% phosphoric acid diluted 1 to 1 with water, and titrate immediately with 0.5 *N* potassium permanganate to the first faint pink color that persists for 1 min. Each ml of 0.5 *N* potassium permanganate is equivalent to 18.02 mg of CaO_2.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead Weigh accurately 4.0 g of the sample into a 250-ml beaker, cautiously add 50 ml of nitric acid, and evaporate just to dryness on a steam bath. Add 20 ml of nitric acid, repeat the evaporation, cool, and dissolve the residue in sufficient water, containing 4 drops of nitric acid, to make 40.0 ml.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.
Fluoride Weigh accurately 1.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.
Heavy Metals A 10-ml portion of the *Sample Solution* meets the requirements of the *Heavy Metals Test*, page 512, using 40 µg of lead ion (Pb) in the control (*Solution A*), and adjusting the solutions to a pH of 2.0, instead of between 3.0 and 4.0.
Lead A 10-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers, and avoid contact with readily oxidizable materials. Observe safety precautions printed on the label of the original container.
Functional Use in Foods Dough conditioner; oxidizing agent.

Calcium Phosphate, Dibasic

Dicalcium Phosphate

$CaHPO_4 \cdot 2H_2O$ Mol wt 172.09

DESCRIPTION

Dibasic calcium phosphate is anhydrous or contains two molecules of water of hydration. It occurs as a white, odorless, tasteless powder that is stable in air. It is practically insoluble in water, but is readily soluble in dilute hydrochloric and nitric acids. It is insoluble in alcohol.

REQUIREMENTS

Identification

A. Dissolve about 100 mg by warming with a mixture of 5 ml

of diluted hydrochloric acid TS and 5 ml of water, add 2.5 ml of ammonia TS, dropwise, with shaking, and then add 5 ml of ammonium oxalate TS. A white precipitate is formed.

B. To 10 ml of a warm solution (1 in 100) in a slight excess of nitric acid add 10 ml of ammonium molybdate TS. A yellow precipitate of ammonium phosphomolybdate is formed.

Assay Not less than 30.0% and not more than 31.7% of calcium (Ca), calculated on the ignited basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 5 ppm.

Loss on Ignition $CaHPO_4$ (anhydrous): between 7.0% and 8.5%; $CaHPO_4.2H_2O$ (dihydrate): between 24.5% and 26.5%.

TESTS

Assay Transfer about 200 mg of the sample, accurately weighed, to a 250-ml beaker equipped with a magnetic stirrer, and dissolve it, with the aid of gentle heat if necessary, in a mixture of 5 ml of hydrochloric acid and 3 ml of water. Cautiously add 125 ml of water. With constant stirring, add in the order named 0.5 ml of triethanolamine, 300 mg of hydroxy naphthol blue indicator, and (from a 50-ml buret) about 23 ml of 0.05 *M* disodium EDTA. Add sodium hydroxide solution (45 in 100) until the initial red color changes to clear blue, then continue to add it dropwise until the color changes to violet, and then add an additional 0.5 ml. The pH should be between 12.3 and 12.5. Continue the titration dropwise with the 0.05 *M* disodium EDTA to the appearance of a clear blue endpoint that persists for not less than 60 s. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of calcium (Ca).

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 1-g sample, dissolved in 10 ml of 1 *N* hydrochloric acid for the dihydrate, or 16 ml for the anhydrous salt, as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution C* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals Warm 1.33 g with 5 ml of diluted hydrochloric acid TS until no more dissolves, dilute to 50 ml with water, and filter. A 25-ml portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 250 mg in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 1.25 µg of lead ion (Pb) in the control.

Loss on Ignition Weigh accurately about 1 g, and ignite, preferably in a muffle furnace, at 800° to 825° to constant weight.

Packaging and Storage Store in well-closed containers.

Labeling Label to indicate whether it is anhydrous or the dihydrate.

Functional Use in Foods Dough conditioner; nutrient; dietary supplement; yeast food.

Calcium Phosphate, Monobasic

Monocalcium Phosphate; Calcium Biphosphate; Acid Calcium Phosphate

$Ca(H_2PO_4)_2$ Mol wt 234.05

DESCRIPTION

Monobasic calcium phosphate is anhydrous or contains one molecule of water of hydration, but, due to its deliquescent nature, more than the calculated amount of water may be present. It occurs as white crystals or granules, or as a granular powder. It is sparingly soluble in water and is insoluble in alcohol.

REQUIREMENTS

Identification

A. Dissolve 100 mg by warming in a mixture of 2 ml of diluted hydrochloric acid TS and 8 ml of water, and add 5 ml of ammonium oxalate TS. A white precipitate forms.

B. To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate forms.

Assay $Ca(H_2PO_4)_2$ (anhydrous): not less than 16.8% and not more than 18.3% of Ca; $Ca(H_2PO_4)_2.H_2O$ (monohydrate): not less than 15.9% and not more than 17.7% of Ca.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.0025%.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 5 ppm.

Loss on Drying $Ca(H_2PO_4)_2.H_2O$ (monohydrate): not more than 1%.

Loss on Ignition $Ca(H_2PO_4)_2$ (anhydrous): between 14.0% and 15.5%.

TESTS

Assay Weigh accurately a portion of the sample equivalent to about 475 mg of $Ca(H_2PO_4)_2$, dissolve it in 10 ml of diluted hydrochloric acid TS, add a few drops of methyl orange TS, and boil for 5 min, keeping the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water, if necessary. Add 2 drops of methyl red TS and 30 ml of ammonium oxalate TS, then add dropwise, with constant stirring, a mixture of equal volumes of ammonia TS and water until the pink color of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through an asbestos mat in a Gooch crucible, using gentle suction. Wash the precipitate in the beaker with about 30 ml of cold (below 20°) wash solution, prepared by diluting 10 ml of ammonium oxalate TS to 1000 ml. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation

three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter with two 10-ml portions of cold (below 20°) water. Place the Gooch crucible in the beaker, and add 100 ml of water and 50 ml of cold dilute sulfuric acid (1 in 6). Add from a buret 35 ml of 0.1 *N* potassium permanganate, and stir until the color disappears. Heat to about 70°, and complete the titration with 0.1 *N* potassium permanganate. Each ml of 0.1*N* potassium permanganate is equivalent to 2.004 mg of Ca.

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 3-g sample, dissolved in 5 ml of 1:1 hydrochloric acid solution, as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.2 ml of *Fluoride Standard Solution*.

Heavy Metals Warm 1.33 g with 5 ml of diluted hydrochloric acid TS until no more dissolves, dilute to 50 ml with water, and filter. A 25-ml portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 250 mg in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 1.25 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry $Ca(H_2PO_4)_2.H_2O$ (monohydrate) at 60° for 3 h.

Loss on Ignition Weigh accurately about 1 g of $Ca(H_2PO_4)_2$ (anhydrous), and ignite, preferably in a muffle furnace, at 800° for 30 min.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Buffer; dough conditioner; firming agent; leavening agent; nutrient; dietary supplement; yeast food; sequestrant.

Calcium Phosphate, Tribasic

Tricalcium Phosphate; Precipitated Calcium Phosphate

DESCRIPTION

Tribasic calcium phosphate consists of a variable mixture of calcium phosphates having the approximate composition of $10CaO.3P_2O_5.H_2O$. It occurs as a white, odorless, tasteless powder that is stable in air. It is insoluble in alcohol and almost insoluble in water, but it dissolves readily in dilute hydrochloric and nitric acids.

REQUIREMENTS

Identification

A. To a warm solution of the sample in a slight excess of nitric acid add ammonium molybdate TS. A yellow precipitate forms.

B. Dissolve about 100 mg by warming with 5 ml of diluted hydrochloric acid TS and 5 ml of water, add 1 ml of ammonia TS, dropwise, with shaking, and then add 5 ml of ammonium oxalate TS. A white precipitate forms.

Assay Not less than 34.0% and not more than 40.0% of calcium (Ca).

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.0075%.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 5 ppm.

Loss on Ignition Not more than 10%.

TESTS

Assay Proceed as directed in the *Assay* under *Calcium Phosphate, Dibasic*, page 58, using a 150-mg sample, accurately weighed. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.004 mg of Ca.

Arsenic A solution of 1 g in 25 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 1-g sample, dissolved in 18 ml of 1 *N* hydrochloric acid, as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution C* and 0.2 ml of *Fluoride Standard Solution*.

Heavy Metals Warm 1.33 g with 7 ml of diluted hydrochloric acid TS until no more dissolves, dilute to 50 ml with water, and filter. A 25-ml portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*). (NOTE: Filter the mixture after pH adjustment.)

Lead A solution of 250 mg in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 1.25 µg of lead ion (Pb) in the control.

Loss on Ignition Weigh accurately about 1 g, and ignite, preferably in a muffle furnace, at 800° to 825° to constant weight.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Anticaking agent; buffer; nutrient; dietary supplement.

Calcium Propionate

$(CH_3CH_2COO)_2Ca$

$C_6H_{10}CaO_4$ Mol wt 186.22

DESCRIPTION

White crystals or crystalline solid, possessing not more than a faint odor of propionic acid. One g dissolves in about 3 ml of water. The pH of a 1 in 10 solution is between 8 and 10.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Calcium*, page 516.
B. Upon ignition at a relatively low temperature, it yields an alkaline residue that effervesces with acids.
C. Warm a small sample with sulfuric acid. Propionic acid, recognizable by its odor, is evolved.

Assay Not less than 98.0% of $C_6H_{10}CaO_4$, calculated on the anhydrous basis.
Arsenic (As As) Not more than 3 ppm.
Fluoride Not more than 0.003%.
Heavy Metals (as Pb) Not more than 10 ppm.
Insoluble Substances Not more than 0.2%.
Magnesium (as MgO) Passes test (about 0.4%).
Water Not more than 5%.

TESTS

Assay Dissolve about 400 mg, accurately weighed, in 100 ml of water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 9.311 mg of $C_6H_{10}CaO_4$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Fluoride Proceed as directed in the *Fluoride Limit Test*, page 510, using *Method I* (1.67-g sample) or *Method III* (1.0-g sample).
Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Insoluble Substances Dissolve 10 g in 100 ml of hot water, filter through a tared filtering crucible, wash the insoluble residue with hot water, and dry at 105° to constant weight.
Magnesium Place 400.0 mg of the sample, 5 ml of diluted hydrochloric acid TS, and about 10 ml of water in a small beaker, and dissolve the sample by heating on a hot plate. Evaporate the solution to a volume of about 2 ml, and cool. Transfer the residual liquid into a 100-ml volumetric flask, dilute to volume with water, and mix. Dilute 7.5 ml of this solution to 20 ml with water, add 2 ml of sodium hydroxide TS and 0.05 ml of a 1 in 1000 solution of Titan yellow (Clayton yellow), mix, allow to stand for 10 min, and shake. Any color does not exceed that produced by 1.0 ml of *Magnesium Standard Solution* (50 µg Mg ion) in the same volume of a control containing 2.5 ml of the sample solution (10-mg sample) and the quantities of the reagents used in the test.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Preservative; mold and rope inhibitor.

Calcium Pyrophosphate

$Ca_2P_2O_7$ Mol wt 254.10

DESCRIPTION

A fine, white, odorless and tasteless powder. It is insoluble in water, but is soluble in dilute hydrochloric and nitric acids.

REQUIREMENTS

Identification

A. Dissolve about 100 mg by warming with a mixture of 5 ml of diluted hydrochloric acid TS and 5 ml of water, add 2.5 ml of ammonia TS, dropwise, with shaking, and then add 5 ml of ammonium oxalate TS. A white precipitate is formed.
B. Dissolve 100 mg of the sample in 100 ml of diluted nitric acid TS. Add 0.5 ml of this solution to 30 ml of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 ml of the solution to 30 ml of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 96.0% of $Ca_2P_2O_7$.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 0.003%.
Lead Not more than 5 ppm.
Loss on Ignition Not more than 1%.

TESTS

Assay Dissolve about 300 mg, accurately weighed, in 10 ml of diluted hydrochloric acid TS, add about 120 ml of water and a few drops of methyl orange TS, and boil for 30 min, keeping the volume and pH of the solution constant during the boiling period by adding hydrochloric acid or water, if necessary. Add 2 drops of methyl red TS and 30 ml of ammonium oxalate TS, then add dropwise, with constant stirring, a mixture of equal volumes of ammonia TS and water until the pink color of the indicator just disappears. Digest on a steam bath for 30 min, cool to room temperature, allow the precipitate to settle, and filter the supernatant liquid through an asbestos mat in a Gooch crucible, using gentle suction. Wash the precipitate in the beaker with about 30 ml of cold (below 20°) wash solution, prepared by diluting 10 ml of ammonium oxalate TS to 1000 ml. Allow the precipitate to settle, and pour the supernatant liquid through the filter. Repeat this washing by decantation three more times. Using the wash solution, transfer the precipitate as completely as possible to the filter. Finally, wash the beaker and the filter

with two 10-ml portions of cold (below 20°) water. Place the Gooch crucible in the beaker, and add 100 ml of water and 50 ml of cold dilute sulfuric acid (1 in 6). Add from a buret 35 ml of 0.1 *N* potassium permanganate, and stir until the color disappears. Heat to about 70°, and complete the titration with 0.1 *N* potassium permanganate. Each ml of 0.1 *N* potassium permanganate is equivalent to 6.35 mg of $Ca_2P_2O_7$.

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Weigh accurately 1.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Heavy Metals Warm 1.33 g with 7 ml of diluted hydrochloric acid TS until no more dissolves, dilute to 50 ml with water, and filter. A 25-ml portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 250 mg in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 1.25 µg of lead ion (Pb) in the control.

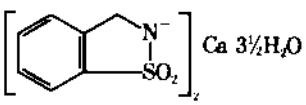
Loss on Ignition Weigh accurately about 1 g, and ignite, preferably in a muffle furnace, at 800° to 825° for 30 min.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Buffer; neutralizing agent; nutrient; dietary supplement.

Calcium Saccharin

1,2-Benzisothiazolin-3-one 1,1-Dioxide Calcium Salt



$C_{14}H_8CaN_2O_6S_2 \cdot 3\text{-}1/2\ H_2O$ Mol wt 467.48

DESCRIPTION

White crystals or a white, crystalline powder. It is odorless or has a faint, aromatic odor. It is intensely sweet even in dilute solutions. One g is soluble in 1.5 ml of water.

REQUIREMENTS

Identification

A. Dissolve about 100 mg in 5 ml of sodium hydroxide solution (1 in 20), evaporate to dryness, and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with diluted hydrochloric acid TS, and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet color.

B. Mix 20 mg with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid results.

C. A 1 in 10 solution gives positive tests for *Calcium*, page 516.

D. To 10 ml of a 1 in 10 solution add 1 ml of hydrochloric acid. A crystalline precipitate of saccharin is formed. Wash the precipitate well with cold water, and dry at 105° for 2 hr. It melts between 226° and 230° (*Class Ia*, page 519).

Assay Not less than 95.0% of $C_{14}H_8CaN_2O_6S_2$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Benzoate and Salicylate Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.

Readily Carbonizable Substances Passes test.

Selenium Not more than 0.003%.

Toluenesulfonamides Not more than 0.0025%.

Water Not more than 15.0%.

TESTS

Assay Weigh accurately about 500 mg, and transfer it quantitatively to a separator with the aid of 10 ml of water. Add 2 ml of diluted hydrochloric acid TS, and extract the precipitated saccharin first with 30 ml, then with five 20-ml portions, of a solvent composed of 9 volumes of chloroform and 1 volume of alcohol. Filter each extract through a small filter paper moistened with the solvent mixture, and evaporate the combined filtrates on a steam bath to dryness with the aid of a current of air. Dissolve the residue in 75 ml of hot water, cool, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination, and make any necessary correction (see page 2). Each ml of 0.1 *N* sodium hydroxide is equivalent to 20.22 mg of $C_{14}H_8CaN_2O_6S_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Benzoate and Salicylate To 10 ml of a 1 in 20 solution previously acidified with 5 drops of acetic acid, add 3 drops of ferric chloride TS. No precipitate or violet color appears.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Readily Carbonizable Substances, page 532 Dissolve 200 mg in 5 ml of sulfuric acid TS, and keep at a temperature of 48° to 50° for 10 min. The color is no darker than *Matching Fluid A*.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Toluenesulfonamides Determine as directed under *Sodium Saccharin*, page 298.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nonnutritive sweetener.

Calcium Silicate

DESCRIPTION

A hydrous or anhydrous silicate with varying proportions of CaO and SiO_2. It occurs as a white to off-white free-flowing powder that remains so after absorbing relatively large amounts of water or other liquids. It is insoluble in water, but forms a gel with mineral acids. The pH of a 1 in 20 aqueous slurry is between 8.4 and 10.2.

REQUIREMENTS

Identification

A. Mix about 500 mg with 10 ml of dilute hydrochloric acid TS, filter, and neutralize the filtrate to litmus paper with ammonia TS. The neutralized filtrate gives positive tests for *Calcium*, page 516.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with a sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.

Assay for Calcium Oxide and Silicon Dioxide Not less than the percentages stated or within the range claimed by the vendor.

Arsenic Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying or Loss on Ignition Not more than the percentages stated or within the range claimed by the vendor.

TESTS

Assay for Silicon Dioxide Transfer about 400 mg of the sample, accurately weighed, into a beaker, add 5 ml of water and 10 ml of perchloric acid, and heat until dense white fumes of perchloric acid are evolved. Cover the beaker with a watch glass, and continue to heat for 15 min longer. Allow to cool, add 30 ml of water, filter, and wash the precipitate with 200 ml of hot water. Retain the combined filtrate and washings for use in the *Assay for Calcium Oxide*. Transfer the filter paper and its contents to a platinum crucible, heat slowly to dryness, and then heat sufficiently to char the filter paper. After cooling, add a few drops of sulfuric acid, and then ignite at about 1300° to constant weight. Moisten the residue with 5 drops of sulfuric acid, add 15 ml of hydrofluoric acid, heat cautiously on a hot plate until all of the acid is driven off, and ignite to constant weight at a temperature not lower than 1000°. Cool in a desiccator and weigh. The loss in weight is equivalent to the SiO_2 in the sample taken.

Assay for Calcium Oxide Using sodium hydroxide TS, neutralize to litmus the combined filtrate and washings retained in the *Assay for Silicon Dioxide*, and add, while stirring, about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret. Add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.804 mg of CaO.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead Transfer 10.0 g of the sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter paper into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Fluoride Prepare a slurry consisting of 5 g of the sample and 45 ml of 0.1 *N* hydrochloric acid, stir for 15 min at room temperature, and filter through a 0.45-μm membrane filter into a 50-ml volumetric flask. Wash the filter with five 1-ml portions of 0.1 *N* hydrochloric acid, collecting the washings in the flask, then dilute to volume with 0.1 *N* hydrochloric acid, and mix. Transfer 5.0 ml of this solution into a 25-ml volumetric flask, add 5.0 ml of a 10% solution of Amadac-F* in 60% isopropanol, dilute to volume with water, mix, and allow to stand for 1 h in diffuse light at room temperature. Determine the absorbance of this solution in a 1-cm cell with a suitable spectrophotometer, at the wavelength of maximum absorption at about 620 nm, against a blank consisting of 5.0 ml of 0.1 *N* hydrochloric acid, 5.0 ml of the Amadac indicator solution, and 15.0 ml of water. The absorbance is not greater than that produced by 5.0 ml of a solution containing 2.21 μg of NaF per ml of 0.1 *N* hydrochloric acid, when treated in the same manner as the sample.

Heavy Metals A 5-ml portion of the *Sample Solution* meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A 10-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Loss on Ignition Transfer about 1 g, previously dried at 105°

*Amadac-F is a product of Burdick & Jackson Laboratories, Inc., Muskegon, Mich. 49442, consisting of a blended solid mixture of partially hydrated sodium acetate, acetic acid, stabilizers, lanthanum nitrate, and 3-amino-methylalizarin-*N,N*-diacetate (alizarin complexan), the lanthanum and complexan being equimolar.

for 2 h and accurately weighed, into a suitable tared crucible, and ignite at 900° to constant weight.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Anticaking agent; filter aid.

Calcium Stearate

DESCRIPTION

Calcium stearate is a compound of calcium with a mixture of solid organic acids obtained from edible sources, and consists chiefly of variable proportions of calcium stearate and calcium palmitate. It occurs as a fine, white to yellowish white, bulky powder having a slight, characteristic odor. It is unctuous, and is free from grittiness. It is insoluble in water, in alcohol, and in ether. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for salts of fatty acids and fatty acids derived from edible fat sources.

REQUIREMENTS

Identification

A. Heat 1 g with a mixture of 25 ml of water and 5 ml of hydrochloric acid. Fatty acids are liberated, floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Calcium*, page 516.

B. Mix 25 g of the sample with 200 ml of hot water, then add 60 ml of diluted sulfuric acid TS, and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see page 538).

Assay Not less than the equivalent of 9.0% and not more than the equivalent of 10.5% of CaO.
Arsenic (as As) Not more than 3 ppm.
Free Fatty Acid (as stearic acid) Not more than 3.0%.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 4%.

TESTS

Assay Boil about 1.2 g, accurately weighed, with 50 ml of 0.1 *N* hydrochloric acid for 10 min, or until the fatty acid layer is clear, adding water if necessary to maintain the original volume. Cool, filter, and wash the filter and flask thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate to litmus with sodium hydroxide TS. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.804 mg of CaO.

Arsenic Mix 1 g of the sample with 10 ml of hydrochloric acid and 8 drops of bromine TS, and heat on a steam bath until a transparent layer of melted fatty acid forms. Add 50 ml of water, boil down to about 25 ml, and filter while hot. Cool, neutralize with a 1 in 2 solution of sodium hydroxide, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Free Fatty Acid Transfer 2 g of the sample, accurately weighed, into a dry 125-ml Erlenmeyer flask containing 50 ml of acetone, fit an air-cooled reflux condenser onto the neck of the flask, boil the mixture on a steam bath for 10 min, and cool. Filter through two layers of Whatman No. 42, or equivalent, filter paper, and wash the flask, residue, and filter with 50 ml of acetone. Add phenolphthalein TS and 5 ml of water to the filtrate, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination, using 100 ml of acetone and 5 ml of water (see page 2). Each ml of 0.1 *N* sodium hydroxide is equivalent to 28.45 mg of stearic acid ($C_{18}H_{36}O_2$).

Heavy Metals, page 512 Place 2.5 g of the sample in a porcelain dish, place 500 mg of the sample in a second dish for the control, and to each add 5 ml of a 1 in 4 solution of magnesium nitrate in alcohol. Cover the dishes with 3-in. short-stem funnels so that the stems are straight up. Heat for 30 min on a hot plate at the low setting, then heat for 30 min at the medium setting, and cool. Remove the funnels, add 20 µg of lead ion (Pb) to the control, and heat each dish over an Argand burner until most of the carbon is burned off. Cool, add 10 ml of nitric acid, and transfer the solutions into 250-ml beakers. Add 5 ml of 70% perchloric acid, evaporate to dryness, then add 2 ml of hydrochloric acid to the residues, and wash down the inside of the beakers with water. Evaporate carefully to dryness again, swirling near the dry point to avoid spattering. Repeat the hydrochloric acid treatment, then cool, and dissolve the residues in about 10 ml of water. To each solution add 1 drop of phenolphthalein TS and sufficient sodium hydroxide TS until the solutions just turn pink, and then add diluted hydrochloric acid TS until the solutions become colorless. Add 1 ml of diluted acetic acid TS and a small amount of charcoal to each solution, and filter through Whatman No. 2, or equivalent, filter paper into 50-ml Nessler tubes. Wash with water, dilute to 40 ml, and add 10 ml of hydrogen sulfide TS to each tube. The color in the solution of the sample does not exceed that produced in the control.

Loss on Drying, page 518 Dry at 105° to constant weight, using 2-h increments of heating.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Anticaking agent; binder; emulsifier.

Calcium Stearoyl Lactylate

DESCRIPTION

A mixture of calcium salts of stearoyl lactic acid, with minor proportions of other salts of related acids. It occurs as a cream-colored powder having a mild, caramellike odor. It is slightly soluble in hot water. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

Calcium stearoyl lactylate responds to the tests for *Identification* under *Calcium Stearate*, page 64.

Acid Value Between 50 and 86.
Arsenic (as As) Not more than 3 ppm.
Calcium Content Between 4.2% and 5.2%.
Ester Value Between 125 and 164.
Heavy Metals (as Pb) Not more than 10 ppm.
Total Lactic Acid Between 32% and 38%.

TESTS

Acid Value Transfer about 1 g, accurately weighed, to a 125-ml volumetric flask, add 25 ml of alcohol, previously neutralized in phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula $56.1V \times N/W$, in which V is the volume, in ml, and N is the normality, respectively, of the sodium hydroxide solution, and W is the weight, in g, of the sample taken. Retain the neutralized solution for the determination of *Ester Value*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Calcium Content

Stock Lanthanum Solution Transfer 5.86 g of lanthanum oxide, La_2O_3, into a 100-ml volumetric flask, wet with a few ml of water, slowly add 25 ml of hydrochloric acid, and swirl until the material is completely dissolved. Dilute to volume with water, and mix.

Stock Calcium Solution Use a solution containing 0.5 mg of Ca in each ml (500 ppm Ca). The solution may be obtained commercially or prepared as follows: Transfer 124.8 mg of calcium carbonate, $CaCO_3$, previously dried at 200° for 4 h, into a 100-ml volumetric flask, carefully dissolve in 2 ml of diluted hydrochloric acid TS, dilute to volume with water, and mix.

Standard Preparations Transfer 10.0 ml of the *Stock Lanthanum Solution* into each of three 50-ml volumetric flasks. Using a microliter syringe, transfer 0.20 ml of the *Stock Calcium Solution* into the first flask, 0.40 ml into the second flask, and 0.50 ml into the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0, and 5.0 µg of Ca per ml, respectively. Prepare these solutions fresh daily.

Sample Preparation Transfer about 250 mg of the sample, accurately weighed, into a 30-ml beaker, dissolve with heating in 10 ml of alcohol, and quantitatively transfer the solution into a 25-ml volumetric flask. Wash the beaker with two 5-ml portions of alcohol, adding the washings to the flask, dilute to volume with alcohol, and mix. Transfer 5.0 ml of the *Stock Lanthanum Solution* to a second 25-ml volumetric flask. Using a microliter syringe, transfer 0.25 ml of the alcoholic solution of the sample to the second flask, dilute to volume with water, and mix.

Procedure Concomitantly determine the absorbance of each *Standard Preparation* and of the *Sample Preparation* at 422.7 nm, with a suitable atomic absorption spectrophotometer, following the operating parameters as recommended by the manufacturer of the instrument. Plot the absorbance of the *Standard Preparations* versus concentration of Ca, in µg per ml, and from the curve so obtained determine the concentration, C, in µg per ml, of Ca in the *Sample Preparation*. Calculate the quantity, in mg, of Ca in the sample taken by the formula $2.5C$.

Ester Value To the neutralized solution retained in the test for *Acid Value* add 10.0 ml of alcoholic potassium hydroxide solution prepared by dissolving 11.2 g of potassium hydroxide in 250 ml of alcohol and diluting with 25 ml of water. Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 h. Cool, add 5 additional drops of phenolphthalein TS, and titrate the excess alkali with 0.1 *N* sulfuric acid. Perform a blank determination using 10.0 ml of the alcoholic potassium hydroxide solution. Calculate the ester value by the formula $56.1(B - S)N/W$, in which $B - S$ represents the difference between the volumes of 0.1 *N* sulfuric acid required for the blank and the sample, respectively, N is the normality of the sulfuric acid, and W is the weight, in g, of the sample taken.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Total Lactic Acid

Standard Curve Dissolve 1.067 g of lithium lactate in sufficient water to make 1000.0 ml. Transfer 10.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 1.0, 2.0, 4.0, 6.0, and 8.0 ml of the diluted standard solution into separate 100-ml volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6, and 8 µg of lactic acid per ml, respectively. Transfer 1.0 ml of each solution into separate test tubes, and continue as directed in the *Procedure*, beginning with "Add 1 drop of cupric sulfate TS. . . . " After color development and reading the absorbance values, construct a *Standard Curve* by plotting absorbance versus µg of lactic acid.

Test Preparation Transfer about 200 mg of the sample, accurately weighed, into a 125-ml Erlenmeyer flask, add 10 ml of 0.5 *N* alcoholic potassium hydroxide and 10 ml of

water, attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 ml of water, and heat on a steam bath until no odor of alcohol remains. Add 6 ml of dilute sulfuric acid (1 in 2), heat until the fatty acids are melted, then cool to about 60°, and add 25 ml of petroleum ether. Swirl the mixture gently, and transfer quantitatively to a separator. Collect the water layer in a 100-ml volumetric flask, and wash the petroleum ether layer with two 20-ml portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 ml of this solution into a second 100-ml volumetric flask, dilute to volume with water, and mix.

Procedure Transfer 1.0 ml of the *Test Preparation* into a test tube, and transfer 1.0 ml of water to a second test tube to serve as the blank. Treat each tube as follows: Add 1 drop of cupric sulfate TS, swirl gently, and then add rapidly from a buret 9.0 ml of sulfuric acid. Loosely stopper the tube, and heat in a water bath at 90° for exactly 5 min. Cool immediately to below 20° in an ice bath for 5 min, add 3 drops of *p*-phenylphenol TS, shake immediately, and heat in a water bath at 30° for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90° for exactly 90 s, and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1-cm cell, at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in μg, of lactic acid in the portion of the *Test Preparation* taken for the *Procedure* by means of the *Standard Curve*.

Packaging and Storage Store in tight containers in a cool dry place.

Functional Use in Foods Dough conditioner; stabilizer; whipping agent.

Calcium Sulfate

$CaSO_4.xH_2O$ Mol wt (anhydrous) 136.14

DESCRIPTION

Calcium sulfate is anhydrous or contains two molecules of water of hydration. It occurs as a fine, white to slightly yellow white, odorless powder.

REQUIREMENTS

Identification

Dissolve about 200 mg by warming with a mixture of 4 ml of diluted hydrochloric acid TS and 16 ml of water. A white precipitate forms when 5 ml of ammonium oxalate TS is added to 10 ml of the solution. Upon the addition of barium chloride TS to the remaining 10 ml, a white precipitate forms that is insoluble in hydrochloric and nitric acids.

Assay Not less than 99.0% of $CaSO_4$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.003%.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying $CaSO_4$ (anhydrous): not more than 1.5%; $CaSO_4.2H_2O$ (dihydrate): between 19% and 23%.

Selenium Not more than 0.003%.

TESTS

Assay Dissolve 250 mg, accurately weighed, in 100 ml of water and 4 ml of diluted hydrochloric acid TS, boil to effect solution, and cool. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 25 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 6.807 mg of $CaSO_4$.

Arsenic Mix 1 g with 10 ml of water, add 12 ml of diluted hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, filter, and dilute the filtrate to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Fluoride Weigh accurately 1.67 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Heavy Metals Mix 2 g with 20 ml of water, add 25 ml of diluted hydrochloric acid TS, and heat to boiling to dissolve the sample. Cool, and add ammonium hydroxide to a pH of 7. Filter, evaporate to a volume of about 25 ml, and refilter if necessary to obtain a clear solution. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 250° to constant weight.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement; yeast food; dough conditioner; firming agent; sequestrant.

Cananga Oil

DESCRIPTION

The oil obtained by distillation from the flowers of the tree *Cananga odorata* Hook f. et Thoms., (Fam. *Anonaceae*). It is a light to deep yellow liquid having a harsh floral odor suggestive of ylang ylang. It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative

maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 588, using the same test conditions as specified therein.

Angular Rotation Between −15° and −30°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.495 and 1.505 at 20°.
Saponification Value Between 10 and 40.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.904 and 0.920.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 95% alcohol, usually becoming cloudy on further dilution.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Candelilla Wax

DESCRIPTION

A purified wax obtained from the leaves of the candelilla plant, *Euphorbia antisyphilitica*. It is a hard, yellowish brown, opaque to translucent wax. Its specific gravity is about 0.983. It is soluble in chloroform and in toluene, but is insoluble in water.

REQUIREMENTS

Identification

Identify candelilla wax by comparing its infrared absorption spectrum with a typical spectrum as shown on page 715. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 12 and 22.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Melting Range Between 68.5° and 72.5°.
Saponification Value Between 43 and 65.

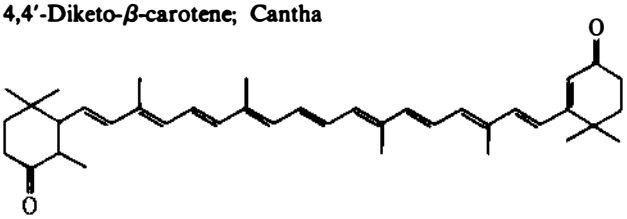
TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Melting Range Determine as directed for *Class II* substances in the general procedure, page 520.
Saponification Value Determine as directed in the general procedure, page 509.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Masticatory substance in chewing gum base; surface-finishing agent.

Canthaxanthin

4,4′-Diketo-β-carotene; Cantha



$C_{40}H_{52}O_2$ Mol wt 564.80

DESCRIPTION

A dark crystalline powder. It is soluble in chloroform, and is very slightly soluble in acetone, but is insoluble in water.

REQUIREMENTS

Identification

The absorbance spectrum of *Sample Solution B*, prepared as directed in the *Assay*, exhibits a maximum between 468 nm and 472 nm.

Assay Not less than 96.0% and not more than 101.0% of $C_{40}H_{52}O_2$.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Melting Range Between 207° and 212°, with decomposition.
Residue on Ignition Not more than 0.2%.

TESTS

Assay

NOTE: Carry out all work in low-actinic glassware and in subdued light.

Sample Solution A Transfer about 50 mg of the sample, accurately weighed, into a 100-ml volumetric flask, dissolve in 10 ml of acid-free chloroform, immediately dilute to volume with cyclohexane, and mix. Pipet 5 ml of this solution into a second 100-ml volumetric flask, dilute to volume with cyclohexane, and mix.

Sample Solution B Pipet 5 ml of *Sample Solution A* into a 50-ml volumetric flask, dilute to volume with cyclohexane, and mix.

Procedure Determine the absorbance of *Sample Solution B* in a 1-cm cell at the wavelength of maximum absorption at about 470 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in mg, of $C_{40}H_{52}O_2$ in the sample taken by the formula $20{,}000A/220$, in which A is the absorbance of the solution and 220 is the absorptivity of pure canthaxantin.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition Ignite 1 g as directed in the general method, page 533, using a silica crucible and moistening the residue with 2 ml of nitric acid and 1 ml of sulfuric acid.

Packaging and Storage Store in tight light-resistant containers under inert gas.
Functional Use in Foods Color.

Caramel

Caramel Color

DESCRIPTION

Caramel is a dark brown to black liquid or solid having the characteristic odor of burnt sugar and a pleasant, bitter taste. At normal usage levels it has little or no taste, however. Liquid caramel spread in a thin layer on a glass plate appears to be homogeneous, is transparent, and has a reddish brown color.

Caramel is prepared by the controlled heat treatment of the following food-grade carbohydrates: dextrose, invert sugar, lactose, malt syrup, molasses, starch hydrolysates and fractions thereof, and sucrose. The following food-grade acids, alkalies, and salts may be used to assist caramelization: acetic acid; citric acid; phosphoric acid; sulfuric acid; sulfurous acid; ammonium hydroxide; calcium hydroxide; potassium hydroxide; sodium hydroxide; ammonium, sodium, or potassium carbonate, bicarbonate, phosphate (including dibasic phosphate and monobasic phosphate), sulfate, bisulfite, and sulfite. Food-grade polyglycerol esters of fatty acids may be used as antifoaming agents in amounts not greater than that required to produce the intended effect.

Caramel consists essentially of colloidal aggregates that are dispersible in water but only partly dispersible in alcohol-water solutions. One part of caramel dissolved in 1000 parts of water yields a clear solution having a distinct yellow brown color that is stable to exposure to sunlight for at least 6 h. Solutions of caramel are only partly dialyzable; they have some reducing capacity and have isoelectric points and pH's varying over a wide range.

REQUIREMENTS

Ammoniacal Nitrogen Not more than 0.5%.*
Arsenic (as As) Not more than 3 ppm.
Color Intensity Meets the representations of the vendor.
Heavy Metals (as Pb) Not more than 0.0025%.
Lead Not more than 5 ppm.
Mercury Not more than 0.1 ppm.
4-Methylimidazole Not more than 0.02%.*
Sulfur Dioxide Not more than 0.1%.*

TESTS

Ammoniacal Nitrogen Add 25 ml of 0.1 *N* sulfuric acid to a 500-ml receiving flask, and connect it to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser such that the condenser delivery tube is immersed beneath the surface of the acid solution in the receiving flask. Transfer about 2 g of the sample, accurately weighed, into an 800-ml long-neck Kjeldahl digestion flask, and to the flask add 2 g of magnesium oxide (carbonate-free), 200 ml of water, and several boiling chips. Swirl the digestion flask to mix the contents, and quickly connect it to the distillation apparatus. Heat the digestion flask to boiling, and collect about 100 ml of distillate in the receiving flask. Wash the tip of the delivery tube with a few ml of water, collecting the washings in the receiving flask, then add 4 or 5 drops of methyl red indicator (500 mg of methyl red in 100 ml of alcohol), and titrate with 0.1 *N* sodium hydroxide, recording the volume, in ml, required as *S*. Conduct a blank determination (see page 2), and record the volume, in ml, of 0.1 *N*

*Based on a product having a color intensity of 0.085 absorbance unit (see *Color Intensity* test); proportionately higher and lower values for these impurities apply to caramels of higher and lower color intensities, respectively.

sodium hydroxide required as *B*. Calculate the percentage of ammoniacal nitrogen in the sample by the formula

$$(B - S) \times 0.0014 \times 100/W,$$

in which *W* is the weight of the sample taken, in g.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Color Intensity Transfer 100 mg of the sample into a 100-ml volumetric flask, dilute to volume with water, and mix. If the solution is hazy, clarify it by centrifugation. (*Caution:* Do not filter.) Determine the absorbance of the clear or clarified solution in a 1-cm cell at 610 nm, with a suitable spectrophotometer previously standardized using water as the reference. (A suitable spectrophotometer for this test is one equipped with a monochromator to provide a bandwidth of 2 nm or less and of such quality that the stray-light characteristic is 0.5% or less. Suitable instruments are the Beckman DU, Beckman DB-G, Bausch & Lomb Spectronic 505, or others equivalent to these.) The observed absorbance value is the color intensity of the sample under test. (NOTE: For the purposes of this specification, color intensity is defined as the absorbance of a 0.1% (w/v) solution at 610 nm.)

Heavy Metals Prepare and test an 800-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Mercury

Standard Preparation Prepare as directed in the *Mercury Limit Test*, page 520, using 1.0 ml of the stock solution, equivalent to 1 µg of Hg, instead of the 2.0 ml specified therein.

Sample Preparation Transfer 5 g of the caramel sample into a 250-ml Erlenmeyer flask, and continue as directed in the second full paragraph under *Sample Solution* in the *Arsenic Test*, page 465, beginning with " . . . add 5 ml of sulfuric acid and a few glass beads. . . . " After the sample has been digested and the solution diluted to 35 ml, as directed therein, add 1 ml of potassium permanganate solution (1 in 25), and mix.

Procedure Continue as directed for *Procedure* in the *Mercury Limit Test*, page 520. Any absorbance produced by the *Sample Preparation* is not more than half that produced by the *Standard Preparation*, indicating not more than 0.1 ppm of Hg in the sample taken.

4-Methylimidazole

Standard Solutions Purify reagent-grade 4-methylimidazole* by redistillation (bp 92° to 93°, 0.05 mm Hg), and then prepare a stock solution by transferring 50 mg of the distillate, accurately weighed, into a 50-ml volumetric flask and diluting to volume with 0.1 *N* sulfuric acid. Store the stock solution in a refrigerator.

To prepare the standard solutions, pipet 1.0-, 1.5-, 2.0-, 2.5-, 3.0-, 3.5-, 4.0-, and 5.0-ml portions of the stock solution into separate 10-ml volumetric flasks, then add solid sodium carbonate to each flask until the evolution of carbon dioxide ceases, dilute each to volume with a 1% solution of sodium carbonate, and mix. The standards thus prepared represent 4-methylimidazole concentrations of 100, 150, 200, 250, 300, 350, 400, and 500 ppm (w/v), respectively. Store the standard solutions in a refrigerator.

Sample Preparation Place a plug of fine glass wool in the base of a 25- × 250-mm chromatographic tube having a Teflon stopcock. Mix intimately chromatographic siliceous earth (Johns-Manville Celite 545 or equivalent) and 2 *N* sodium hydroxide at a ratio of 3:2 (w/v), corresponding to 1.33 meq of NaOH per g of chromatographic siliceous earth. Add 5 g of the mixture to the tube, and tamp gently to produce a uniform mass.

Transfer 10 g of the caramel sample, previously mixed by shaking or stirring and accurately weighed, into a 150-ml beaker containing 6.0 g of a 20% solution (w/v) of sodium carbonate, and mix well. Add 15 g of chromatographic siliceous earth, mix intimately with the sample solution, and then transfer the mixture quantitatively to the column. Place a plug of glass wool on top of the column, and then allow the column to fall a short distance vertically to help settle the contents. The layers should be of uniform consistency yet open enough to allow for elution to occur readily.

Elute the column with an 80:20 chloroform-ethanol mixture (v/v), using a sufficient amount of the mixture to collect 125 ml of eluate, received in a 250-ml separator. Adjust the stopcock so that the eluate is collected at the rate of about 5 ml per min. Extract the contents of the separator with one 25-ml portion followed by one 10-ml portion of 0.05 *N* sulfuric acid. The extract should be strongly acidic (pH below 3). (NOTE: More than 35 ml of 0.05 *N* sulfuric acid may be required when extracting caramel samples having a high content of ammonia.) Quantitatively transfer the aqueous acidic extracts to a 200-ml round-bottom flask, and concentrate the solution to a volume of 5 to 6 ml by means of a suitable rotary vacuum evacuator (consisting of a water aspirator and water bath at 55°). (*Caution:* During the concentration step watch the flask carefully to ensure that no bumping occurs and that the volume is not reduced below 3 ml.) Transfer the aqueous concentrate into a 10-ml volumetric flask, washing the 200-ml round-bottom flask with 1-ml portions of water and adding the washings to the volumetric flask until the 10-ml dilution mark is reached. Mix the contents of the flask by inverting it several times, then transfer the solution into a suitable sample vial (with Teflon-lined stopper), and treat with small portions of solid sodium carbonate until the evolution of carbon dioxide ceases and the pH is above 9, as determined by test with pH indicator paper.

Gas Chromatography Apparatus Use a suitable gas chromatograph (page 475) equipped with a hydrogen flame ionization detector, containing a 1.23-m (4-ft) × 6.35-mm (od) stainless steel column (previously rinsed with 5% alcoholic potassium hydroxide solution then dried with a current of air drawn through it), and packed with 5% Carbowax 20M on 80/90-mesh Anakrom AB, or equivalent materials. Make the column support basic by the application of 2% alcoholic potassium hydroxide solution.

*A suitable grade may be obtained from Research Organic/Inorganic Chemical Corp., Sun Valley, Calif. 91352.

Operating Conditions The operating conditions may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained by using the following conditions: *column temperature*, 180° (isothermal); *injection port temperature*, 200°; *carrier gas*, helium, flowing at a rate of 75 ml per min; *detector temperature*, 250°.

Procedure Equilibrate the column prior to analysis of the sample by injecting several 10-µl portions of a solution containing 1% (w/v) of 4-methylimidazole (redistilled) in 1% sodium carbonate solution.

Inject 5.0 µl of each standard solution and obtain the chromatograms. (NOTE: To avoid fractionation in the syringe needle, and to ensure that 5.0 µl is injected, use the solvent-flush technique, with distilled water as the solvent.) For each standard chromatogram, calculate the corrected peak area by multiplying the peak height, in mm, by the peak width at one-half height, in mm, by the proper attenuation and range factors, depending upon the particular apparatus and operating parameters used. Plot the corrected peak areas thus obtained versus the respective concentrations of 4-methylimidazole in the standards to obtain the standard curve.

In the same manner, chromatograph a 5.0-µl portion of the *Sample Preparation*, calculate the peak area corresponding to any 4-methylimidazole contained in the sample, and by reference to the standard curve obtain the content of the 4-methylimidazole in the sample.

Sulfur Dioxide Determine as directed in the general test for *Sulfur Dioxide* (in *Starches and Related Substances*), page 546, using about 25 g of sample.

Packaging and Storage Store in well-closed containers and avoid exposure to excessive heat.

Functional Use in Foods Color.

Caraway Oil

DESCRIPTION

A volatile oil distilled from the dried, ripe fruit of *Carum carvi* L. (Fam. *Umbelliferae*). It is a colorless to pale yellow liquid having the characteristic odor and taste of caraway.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 588, using the same test conditions as specified therein.

Assay Not less than 50%, by volume, of ketones as carvone.

Angular Rotation Between +70° and +80°.

Heavy Metals (as Pb) Passes tests.

Refractive Index Between 1.484 and 1.488 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.900 and 0.910.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 8 ml of 80% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Carbon, Activated

Activated Charcoal; Decolorizing Carbon; Active Carbon

DESCRIPTION

A solid, porous, carbonaceous material prepared by carbonizing and activating organic substances. The raw materials, which include sawdust, peat, lignite, coal, cellulose residues, coconut shells, petroleum coke, etc., may be carbonized and activated at a high temperature with or without the addition of inorganic salts in a stream of activating gases such as steam or carbon dioxide. Alternatively, carbonaceous matter may be treated with a chemical activating agent such as phosphoric acid or zinc chloride and the mixture carbonized at an elevated temperature, followed by removal of the chemical activating agent by water washing. Activated carbon occurs as a black, tasteless substance, varying in particle size from coarse granules to a fine powder, depending upon its intended use. It is insoluble in water and in organic solvents.

REQUIREMENTS

Identification

A. Place about 3 g of powdered sample in a glass-stoppered Erlenmeyer flask containing 10 ml of dilute hydrochloric acid (5%), boil for 30 s, and cool to room temperature. Add 100 ml of iodine TS, stopper, and shake vigorously for 30 s. Filter through Whatman No. 12 filter paper, or equivalent,

discarding the first portion of filtrate. Compare 50 ml of the subsequent filtrate with a reference solution prepared by diluting 10 ml of iodine TS to 50 ml with water, but not treated with carbon. The color of the carbon-treated iodine solution is no darker than that of the reference solution, indicating the adsorptivity of the sample.

B. Ignite a portion of the sample in air. Carbon monoxide and carbon dioxide are produced, and an ash remains.

Arsenic (as As) Not more than 3 ppm.
Cyanogen Compounds Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Higher Aromatic Hydrocarbons Passes test.
Lead Not more than 10 ppm.
Water Extractables Not more than 4%.

The following additional REQUIREMENTS should conform to the representations of the vendor: **Loss on Drying** and **Residue on Ignition**.

TESTS

Arsenic A 20-ml portion of the filtrate obtained in the test for *Water Extractables*, diluted to 35 ml with water, meets the requirements of the *Arsenic Test*, page 464.

Cyanogen Compounds Mix 5 g of the sample with 50 ml of water and 2 g of tartaric acid, and distil the mixture, collecting 25 ml of distillate below the surface of a mixture of 2 ml of sodium hydroxide TS and 10 ml of water contained in a small flask placed in an ice bath. Dilute the distillate to 50 ml with water, and mix. Add 12 drops of ferrous sulfate TS to 25 ml of the diluted distillate, heat almost to boiling, cool, and add 1 ml of hydrochloric acid. No blue color is produced.

Heavy Metals A 10-ml portion of the filtrate obtained in the test for *Water Extractables* meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Higher Aromatic Hydrocarbons Extract 1 g of the sample with 12 ml of cyclohexane in a continuous-extraction apparatus for 2 h. Using matched Nessler tubes, the extract shows no more color or fluorescence than does a solution of 100 µg of quinine sulfate in 1000 ml of 0.1 *N* sulfuric acid when observed in ultraviolet light.

Lead A 20-ml portion of the filtrate obtained in the test for *Water Extractables* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 120° for 4 h.

Residue on Ignition Ignite 500 mg as directed in the general method, page 533.

Water Extractables Transfer 5.00 g of the sample into a 250-ml flask provided with a reflux condenser and a Bunsen valve. Add 100 ml of water and several glass beads, and reflux for 1 h. Cool slightly, and filter through Whatman No. 12 or equivalent filter paper, discarding the first 10 ml of filtrate. Cool the subsequent filtrate to room temperature, and pipet 25.0 ml into a tared crystallization dish. (NOTE: Retain the remainder of the filtrate for the *Arsenic, Heavy Metals,* and *Lead* tests.) Evaporate the filtrate in the dish to incipient dryness on a hot plate, never allowing the solution to boil. Dry for 1 h at 100° in a vacuum oven, cool, and weigh.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Decolorizing agent; taste- and odor-removing agent; purification agent in food processing.

Cardamom Oil

DESCRIPTION

The volatile oil distilled from the seed of *Elettaria cardamomum* (L.) Maton (Fam. *Zingiberaceae*). It is a colorless or very pale yellow liquid with the aromatic, penetrating, and somewhat camphoraceous odor of cardamom and a pungent, strongly aromatic taste. It is affected by light. It is miscible with alcohol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 588, using the same test conditions as specified therein.

Angular Rotation Between +22° and +44°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.462 and 1.466 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.917 and 0.947.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

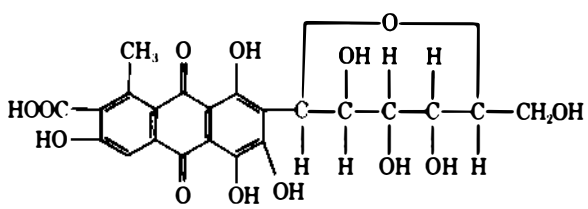
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 5 ml of 70% alcohol. The solution may be clear or hazy.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Carmine

Carminic Acid



$C_{22}H_{20}O_{13}$ Mol wt 492.39

DESCRIPTION

Carmine is the aluminum or the calcium-aluminum lake, on an aluminum hydroxide substrate, of the coloring principles obtained by an aqueous extraction of cochineal. Cochineal consists of the dried female insects *Dactylopius coccus costa* (*Cossus cacti L.*), enclosing young larvae; the coloring principles derived therefrom consist chiefly of carminic acid ($C_{22}H_{20}O_{13}$).

Carminic acid crystallizes from water as bright red crystals that darken at 130° and decompose at 250°; it is freely soluble in water, in alcohol, in ether, in concentrated sulfuric acid, and in solutions of alkali hydroxides; it is insoluble in petroleum benzin, in benzene, and in chloroform; its solutions at pH 4.8 are yellow, and at 6.2 are violet.

Carmine occurs as bright red, friable pieces or as a dark red powder. It is soluble in alkali solutions, slightly soluble in hot water, and practically insoluble in cold water and in dilute acids.

Before use in food, carmine must have been pasteurized or otherwise treated to destroy all viable *Salmonella* microorganisms. According to the pertinent federal color additive regulation (21 CFR 73.100), pasteurization or such other treatment is deemed to permit the addition of safe and suitable substances (other than chemical preservatives) that are essential to the method of pasteurization or other treatment used.

REQUIREMENTS

Identification

Mix 333 mg of carmine with 44 ml of water, 0.15 ml of sodium hydroxide solution (1 in 10), and 0.2 ml of ammonium hydroxide, warm to dissolve, and dilute to volume with water in a 500-ml volumetric flask. Pipet 10.0 ml of this solution into a 250-ml volumetric flask, dilute to volume with water, and mix. The resulting solution exhibits absorption maxima at 520 nm and 550 nm, when determined in a 1-cm cell with a suitable spectrophotometer against a water blank, and the absorbance at 520 nm is not less than 0.30.

Assay Not less than 42.0% of carminic acid ($C_{22}H_{20}O_{13}$).*

Arsenic (as As) Not more than 1 ppm.

Ash Not more than 12.0%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 20.0%.

Salmonella Negative by test.

TESTS

Assay Weigh accurately about 30 mg of the sample, dissolve it in 30 ml of boiling 2 *N* hydrochloric acid, and cool. Transfer quantitatively to a 1000-ml volumetric flask, dilute to volume with water, and mix. Determine the absorbance of this solution in a 1-cm cell at the wavelength of maximum absorbance at about 494 nm with a suitable spectrophotometer, using 0.06 *N* hydrochloric acid as the blank. If the measured absorbance of the solution is not within the range of 0.20 to 0.25, prepare another sample solution and adjust the weight accordingly. Calculate the percentage of carminic acid in the carmine taken for analysis by the formula

$$15 \times A \times 100/0.262W,$$

in which A is the absorbance of the sample solution, 0.262 is the absorbance of a solution of carminic acid having a concentration of 15 mg per 1000 ml, and W is the weight of sample taken, in mg.

Arsenic, page 464 Transfer 3.0 g of the sample into a 500-ml Kjeldahl flask equipped with a steam trap, add 5 g of ferrous sulfate and 75 ml of hydrochloric acid, and mix. Connect the flask with the steam trap and with a condenser, the delivery tube of which consists of a large-size straight adapter and extends to slightly above the bottom of a 500-ml Erlenmeyer flask containing 100 ml of water. Begin heating the Kjeldahl flask and collect about 40 ml of distillate in the Erlenmeyer flask. Pour the distillate mixture into a 600-ml beaker, add 20 ml of bromine water, and heat on a hot plate until the volume is reduced to about 2 ml. Transfer the residual liquid into a 125-ml arsine generator flask (see page 463) with the aid of 35 ml of water, and continue as directed in the *Procedure*, page 465, beginning with "Add 20 ml of dilute sulfuric acid (1 in 5). . . . "

Ash Transfer about 1 g of the sample into a tared, previously ignited and cooled porcelain crucible, and ignite with a Meker burner (red hot) to constant weight.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry a 1-g sample at 135° for 3 h.

Salmonella Determine as directed in Chapter VI, *Bacterial Analytical Manual*, Fifth Edition (1978) or later, Food and Drug Administration.

Packaging and Storage Store in well-closed containers in a cool, dry place.

Functional Use in Foods Color.

*The 42.0% minimum content of carminic acid specified herein does not indicate that the FCC-grade product is of any lower quality than that described in the second supplement to FCC II (page 18), which specified 50.0%. Rather, the revised *Assay* procedure used for this edition gives a more accurate indication of the true carminic acid content.

Carnauba Wax

DESCRIPTION

A purified wax obtained from the leaf buds and leaves of the Brazilian wax palm *Copernicia cereferia* (Arruda) Mart. It is hard and brittle, has a resinous fracture, and ranges in color from light brown to pale yellow. Its specific gravity is about 0.997. It is partially soluble in boiling alcohol, is soluble in chloroform and in ether, but is insoluble in water.

REQUIREMENTS

Acid Value Between 2 and 7.
Arsenic (as As) Not more than 3 ppm.
Ester Value Between 75 and 85.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Melting Range Between 82° and 86°.
Unsaponifiable Matter Between 50% and 55%.

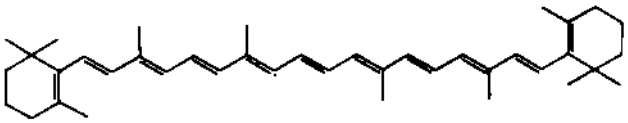
TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Ester Value Weigh accurately about 5 g, and determine the *Saponification Value* as directed in the general procedure, page 509. Subtract the *Acid Value* from the *Saponification Value* to obtain the *Ester Value*.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Melting Range, page 520 Determine as directed in *Procedure for Class II*.
Unsaponifiable Matter Determine as directed in the general method, page 509.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Candy glaze and polish.

β-Carotene

Carotene



$C_{40}H_{56}$ Mol wt 536.88

DESCRIPTION

Red crystals or crystalline powder. It is insoluble in water and in acids and alkalies, but is soluble in carbon disulfide, in benzene, and in chloroform. It is sparingly soluble in ether, in solvent hexane, and in vegetable oils, and is practically insoluble in methanol and in ethanol.

REQUIREMENTS

Identification

A. Determine the absorbance of *Sample Solution B* (prepared for the *Assay*) at 455 nm and at 483 nm. The ratio A_{455}/A_{483} is between 1.14 and 1.18.
B. Determine the absorbance of *Sample Solution B* at 455 nm and that of *Sample Solution A* at 340 nm. The ratio $A_{455} \times 10/A_{340}$ is not lower than 15.

Assay Not less than 96.0% and not more than the equivalent of 101.0% of $C_{40}H_{56}$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Melting Range Between 176° and 182°, with decomposition.
Residue on Ignition Not more than 0.2%.
Solution in Chloroform Passes test.

TESTS

Assay

NOTE: Carry out all work in low-actinic glassware and in subdued light.

Sample Solution A Transfer about 50 mg, accurately weighed, into a 100-ml volumetric flask, dissolve in 10 ml of acid-free chloroform, immediately dilute to volume with cyclohexane, and mix. Pipet 5 ml of this solution into a second 100-ml volumetric flask, dilute to volume with cyclohexane, and mix.

Sample Solution B Pipet 5 ml of *Sample Solution A* into a 50-ml volumetric flask, dilute to volume with cyclohexane, and mix.

Procedure Determine the absorbance of *Sample Solution B* in a 1-cm cell at the wavelength of maximum absorption at about 455 nm, with a suitable spectrophotometer, using cyclohexane as the blank. Calculate the quantity, in mg, of $C_{40}H_{56}$ in the sample taken by the formula $20{,}000A/250$, in

which *A* is the absorbance of the solution, and 250 is the absorptivity of pure β-carotene.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Melting Range, page 519 Determine as directed under *Procedure for Class Ia.*

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Solution in Chloroform A 1 in 100 solution in chloroform is complete and clear.

Packaging and Storage Store in a cool place in tight, light-resistant containers under inert gas.

Functional Use in Foods Nutrient; dietary supplement; color.

Carrageenan

DESCRIPTION

Carrageenan is obtained by extraction with water or aqueous alkali from certain members of the class Rhodophyceae (red seaweeds). It is a hydrocolloid consisting mainly of the potassium, sodium, magnesium, calcium, and ammonium sulfate esters of galactose and 3,6-anhydrogalactose copolymers. These hexoses are alternately linked α-1,3 and β-1,4 in the polymer. The relative proportion of cations existing in carrageenan may be changed during processing to the extent that one may become predominant.

The prevalent copolymers in the hydrocolloid are designated *kappa*-, *iota*-, and *lambda*-carrageenan. *Kappa*-carrageenan is mostly the alternating polymer of D-galactose-4-sulfate and 3,6-anhydro-D-galactose; *iota*-carrageenan is similar, except that the 3,6-anhydrogalactose is sulfated at carbon 2. Between *kappa*-carrageenan and *iota*-carrageenan there is a continuum of intermediate compositions differing in degree of sulfation at carbon 2. In *lambda*-carrageenan, the alternating monomeric units are mostly D-galactose-2-sulfate (1,3-linked) and D-galactose-2,6-disulfate (1,4-linked).

The ester sulfate content of carrageenan ranges from 18% to 40% (see REQUIREMENTS). In addition, it contains inorganic salts that originate from the seaweed and the process of recovery from the extract. Carrageenan is recovered by alcohol precipitation, by drum drying, or by freezing. The alcohols used during recovery and purification are restricted to methanol, ethanol, and isopropanol. When carrageenan is recovered by drum roll drying, it may contain mono- and diglycerides or up to 5% polysorbate 80 used as roll-stripping agents.

Carrageenan is a yellowish or tan to white, coarse to fine powder that is practically odorless and has a mucilaginous taste. It is soluble in water at a temperature of about 80°, forming a viscous, clear or slightly opalescent solution that flows readily. It disperses in water more readily if first moistened with alcohol, glycerin, or a saturated solution of sucrose in water.

REQUIREMENTS

Identification

A. Add 4 g of sample to 200 ml of water, and heat the mixture in a water bath at 80°, with constant stirring, until dissolved. Replace any water lost by evaporation, and allow the solution to cool to room temperature. It becomes viscous and may form a gel.

B. To 50 ml of the solution or gel obtained in *Identification Test A* add 200 mg of potassium chloride, then reheat, mix well, and cool. A short-textured ("brittle") gel indicates a carrageenan of a predominantly *kappa* type; a compliant ("elastic") gel indicates a predominantly *iota* type. If the solution does not gel, the carrageenan is of a predominantly *lambda* type.

C. To 5 ml of the solution obtained in *Identification Test A* add 1 drop of a 1 in 100 solution of methylene blue. A fibrous precipitate forms.

D. Obtain infrared absorption spectra on the gelling and nongelling fractions of the sample by the following procedure: Disperse 2 g of the sample in 200 ml of 2.5% potassium chloride solution, and stir for 1 h. Let stand overnight, stir again for 1 h, and transfer into a centrifuge tube. (If the transfer cannot be made because the dispersion is too viscous, dilute with up to 200 ml of the potassium chloride solution.) Centrifuge for 15 min at approximately 1000 *g*'s.

Remove the clear supernatant, resuspend the residue in 200 ml of 2.5% potassium chloride solution, and centrifuge again. Coagulate the combined supernatants by adding 2 volumes of 85% ethanol or isopropanol. (NOTE: Retain the sediment for use as directed below.) Recover the coagulum, and wash it with 250 ml of the alcohol. Press the excess liquid from the coagulum, and dry it at 60° for 2 h. The product obtained is the nongelling fraction (*lambda*-carrageenan).

Disperse the sediment (retained above) in 250 ml of cold water, heat at 90° for 10 min, and cool to 60°. Coagulate the mixture, and then recover, wash, and dry the coagulum as described above. The product obtained is the gelling fraction (*kappa*- and *iota*-carrageenan).

Prepare a 0.2% aqueous solution of each fraction, cast films 0.0005 cm thick (when dry) on a suitable nonsticking surface such as Teflon, and obtain the infrared absorption spectrum of each film. (Alternatively, the spectra may be obtained on potassium bromide pellets if care is taken to avoid moisture.)

Carrageenan has strong, broad absorption bands, typical of all polysaccharides, in the 1000 to 1100 cm^{-1} region. Absorption maxima are 1065 and 1020 cm^{-1} for gelling

and nongelling types, respectively. Other characteristic absorption bands and their intensities relative to the absorbance at 1050 cm^{-1} are as follows:

Wave Number (cm^{-1})	Molecular Assignment	Absorbance Relative to 1050 cm^{-1}		
		Kappa	Iota	Lambda
1220–1260	ester sulfate	0.7–1.2	1.2–1.6	1.4–2.0
928–933	3,6-anhydrogalactose	0.3–0.6	0.2–0.4	0–0.2
840–850	galactose-4-sulfate	0.3–0.5	0.2–0.4	–
825–830	galactose-2-sulfate	–	–	0.2–0.4
810–820	galactose-6-sulfate	–	–	0.1–0.3
800–805	3,6-anhydrogalactose-2-sulfate	0–0.2	0.2–0.4	–

Arsenic (as As) Not more than 3 ppm.

Ash (Acid-Insoluble) Not more than 1.0%.

Ash (Total) Not more than 35.0%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 12%.

Sulfate Between 18% and 40% on the dry weight basis.

Viscosity of a 1.5% Solution Not less than 5 centipoises at 75°.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Acid-Insoluble) Proceed as directed in the general method, page 466.

Ash (Total) Transfer about 2 gm, accurately weighed, into a previously ignited, tared, silica or platinum crucible. Heat the sample with a suitable infrared heat lamp, increasing the intensity gradually, until it is completely charred, and then continue for an additional 30 min. Transfer the crucible and charred sample into a muffle furnace and ignite at about 550° for 1 h, then cool in a desiccator and weigh. Repeat the ignition in the muffle furnace until a constant weight is attained. If a carbon-free ash is not obtained after the first ignition, moisten the charred spots with a 1 in 10 solution of ammonium nitrate and dry under an infrared heat lamp before reigniting.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518 using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Sulfate Transfer about 500 mg, previously dried at 105° for 12 h and accurately weighed, into a 100-ml Kjeldahl flask. Add 10 ml of nitric acid and heat gently for 30 min, adding more of the acid, if necessary, to prevent evaporation to dryness, and to yield a volume of about 3 ml at the end of the heating. Cool the mixture to room temperature and decompose the excess nitric acid by the addition of formaldehyde TS, dropwise, heating, if necessary, until no brown fumes continue to be evolved. Continue the heating until the volume of the reaction mixture is reduced to about 5 ml, and then cool. Transfer the residue quantitatively with the aid of water into a 400-ml beaker, dilute it to about 100 ml, and filter, if necessary, to produce a clear solution. Dilute the solution to about 200 ml, and add 1 ml of hydrochloric acid. Heat to boiling and add, dropwise, with constant stirring, an excess (about 6 ml) of hot barium chloride TS. Heat the mixture for 1 h on a steam bath, collect the precipitate of barium sulfate on a filter, wash it until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.4116, gives the equivalent of sulfate (SO_4).

Viscosity of a 1.5% Solution Transfer 7.5 g of the sample into a tared, 600-ml tall-form (Berzelius) beaker, and disperse with agitation for 10 to 20 min in 450 ml of deionized water. Add sufficient water to bring the final weight to 500 g, and heat in a water bath, with continuous agitation, until a temperature of 80° is reached (20 to 30 min). Add water to adjust for loss by evaporation, cool to 76° to 77°, and place in a constant-temperature bath at 75°. Preheat the bob and guard of a Brookfield LVF or LVT viscometer to approximately 75° in water, then dry the bob and guard and attach them to the viscometer, which should be equipped with a No. 1 spindle (19 mm in diameter, approximately 65 mm in length) and capable of rotating at 30 rpm. Adjust the height of the bob in the sample solution, start the viscometer rotating at 30 rpm, and, after six complete revolutions, take the reading on the 0–100 scale. Record the results in centipoises by multiplying the reading by 2.

NOTE: Some samples of carrageenan may be too viscous to be read when a No. 1 spindle is used. Such samples obviously pass the specification, but if a viscosity reading is desired for other reasons, use a No. 2 spindle, take the reading on the 0–100 scale, and multiply the reading by 10 to obtain the viscosity in centipoises, or read on the 0–500 scale and multiply by 2. If the viscosity is very low, increased precision may be obtained by using the Brookfield UL (ultra low) adapter, in which case the viscometer reading on the 0–100 scale should be multiplied by 0.2 to obtain the viscosity in centipoises.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; stabilizer; thickener; gelling agent.

Carrot Seed Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the crushed seeds of *Daucus carota* L. (Fam. *Umbelliferae*). It is a light yellow to amber liquid having a pleasant aromatic odor. It is soluble in most fixed oils, and is soluble, with opalescence, in mineral oil. It is practically insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 589, using the same test conditions as specified therein.

Acid Value Not more than 5.0.
Angular Rotation Between $-4°$ and $-30°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.480 and 1.491 at 20°.
Saponification Value Between 9 and 58.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.900 and 0.943.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 90% alcohol. The solution may become opalescent upon further dilution up to 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Cascarilla Oil

Sweetwood Bark Oil

DESCRIPTION

The volatile oil obtained by steam distillation of the dried bark of *Croton cascarilla* Benn. and of *Croton eluteria* Benn. (Fam *Euphorbiaceae*). It is a light yellow to brown amber liquid having a pleasant spicy odor. It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 589, using the same test conditions as specified therein.

Acid Value Between 3 and 10.
Angular Rotation Between $-1°$ and $+8°$.
Ester Value after Acetylation Between 62 and 88.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.488 and 1.494 at 20°.
Saponification Value Between 8 and 20.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.892 and 0.914.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value after Acetylation Proceed as directed under *Total Alcohols*, page 499, using about 2 g of the dried acetylized oil, accurately weighed. Calculate the *Ester Value after Acetylation* by the formula $A \times 28.05/B$, in which A is the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the saponification, and B is the weight of the sample of acetylized oil, in g.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using 5 g, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 90% alcohol and remains in solution on dilution to 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Cassia Oil

Cinnamon Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the leaves and twigs of *Cinnamomum cassia* Blume (Fam. *Lauraceae*), rectified by distillation. It is a yellowish or brownish liquid having the characteristic odor and taste of cassia cinnamon. Upon aging or exposure to air it darkens and thickens. It is soluble in glacial acetic acid and in alcohol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 589, using the same test conditions as specified therein.

Assay Not less than 80%, by volume, of total aldehydes.
Angular Rotation Between $-1°$ and $+1°$.
Chlorinated Compounds Passes test.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.602 and 1.614 at 20°.
Rosin or Rosin Oils Passes test.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.045 and 1.063.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Chlorinated Compounds Proceed as directed in the general method, page 500.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Rosin or Rosin Oils Shake a 2-ml sample in a test tube with 5 to 10 ml of solvent hexane, allow the liquids to separate, decant the hexane layer, which is but slightly colored, into another test tube, and shake it with an equal volume of cupric acetate solution (1 in 1000). The mixture does not assume a green color.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, light-resistant containers. Avoid exposure to excessive heat.

Functional Use in Foods Flavoring agent.

Castor Oil

DESCRIPTION

The fixed oil obtained from the seed of *Ricinus communis* L. (Fam. *Euphorbiaceae*). It is a pale yellowish or almost colorless, transparent, viscid liquid and has a faint, mild odor and a bland, characteristic taste. It is soluble in alcohol, and is miscible with absolute alcohol, with glacial acetic acid, with chloroform, and with ether.

REQUIREMENTS

Identification

It is only partly soluble in solvent hexane (distinction from *most other fixed oils*), but it yields a clear liquid with an equal volume of alcohol (*foreign fixed oils*).

Arsenic (as As) Not more than 3 ppm.
Free Fatty Acids Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Hydroxyl Value Between 160 and 168.
Iodine Value Between 83 and 88.
Saponification Value Between 176 and 182.
Specific Gravity Between 0.945 and 0.965.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Free Fatty Acids Dissolve about 10 g, accurately weighed, in 50 ml of a mixture of equal volumes of alcohol and ether (which has been neutralized to phenolphthalein with 0.1 *N* sodium hydroxide) contained in a flask. Add 1 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains pink after shaking for 30 s. Not more than 7 ml of 0.1 *N* sodium hydroxide is required for a 10.0-g sample.

Heavy Metals Prepare and test a 2-g sample as directed in

Method II under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Iodine Value Determine by the *Wijs Method*, page 505, using about 300 mg, accurately weighed.

Saponification Value Determine as directed in the general method, page 509, using about 3 g, accurately weighed.

Specific Gravity Determine as directed in the general method, page 3.

Packaging and Storage Store in tight containers, and avoid exposure to excessive heat.

Functional Use in Foods Antisticking agent; release agent; component of protective coatings.

Cedar Leaf Oil

Thuja Oil; White Cedar Leaf Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh leaves and branch ends of the eastern arborvitae, *Thuja occidentalis* L. (Fam. *Cupressaceae*). It is a colorless to yellow liquid having a strong camphoraceous and sagelike odor. It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 589, using the same test conditions as specified therein.

Assay Not less than 60.0% of ketones, calculated as thujone ($C_{10}H_{16}O$).

Angular Rotation Between −10° and −14°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.456 and 1.459 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.910 and 0.920.

TESTS

Assay Weigh accurately about 1 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, using 76.10 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 70% alcohol, occasionally becoming cloudy on dilution to 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Celery Seed Oil

DESCRIPTION

The volatile oil obtained by steam distillation of the fruit or seed of *Apium graveolens* L. It is a yellow to greenish brown liquid having a pleasant aromatic odor. It is soluble in most fixed oils with the formation of a flocculent precipitate, and in mineral oil with turbidity. It is partly soluble in propylene glycol, and it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 590, using the same test conditions as specified therein.

Acid Value Not more than 3.5.

Angular Rotation Between +48° and +78°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.480 and 1.490 at 20°.

Saponification Value Between 35 and 75.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.872 and 0.910.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added,

and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using 5 g, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 8 ml of 90% alcohol, usually with turbidity.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass, tin-lined, or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Cellulose, Microcrystalline

Cellulose Gel

DESCRIPTION

Microcrystalline cellulose is purified, partially depolymerized cellulose prepared by treating alpha cellulose, obtained as a pulp from fibrous plant material, with mineral acids. It occurs as a fine, white, odorless, crystalline powder. It consists of free-flowing, nonfibrous particles that may be compressed into self-binding tablets that disintegrate rapidly in water. It is insoluble in water, in dilute acids, in dilute sodium hydroxide solutions, and in most organic solvents.

REQUIREMENTS

Identification

A. Determine the particle size of the sample by sieving 20 g for 5 min on an Air Jet Sieve, or equivalent, equipped with a screen having 37-μm openings. If more than 5% is retained on the screen, mix 30 g of the sample with 270 ml of water; otherwise, mix 45 g with 255 ml. Perform the mixing in a high-speed (18,000 rpm) power blender for 5 min. Transfer 100 ml of the dispersion into a 100-ml graduate, and allow to stand for 3 h. A white, opaque, bubble-free dispersion, which does not form a supernatant liquid at the surface, is obtained.

B. To 20 ml of the dispersion obtained in *Identification Test A* add a few drops of iodine TS, and mix. No purplish to blue or blue color is produced.

Assay Not less than 97.0% and not more than the equivalent of 102.0% of carbohydrate, calculated as cellulose on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 5%.

pH Between 5.5 and 7.0 for samples having a sieve fraction greater than 5% retained on a 37-μm screen; between 5.0 and 7.0 for other samples.

Residue on Ignition Not more than 0.05%.

Water-Soluble Substances Not more than 0.16%.

TESTS

Assay Transfer about 125 mg of the sample, accurately weighed, to a 300-ml Erlenmeyer flask, using about 25 ml of water. Add 50.0 ml of 0.5 *N* potassium dichromate, mix, then carefully add 100 ml of sulfuric acid, and heat to boiling. Remove from heat, allow to stand at room temperature for 15 min, cool in a water bath, and transfer into a 250-ml volumetric flask. Dilute with water almost to volume, cool to 25°, then dilute to volume with water, and mix. Titrate a 50.0-ml aliquot with 0.1 *N* ferrous ammonium sulfate, using 2 or 3 drops of orthophenanthroline TS as the indicator, and record the volume required as *S*, in ml. Perform a blank determination, and record the volume of 0.1 *N* ferrous ammonium sulfate required as *B*, in ml. Calculate the percentage of cellulose in the sample by the formula

$$(B - S) \times 338/W,$$

in which *W* is the weight of sample taken, in mg, corrected for *Loss on Drying*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements for the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry to constant weight at 105°.

pH Shake about 5 g with 40 ml of water for 20 min, centrifuge, and determine the pH of the supernatant liquid by the *Potentiometric Method*, page 531.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Water-Soluble Substances Shake 5 g with 80 ml of water for 10 min. Filter the mixture through Whatman No. 42 or equivalent filter paper into a tared beaker, evaporate the filtrate to dryness on a steam bath, dry at 105° for 1 h, cool, and weigh.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Anticaking agent; binding agent; disintegrating agent; dispersing agent; tableting aid.

Cellulose, Powdered

DESCRIPTION

Powdered cellulose is purified, mechanically disintegrated cellulose prepared by processing bleached cellulose obtained as a pulp from such fibrous materials as wood or cotton. It occurs as a white, odorless substance and consists of fibrous particles that may be compressed into self-binding tablets that disintegrate rapidly in water. It exists in various grades, exhibiting degrees of fineness ranging from a dense, free-flowing powder to a coarse, fluffy, nonflowing material. It is insoluble in water, in dilute acids, and in nearly all organic solvents. It is slightly soluble in sodium hydroxide TS.

REQUIREMENTS

Identification

A. Mix approximately 30 g of the sample with 270 ml of water in a high-speed (approximately 12,000 rpm) power blender for 5 min. The mixture will be either a free-flowing suspension or a heavy, lumpy suspension that flows poorly (if at all), settles only slightly, and contains many trapped air bubbles. The mixture is not slimy. If a free-flowing suspension is obtained, transfer 100 ml of it into a 100-ml graduate, and allow to settle for 1 h: The solids settle in the cylinder and a supernatant liquid appears above the layer of the cellulose.

B. Boil 10 g of the sample with 90 ml of water for 5 min, filter while hot through ashless fine-quantitative paper (S & S 589 Blue Ribbon, or equivalent), and add 2 drops of iodine TS to the filtrate. No change in color from the yellow red is produced.

C. To 20 ml of a 0.1% solution of anthrone in 75% sulfuric acid add from 2 to 5 mg of the sample, and heat on a steam bath. The solution turns blue green within 5 min.

D. Place a few drops of the mixture from test *A* on a microscope slide, and insert a coverglass. Observe at 100 magnifications with a microscope. Fibers and fiber fragments are visible, regardless of the degree of fineness of the sample.

E. Dilute 10 ml of the mixture from test *A* to 1000 ml with water, and filter 125 ml of the dilution through a Buchner funnel. Rinse the pad with 25 ml of acetone, and dry (paper included) at 105°. Transfer the powder to a tared weighing bottle, weigh, then transfer to a 50-ml Erlenmeyer flask, and seal with a rubber stopper. Record the weight of the sample as *w*, in mg. Dissolve the sample in 0.167 *M* and 1.0 *M* solutions of cupriethylenediamine (CED), the volumes of which are determined as follows: 0.12 × *w* equals the ml of 0.167 *M* CED, and 0.08 × *w* equals the ml of 1.0 *M* CED. Add a few 3-mm glass beads and the calculated volume of 0.167 *M* CED, blow nitrogen over the surface of the solution, and shake for 2 min. Add the calculated volume of 1.0 *M* CED, again introduce the nitrogen, and shake vigorously for at least 3 min. A dark blue solution, clear under microscopic examination, is produced.

Assay Not less than 97.0% and not more than the equivalent of 102.0% of carbohydrate, calculated as cellulose.

Arsenic (as As) Not more than 1 ppm.

Ash (Total) Not more than 0.3%.

Chloride Not more than 0.05%.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 7%.

pH Between 5.0 and 7.5.

Sulfur (Total) Not more than 0.01%.

Water-Soluble Substances Not more than 1.5%.

TESTS

Assay Transfer about 125 mg of the sample, accurately weighed, to a 300-ml Erlenmeyer flask, using about 25 ml of water. Add 50.0 ml of 0.5 *N* potassium dichromate, mix, then carefully add 100 ml of sulfuric acid, and heat to boiling. Remove from heat, allow to stand at room temperature for 15 min, then cool in a water bath, and transfer the solution to a 250-ml volumetric flask. Dilute with water almost to volume, cool to 25°, dilute to volume with water, and mix. Titrate a 50-ml aliquot with 0.1 *N* ferrous ammonium sulfate, using 2 or 3 drops of orthophenanthroline TS. Perform a blank determination, and calculate the normality, *N*, of the ferrous ammonium sulfate solution by the formula $(0.1 \times 50)/B$, in which *B* is the volume, in ml, of ferrous ammonium sulfate solution required in the blank titration. Calculate the percentage of cellulose in the sample by the formula

$$6.75(B - S) \times N/2W,$$

in which *S* is the volume, in ml, of ferrous ammonium sulfate solution used in the sample titration, and *W* is the weight of the sample taken, in g, corrected for moisture content (see *Loss on Drying*).

Arsenic A *Sample Solution* prepared from a 3-g sample as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Total) Heat 3 g at 550° ± 50° until completely charred, then ignite at 800° ± 25° until free from carbon, cool in a desiccator, and weigh.

Chloride Transfer about 5 g of the sample, accurately weighed, to a 500-ml conical flask, add 250 ml of water, and reflux the mixture for 1 h. Filter through paper, and again reflux the sample with 200 ml of water for 30 min. Filter and combine the filtrates and hot water rinses. Add 1 ml of nitric acid, heat to boiling, and slowly add 5 ml of a 5% solution of silver nitrate. After the precipitate has coagulated, cool, and filter through a Gooch crucible. Wash with nitric acid solution (1 in 100) until free from silver nitrate, then rinse with water, dry at 130°, and weigh. Perform a blank determination to obtain the corrected weight of the sample precipitate, each mg of which is equivalent to 0.247 mg of chloride.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry to constant weight at 105°.

pH Mix 10 g of the sample with 90 ml of water, allow to stand with occasional stirring for 1 h, and determine the pH of the supernatant liquid by the *Potentiometric Method*, page 531.

Sulfur (Total) Transfer about 5 g of the sample, previously dried at 105° to constant weight and accurately weighed, to a 300-ml conical flask, and add 50 ml of a 2:3 mixture, v/v, of perchloric acid and nitric acid. Heat on a hot plate under a hood, and boil until all organic matter has been destroyed and copious fumes of perchloric acid are evolved. If the organic matter chars and cannot be destroyed quickly by further heating for a short time, add 10 to 20 ml of the acid mixture and continue the treatment until a clear, syrupy residue is obtained. (NOTE: It is absolutely necessary that all of the nitric acid be driven from the flask, as it will form a double salt with the barium sulfate formed later.) Allow the mixture to cool for a few min, then add 200 ml of hot water, and heat again to boiling. (If the solution is cloudy, filter and rinse the filter with a small amount of hot water before boiling.) As soon as the mixture is boiling gently, carefully run in 20 ml of barium chloride TS, boil for a few min longer, and allow to stand for at least 12 h on a steam bath. Filter any barium sulfate onto an ashless filter paper, and rinse with five portions of boiling water to remove traces of perchloric acid. Place the paper in a tared platinum dish, dry in an oven at 105°, and ignite at 800° ± 25° for 1 h. Perform a blank determination to obtain the corrected weight of the sample precipitate, each mg of which is equivalent to 0.137 mg of sulfur.

Water-Soluble Substances Mix 6 g of the sample with 90 ml of recently boiled and cooled water, and allow to stand with occasional stirring for 10 min. Filter, discard the first 10 ml of filtrate, and pass the filtrate through the same filter a second time, if necessary, to obtain a clear filtrate. Evaporate a 15-ml portion of the filtrate to dryness in a tared evaporating dish on a steam bath, dry at 105° for 1 h, cool in a desiccator, and weigh.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Anticaking agent; binding agent; bulking agent; disintegrating agent; dispersing agent; filter aid; texturizing agent; thickening agent.

Chamomile Oil, English Type

DESCRIPTION

The oil obtained by steam distillation of the dried flowers of the so-called English or Roman Chamomile, *Anthemis nobilis* L. It is a light blue or light greenish blue liquid with a strong aromatic odor, characteristic of the flowers. The color may change with age to greenish yellow or yellow brown. It is soluble in most fixed oils, and it is almost completely soluble in mineral oil. It is soluble, with slight haziness, in propylene glycol, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 590, using the same test conditions as specified therein.

Acid Value Not more than 15.0.
Ester Value Between 250 and 310.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.440 and 1.450 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.892 and 0.910.

TESTS

Acid Value Determine as directed in the general method, page 499.

Ester Value Determine as directed in the general method, page 501, using about 1 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, (page 533) Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 80% alcohol, sometimes with a slight precipitate.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Chamomile Oil, German Type

Chamomile Oil, Hungarian Type

DESCRIPTION

The oil obtained by steam distillation of the flowers and stalks of *Matricaria chamomilla* L. It is a deep blue or bluish green

liquid with a strong and characteristic odor and a bitter aromatic taste. When exposed to light or air, the blue color changes to green and finally to brown. Upon cooling, the oil may become viscous. It is soluble in most fixed oils and in propylene glycol. It is insoluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 590, using the same test conditions as specified therein.

Acid Value Between 5 and 50.
Ester Value Not more than 40.
Ester Value after Acetylation Between 65 and 155.
Heavy Metals (as Pb) Passes test.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.910 and 0.950.

TESTS

Acid Value Determine as directed in the general method, page 499.

Ester Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Ester Value after Acetylation Acetylate a 10-ml sample as directed under *Total Alcohols*, page 499. Weigh accurately about 1.5 g of the dried, acetylated oil, and proceed as directed under *Ester Value*, page 501.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Solubility in Alcohol Proceed as directed in the general method, page 502. The oil does not usually dissolve clearly in 95% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass or aluminum containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Chlorine

Cl_2 Mol wt 70.91

DESCRIPTION

A greenish yellow gas, normally packaged as a liquid under pressure in containers approved by the U.S. Department of Transportation. At 60°F, it has a vapor pressure of 70.91 psig. Its vapor density is about 2.5 times that of air. About 0.8 lb (0.362 kg) is soluble in 100 lb (45.4 kg) of water at 60°F under atmospheric pressure.

Caution: Chlorine gas is a respiratory irritant. Large amounts cause coughing, labored breathing, and irritation of the eyes. In extreme cases, the difficulty in breathing may cause death due to suffocation. Liquid chlorine causes skin and eye burns on contact. (Safety precautions to be observed in handling the material are specified in the *Chlorine Manual*, available from the Chlorine Institute, 342 Madison Avenue, New York, N.Y. 10017.)

REQUIREMENTS

Identification

Cautiously pass a few ml of chlorine gas through 10 ml of sodium hydroxide TS that has been previously chilled in an ice bath. The resulting solution gives positive tests for *Chloride*, page 516, and it darkens starch iodide paper.

Assay Not less than 99.5%, by volume.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.003%.
Lead Not more than 10 ppm.
Mercury Not more than 1 ppm.
Moisture Not more than 0.015%, by weight.
Residue Not more than 0.015%, by weight, of nonvolatile matter.

TESTS

Assay Determine by ASTM Method E 412-70, "Assay of Liquid Chlorine (Zinc Amalgam Method)."

Sample Solution for the Determination of Arsenic, Heavy Metals, Lead, and Mercury Dissolve the residue, obtained in the test for *Residue*, in 2.5 ml of freshly prepared aqua regia, and dilute with water to a volume, in ml, equivalent to the weight, in g, of the initial chlorine sample, so that 1 ml of the final dilution is equivalent to 1 g of chlorine.

Arsenic A 1.0-ml portion of the *Sample Solution*, diluted to 35 ml with water, meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A 0.67-ml portion of the *Sample Solution*, diluted to 25 ml with water, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 1.0-ml portion of the *Sample Solution*, mixed with 5 ml of water and 11 ml of diluted hydrochloric acid TS, meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Mercury Transfer 2.0 ml of the *Sample Solution* into a 50-ml beaker, add 10 ml of water, 1 ml of dilute sulfuric acid (1 in 5), and 1 ml of potassium permanganate solution (1 in 25),

cover with a watch glass, boil for a few seconds, and cool. Use the resulting solution as the *Sample Preparation* as directed under the *Mercury Limit Test*, page 520.

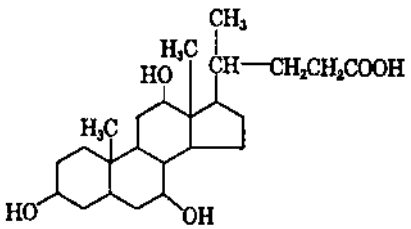
Moisture and Residue Determine by ASTM Method E 410-70, "Moisture and Residue in Liquid Chlorine."

Packaging and Storage Store in suitable pressure containers, observing applicable federal regulations pertaining to shipping containers.

Functional Use in Foods Antimicrobial agent; bleaching agent; oxidizing agent.

Cholic Acid

Cholalic Acid; 3,7,12-Trihydroxycholanic Acid



$C_{24}H_{40}O_5$ Mol wt 408.58

DESCRIPTION

Colorless plates or a white, crystalline powder having a bitter taste with a sweetish aftertaste. One g dissolves in about 30 ml of alcohol or acetone and in about 7 ml of glacial acetic acid. It is very slightly soluble in water.

REQUIREMENTS

Identification

To 1 ml of a 1 in 5000 solution in 50% acetic acid add 1 ml of a solution of furfural (1 in 100). Cool in an ice bath for 5 min, add 15 ml of dilute sulfuric acid (1 in 2), mix, and warm in a water bath at 70° for 10 min. Immediately cool in an ice bath and stir for 2 min. A blue color develops.

Assay Not less than 98.0% of $C_{24}H_{40}O_5$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Melting Range Between 197° and 202°.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{25°}$: Not less than +37°.

TESTS

Assay Transfer about 400 mg, accurately weighed, into a 250-ml Erlenmeyer flask, add 20 ml of water and 40 ml of alcohol, cover with a watch glass, heat gently on a steam bath until dissolved, and cool. Add 5 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide, using a 10-ml microburet, to the first pink color that persists for 15 s. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* sodium hydroxide is equivalent to 40.86 mg of $C_{24}H_{40}O_5$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 140° under a vacuum of not more than 5 mm of Hg for 4 h.

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution in alcohol containing 200 mg in each 10 ml.

Packaging and Storage Store in tight containers.
Functional Use in Foods Emulsifier.

Choline Bitartrate

(2-Hydroxyethyl)trimethylammonium Bitartrate

$[HOCH_2CH_2N^+(CH_3)_3]HC_4H_4O_6^-$

$C_9H_{19}NO_7$ Mol wt 253.25

DESCRIPTION

A white, hygroscopic, crystalline powder having an acidic taste. It is odorless or may have a faint trimethylaminelike odor. It is freely soluble in water, slightly soluble in alcohol, and insoluble in ether, chloroform, and benzene.

REQUIREMENTS

Identification

A. Dissolve 500 mg in 2 ml of water, add 3 ml of sodium hydroxide TS, and heat to boiling. The odor of trimethylamine is detectable.

B. Dissolve 500 mg in 2 ml of iodine TS. A reddish brown precipitate is immediately formed. Add 5 ml of sodium hydroxide TS. The precipitate dissolves and the solution becomes clear yellow. Heat the solution. A pale yellow precipitate forms and the odor of iodoform may be detected.

C. To 2 ml of cobaltous chloride TS add 1 ml of a 1 in 100 solution of the sample and 2 ml of potassium ferrocyanide solution (1 in 50). An emerald green color develops immediately.

Assay Not less than 98.0% of $C_9H_{19}NO_7$, calculated on the anhydrous basis.
Arsenic (as As) Not more than 3 ppm.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Residue on Ignition Not more than 0.1%.
Water Not more than 0.5%.

TESTS

Assay Transfer about 500 mg, accurately weighed, into a 250-ml Erlenmeyer flask, add 50 ml of glacial acetic acid, and warm on a steam bath until solution is complete. Cool, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a green endpoint. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 25.36 mg of $C_9H_{19}NO_7$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Water Determine by drying in a vacuum desiccator over phosphorus pentoxide for 4 h or by the *Karl Fischer Titrimetric Method*, page 552, using a 2-g sample dissolved in 50 ml of methanol.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Choline Chloride

(2-Hydroxyethyl)trimethylammonium Chloride

$$[HOCH_2CH_2N^+(CH_3)_3]Cl^-$$

$C_5H_{14}ClNO$ Mol wt 139.62

DESCRIPTION

Colorless or white crystals or crystalline powder, usually having a slight odor of trimethylamine. It is hygroscopic, and is very soluble in water and in alcohol.

REQUIREMENTS

Identification

A. It responds to *Identification Tests A, B,* and *C* under *Choline Bitartrate*, page 83.
B. A 1 in 20 solution gives positive tests for *Chloride*, page 516.

Assay Not less than 98.0% of $C_5H_{14}ClNO$, calculated on the anhydrous basis.
Arsenic (as As) Not more than 3 ppm.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Residue on Ignition Not more than 0.05%.
Water Not more than 0.5%.

TESTS

Assay Transfer about 300 mg, accurately weighed, into a 250-ml Erlenmeyer flask, add 50 ml of glacial acetic acid, and warm on a steam bath until solution is complete. Cool, add 10 ml of mercuric acetate TS and 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a green endpoint. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 13.96 mg of $C_5H_{14}ClNO$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Residue on Ignition Ignite 4 g as directed in the general method, page 533.

Water Determine by drying in a vacuum desiccator for 4 h over phosphorus pentoxide or by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Cinnamon Bark Oil, Ceylon Type

DESCRIPTION

The volatile oil obtained by steam distillation from the dried inner bark of the clipped cinnamon shrub *Cinnamomum zeylanicum* Nees (Fam. *Lauraceae*). It is a yellow liquid with an odor of cinnamon and a spicy burning taste. It is soluble in most fixed oils and in propylene glycol. It is insoluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 590, using the same test conditions as specified therein.

Assay Not less than 55.0% and not more than 78.0% of aldehydes, calculated as cinnamic aldehyde (C_9H_8O).
Angular Rotation Between $-2°$ and 0°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.573 and 1.591 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.010 and 1.030.

TESTS

Assay Weigh accurately about 2.5 g, and proceed as directed under *Aldehydes*, page 499, using 66.10 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 70% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, light-resistant glass, aluminum, or tin-lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Cinnamon Leaf Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the leaves and twigs of the true cinnamon shrub *Cinnamomum zeylanicum* Nees. The commercial oils, according to the geographical origin, are designated as either cinnamon leaf oil, Ceylon, or cinnamon leaf oil, Seychelles, and the two types differ in physical and chemical properties. The oil is a light to dark brown liquid having a spicy cinnamon, clovelike odor and taste. It is soluble in most fixed oils and in propylene glycol. It is soluble, with cloudiness, in mineral oil, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 591, using the same test conditions as specified therein.

Assay *Ceylon type:* not less than 80% and not more than 88%, by volume, of phenols as eugenol; *Seychelles type:* not less than 87% and not more than 96%, by volume, of phenols as eugenol.
Angular Rotation *Ceylon type:* between $-2°$ and $+1°$; *Seychelles type:* between $-2°$ and 0°.
Heavy Metals (as Pb) Passes test.
Refractive Index *Ceylon type:* between 1.529 and 1.537; *Seychelles type:* between 1.533 and 1.540 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity *Ceylon type:* between 1.030 and 1.050; *Seychelles type:* between 1.040 and 1.060.

TESTS

Assay Shake a suitable quantity of the oil with about 2% of powdered tartaric acid, and filter. Proceed with a sample of the filtered oil as directed under *Phenols*, page 502.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml of the Ceylon type oil dissolves in 1.5 ml of 70% alcohol. One ml of the Seychelles type oil dissolves in 1 ml of 70% alcohol. The solutions may cloud upon further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, light-resistant, glass, aluminum, or tin-lined containers in a cool place protected from light.

Labeling Label cinnamon leaf oil to indicate whether it is the Ceylon or Seychelles type.

Functional Use in Foods Flavoring agent.

Citric Acid

$CH_2(COOH)C(OH)(COOH)CH_2COOH$

$C_6H_8O_7$ Mol wt 192.12

DESCRIPTION

Citric acid is anhydrous or contains one molecule of water of hydration. It occurs as colorless, translucent crystals or as a white, granular to fine crystalline powder. It is odorless and has a strongly acid taste, and the hydrous form is efflorescent in dry air. One g is soluble in about 0.5 ml of water, in about 2 ml of alcohol, and in about 30 ml of ether.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Citrate*, page 516.

Assay Not less than 99.5% of $C_6H_8O_7$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Oxalate Passes test.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.05%.

Tridodecylamine Not more than 0.1 ppm.

Ultraviolet Absorbance (polynuclear hydrocarbons) 280–289 nm, not more than 0.25; 290–299 nm, not more than 0.20; 300–359 nm, not more than 0.13; 360–400 nm, not more than 0.03.

Water *Anhydrous form:* not more than 0.5%; *hydrous form:* not more than 8.8%.

TESTS

Assay Dissolve about 3 g, accurately weighed, in 40 ml of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 64.04 mg of $C_6H_8O_7$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Tests*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Oxalate Neutralize 10 ml of a 1 in 10 solution with ammonia TS, add 5 drops of diluted hydrochloric acid TS, cool, and add 2 ml of calcium chloride TS. No turbidity is produced.

Readily Carbonizable Substances, page 532 Transfer 1.0 g, finely powdered, to a 22- × 175-mm test tube, previously rinsed with 10 ml of sulfuric acid TS and allowed to drain for 10 min. Add 10 ml of sulfuric acid TS, agitate the tube until solution is complete, and immerse the tube in a water bath at 90° ± 1° for 60 ± 0.5 min, keeping the level of the acid below the level of the water during the heating period. Cool the tube in a stream of water, and transfer the acid solution to a color-comparison tube. The color of the acid solution is not darker than that of the same volume of *Matching Fluid K* in a similar matching tube, viewing the tubes vertically against a white background.

Residue on Ignition, page 533 Ignite 4 g as directed in the general method.

Tridodecylamine

Buffered Indicator Solution Prepare a mixture consisting of 700 ml of 0.1 *M* citric acid (anhydrous, reagent grade), 200 ml of 0.2 *M* disodium phosphate, and 50 ml each of 0.2% bromophenol blue and of 0.2% bromocresol green in spectro-grade methanol.

No-Indicator Buffer Solution Prepare a mixture consisting of 700 ml of 0.1 *M* citric acid (anhydrous, reagent grade), 200 ml of 0.2 *M* disodium phosphate, and 100 ml of spectro-grade methanol.

Amine Stock Solution Transfer between 40 and 45 mg of tridodecyl(trilauryl)amine, accurately weighed, into a 500-ml volumetric flask, dilute to volume with isopropyl alcohol, and mix. Discard after three weeks.

Standard Amine Solution Using a graduated 5-ml pipet, transfer into a 100-ml volumetric flask an amount of *Amine Stock Solution* equivalent to 400 µg of tridodecylamine, dilute to volume with isopropyl alcohol, and mix. Prepare this solution fresh on the day of use.

Procedure Dissolve 160 g of anhydrous reagent-grade citric acid (not the sample to be tested) in 320 ml of water, and divide the solution equally between two 250-ml separators, S_1 and S_2. To S_1 add 5 ml of *No-Indicator Buffer Solution*. To S_2 add 2.0 ml of *Standard Amine Solution* and 5 ml of *Buffered Indicator Solution*.

To prepare solutions to the sample being tested, dissolve 160 g of anhydrous citric acid sample in 320 ml of water (or 174 g of citric acid monohydrate sample in 306 ml of water). Divide the test solution equally between two 250-ml separators, S_3 and S_4. Add 5 ml of *No-Indicator Buffer Solution* to S_3, and 5 ml of *Buffered Indicator Solution* to S_4.

To each of the four separators add 20 ml of a 1 to 1 mixture (v/v) prepared from spectro-grade chloroform and *n*-heptane, shake for 15 min on a mechanical shaker, and allow the phases to separate for 45 min. Drain all except the last few drops of the lower (aqueous) phases, and discard. Hand-shake the organic phases with 25 ml each of 0.05 *N* sulfuric acid for 30 s, and allow the phases to separate for 30 min. Drain all except the last few drops of the lower (organic)

phases through dry Whatman No. 40 (or equivalent) paper, and collect the aqueous filtrates in separate small glass-stoppered containers.

Determine the absorbance of each solution in a 5-cm cell at 400 nm, with a suitable spectrophotometer standardized prior to analysis, against chloroform-heptane (1:1 v/v). The net absorbance of the sample ($S_4 - S_3$) is not greater than that of the standard ($S_2 - S_1$).

Ultraviolet Absorbance Determine as directed in the federal food additive regulation pertaining to the use of *Candida lipolytica* in the production of citric acid (21 **CFR** 173.165).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Labeling Label to indicate whether it is anhydrous or hydrous.

Functional Use in Foods Sequestrant; dispersing agent; acidifier; flavoring agent.

Clary Oil

Clary Sage Oil; Oil of Muscatel

DESCRIPTION

The oil obtained by steam distillation from the flowering tops and leaves of the clary sage plant, *Salvia sclarea* L. (Fam. *Labiatae*). It is a pale yellow to yellow liquid having a herbaceous odor and a winy bouquet. It is soluble in most fixed oils, and in mineral oil up to 3 volumes, but becomes opalescent on further dilution. It is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 591, using the same test conditions as specified therein.

Assay Not less than 48.0% and not more than 75.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).

Acid Value Not more than 2.5.

Angular Rotation Between $-6°$ and $-20°$.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.458 and 1.473 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.886 and 0.929.

TESTS

Assay Weigh accurately about 2 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 90% alcohol, becoming opalescent upon further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or galvanized iron containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Clove Leaf Oil

DESCRIPTION

The volatile oil obtained by steam distillation of the leaves of *Eugenia caryophyllata* Thunberg (*Eugenia aromatica* L. Baill.) (Fam. *Myrtaceae*). It is a pale yellow liquid. It is soluble in propylene glycol, and in most fixed oils with slight opalesence, and it is relatively insoluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 591, using the same test conditions as specified therein.

Assay Not less than 84% and not more than 88%, by volume, of phenols as eugenol.

Angular Rotation Between $-2°$ and 0°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.531 and 1.535 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 1.036 and 1.046.

TESTS

Assay Shake a suitable quantity of the oil with 2% of

powdered tartaric acid for about 2 min, and filter. Then, using a sample of the filtered oil, proceed as directed under *Phenols*, page 502, modified by heating the flask in a boiling water bath for 10 min, after shaking the oil with potassium hydroxide TS. Remove from the boiling water bath, cool, and proceed as directed.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol. A slight opalescence may occur when additional solvent is added.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, light-resistant, glass, tin-lined, stainless, or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Clove Oil

Clove Bud Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried flowerbuds of *Eugenia caryophyllata* Thunberg (*Eugenia aromatica* L. Baill.) (Fam. *Myrtaceae*). It is a colorless or pale yellow liquid having the characteristic clove odor and taste. It darkens and thickens upon aging or exposure to air.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 591, using the same test conditions as specified therein.

Assay Not less than 85%, by volume, of phenols as eugenol.
Angular Rotation Between −1°30′ and 0°.
Heavy Metals (as Pb) Passes test.
Phenol Passes test.
Refractive Index Between 1.527 and 1.535 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.038 and 1.060.

TESTS

Assay Proceed as directed under *Phenols*, page 502.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Phenol Shake 1 ml of sample with 20 ml of hot water. The water shows no more than a scarcely perceptible acid reaction with blue litmus paper. Cool the mixture, pass the water layer through a wetted filter, and treat the clear filtrate with 1 drop of ferric chloride TS. The mixture has only a transient grayish green color, but not a blue or violet color.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, light-resistant containers and avoid exposure to excessive heat.

Functional Use in Foods Flavoring agent.

Clove Stem Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried stems of the buds of *Eugenia caryophyllata* Thunberg (*Eugenia aromatica* L. Baill.) (Fam. *Myrtaceae*). It is a yellow to light brown liquid with a characteristic odor and taste. It is soluble in fixed oils and in propylene glycol, but it is relatively insoluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 592, using the same test conditions as specified therein.

Assay Not less than 89% and not more than 95%, by volume, of phenols as eugenol.
Angular Rotation Between −1.5° and 0°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.534 and 1.538 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.048 and 1.056.

TESTS

Assay Shake a suitable quantity of the oil with about 2% of powdered tartaric acid for about 2 min, and filter. Then, using a sample of the filtered oil, proceed as directed under *Phenols*, page 502, modifed by heating the flask in a boiling water bath for 10 min, after shaking the oil with potassium hydroxide TS. Remove from the boiling water bath, cool, and proceed as directed.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store preferably in full, tight, light-resistant glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Cognac Oil, Green

Wine Yeast Oil

DESCRIPTION

The volatile oil obtained by steam distillation from wine lees. It is a green to bluish green liquid with the characteristic aroma of cognac. It is soluble in most fixed oils and in mineral oil. It is very slightly soluble in propylene glycol, and it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 592, using the same test conditions as specified therein.

Acid Value Between 32 and 70.
Angular Rotation Between $-1°$ and $+2°$.
Ester Value Between 200 and 245.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.427 and 1.430 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.864 and 0.870.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Proceed as directed in the general method, page 501, using about 1 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 80% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Copaiba Oil

DESCRIPTION

The volatile oil obtained by steam distillation of copaiba balsam, an exudate from the trunk of various South American species of *Copaifera* L. (Fam. *Leguminosae*). It is a colorless to slightly yellow liquid having the characteristic odor of copaiba balsam and an aromatic, slightly bitter, and pungent taste. It is soluble in alcohol, in most fixed oils, and in mineral oil. It is insoluble in glycerin and practically insoluble in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 592, using the same test conditions as specified therein.

Angular Rotation Between $-7°$ and $-33°$.
Gurjun Oil Passes test.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.493 and 1.500 at 20°.
Specific Gravity Between 0.880 and 0.907.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Gurjun Oil Add 5 or 6 drops of the sample to 10 ml of glacial acetic acid containing 5 drops of nitric acid. No purple color develops in 2 min, indicating the absence of gurjun oil.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin, aluminum, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Copper Gluconate

$$[CH_2OH(CHOH)_4COO]_2Cu$$

$C_{12}H_{22}CuO_{14}$ Mol wt 453.84

DESCRIPTION

A fine, light blue powder. It is very soluble in water, and is very slightly soluble in alcohol.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Copper*, page 516.

B. To 5 ml of a warm solution (1 in 10) add 0.7 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, heat on a steam bath for 30 min, and allow to cool. Induce crystallization by scratching the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{12}H_{22}CuO_{14}$.

Arsenic (as As) Not more than 3 ppm.

Lead Not more than 10 ppm.

Reducing Substances Not more than 1%.

TESTS

Assay Dissolve about 1.5 g, accurately weighed, in 100 ml of water in a 250-ml Erlenmeyer flask, add 2 ml of glacial acetic acid and 5 g of potassium iodide, mix well, and titrate with 0.1 *N* sodium thiosulfate to a light yellow color. Add 2 g of ammonium thiocyanate, mix, then add 3 ml of starch TS and continue titrating to a milk-white endpoint. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 45.38 mg of $C_{12}H_{22}CuO_{14}$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Lead A solution of 1 g in 25 ml of water meets the requirements of the *Lead Limit Test*, page 518.

Reducing Substances Transfer about 1 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, dissolve in 10 ml of water, add 25 ml of alkaline cupric citrate TS, and cover the flask with a small beaker. Boil gently for exactly 5 min and cool rapidly to room temperature. Add 25 ml of a 1 in 10 solution of acetic acid, 10.0 ml of 0.1 *N* iodine, 10 ml of diluted hydrochloric acid TS, and 3 ml of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in mg, of reducing substances (as D-glucose) by the formula $(V_1N_1 - V_2N_2)27$, in which V_1 and N_1 are the volume and normality, respectively, of the iodine solution, V_2 and N_2 are the volume and normality, respectively, of the sodium thiosulfate solution, and 27 is an empirically determined equivalence factor for D-glucose.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Coriander Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried ripe fruit of *Coriandrum sativum* L. (Fam. *Umbelliferae*). It is a colorless or pale yellow liquid having the characteristic odor and taste of coriander.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 592, using the same test conditions as specified therein.

Angular Rotation Between +8° and +15°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.462 and 1.472 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.863 and 0.875.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers protected from light. Avoid exposure to excessive heat.

Functional Use in Foods Flavoring agent.

Costus Root Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried, triturated roots of the herbaceous perennial plant *Saussurea lappa* Clarke (Fam. *Compositae*), or by a solvent extraction procedure followed by vacuum distillation of the resinoid extract. It is a light yellow to brown, viscous liquid having a peculiar, persistent odor reminiscent of violet, orris, and vetivert. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 593, using the same test conditions as specified therein.

Acid Value Not more than 42.
Angular Rotation Between +10° and +36°.
Ester Value Between 90 and 150.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.512 and 1.523 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.995 and 1.039.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Determine as directed in the general method, page 501, using about 1 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 90% alcohol, but the solution becomes cloudy upon further dilution and occasionally paraffin crystals may separate.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Cubeb Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the mature, unripe, sun-dried fruit of the perennial vine *Piper cubeba* L. (Fam. *Piperaceae*). It is a colorless or light green to bluish green liquid having a spicy odor and a slightly acrid taste. It is soluble in most fixed oils and in mineral oil, but it is insoluble in glycerin and propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 593, using the same test conditions as specified therein.

Acid Value Not more than 2.0.
Angular Rotation Between −12° and −43°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.492 and 1.502 at 20°.
Saponification Value Not more than 8.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.898 and 0.928.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is

saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 10 ml of 90% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Cumin Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the plant *Cuminum cyminum* L. It is a light yellow to brown liquid having a strong and somewhat disagreeable odor. It is relatively soluble in most fixed oils and in mineral oil. It is very soluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 593, using the same test conditions as specified therein.

Assay Not less than 45.0% and not more than 52.0% of aldehydes, calculated as cuminaldehyde ($C_{10}H_{12}O$).

Angular Rotation Between +3° and +8°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.501 and 1.506 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.905 and 0.925.

TESTS

Assay Weigh accurately about 1 g, and proceed as directed under *Aldehydes*, page 499, using 74.10 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 30 min at room temperature before titrating.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 8 ml of 80% alcohol. The solution may become hazy upon the addition of more alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin, or suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

L-Cysteine Monohydrochloride

L-2-Amino-3-mercaptopropanoic Acid Monohydrochloride

$$\begin{array}{l} HSCH_2CHCOOH \cdot H_2O \\ \quad\quad | \\ \quad NH_2 \cdot HCl \end{array}$$

$C_3H_7NO_2S.HCl.H_2O$ Mol wt 175.63

DESCRIPTION

A white, odorless, crystalline powder having a characteristic acidic taste. It is freely soluble in water and in alcohol. The anhydrous form melts with decomposition at about 175°.

REQUIREMENTS

Identification

A. Dissolve 100 mg in 5 ml of water and add 10 ml of cupric nitrate TS. A bluish gray precipitate is formed.

B. A 1 in 20 solution gives positive tests for *Chloride*, page 516.

Assay Not less than 98.0% and not more than 102.0% of $C_3H_7NO_2S.HCl$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not less than 8% and not more than 12%.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]_D^{20}$: Between +5.0° and +8.0°.

TESTS

Assay Transfer about 300 mg, previously dried as directed under *Loss on Drying* and accurately weighed, into a 250-ml glass-stoppered flask. Add 20 ml of water, 4 g of potassium iodide, 5 ml of diluted hydrochloric acid TS, and 25.0 ml of 0.1 *N* iodine. Stopper the flask, allow the mixture to stand for 30 min in a dark place, and titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a blank determination (see page 2). Each ml of 0.1 *N* iodine is equivalent to 15.76 mg of $C_3H_7NO_2S.HCl$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at room temperature for 24 h in a vacuum desiccator using a suitable desiccant and maintaining a pressure of not more than 5 mm of Hg.

Residue on Igultion, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution containing 8 g of undried sample in sufficient 1 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Cystine

3,3'-Dithiobis(2-aminopropanoic acid)

$$HOOCCH(NH_2)CH_2SSCH_2CH(NH_2)COOH$$

$C_6H_{12}N_2O_4S_2$ Mol wt 240.29

DESCRIPTION

Colorless, practically odorless, white crystals. It is soluble in diluted mineral acids and in alkaline solutions. It is very slightly soluble in water and in alcohol.

REQUIREMENTS

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_6H_{12}N_2O_4S_2$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Iron Not more than 0.005%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.2%.

Nitrogen (Total) Between 11.5% and 11.9%.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]_D^{20}$: Between −215° and −225°.

TESTS

Assay

Sodium Cyanide Solution On the day of use, dissolve 2.5 g of sodium cyanide in 25 ml of sodium hydroxide TS, and dilute to 50 ml with water.

Sodium Hydrosulfite Solution Within 1 h of use, dissolve 1 g of sodium hydrosulfite, $Na_2S_2O_4$, in 25 ml of sodium hydroxide TS, and dilute to 50 ml with water.

Sodium Naphthoquinone-4-sulfonate Solution Within 1 h of use, dissolve 150 mg of sodium β-naphthoquinone-4-sulfonate, $C_{10}H_7NaO_4S$, in sufficient water to make 50 ml.

Sodium Sulfite Solution On the day of use, dissolve 5 g of sodium sulfite in 25 ml of sodium hydroxide TS, and dilute to 50 ml with water.

Standard Preparation Transfer about 100 mg of USP L-Cystine Reference Standard, previously dried for 3 h over phosphorus pentoxide and accurately weighed, into a 250-ml volumetric flask, dissolve in 100 ml of 0.1 *N* hydrochloric acid, dilute to volume with water, and mix. Transfer 20.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with water, and mix.

Assay Preparation Transfer about 100 mg of the sample, previously dried for 3 h over phosphorus pentoxide and accurately weighed, into a 250-ml volumetric flask, dissolve in 100 ml of 0.1 *N* hydrochloric acid, dilute to volume with water, and mix. Transfer 20.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with water, and mix.

Procedure Pipet 5 ml each of the *Standard Preparation* and of the *Assay Preparation* into separate 25-ml volumetric flasks. To each flask add 2 ml of the *Sodium Cyanide Solution*, allow to stand for 10 min, then add 1 ml of the *Sodium Naphthoquinone-4-sulfonate Solution*, followed in 10 s by 5 ml of the *Sodium Sulfite Solution*. Allow the color to develop for 25 min, then add to each flask 2 ml of 5 *N* sodium hydroxide and 1 ml of the *Sodium Hydrosulfite Solution*. Dilute each flask to volume with water, mix, and concomitantly determine the absorbance of each solution at 500 nm in 1-cm cells, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_6H_{12}N_2O_4S_2$ in the sample taken by the formula $1.25C(A_U/A_S)$, in which C is the concentration, in µg per ml, of the *Standard Preparation*, and A_U and A_S are the absorbances of the solutions from the *Assay Preparation* and the *Standard Preparation*, respectively.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron To the ash obtained in the test for *Residue on Ignition* add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, and dilute with water to 50 ml. Dilute 5 ml of this solution to 40 ml with water, and add 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 µg Fe) in an equal volume of a solution containing the quantities of the reagents used in the test.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry over silica gel for 4 h.

Nitrogen (Total) Determine as directed under *Nitrogen Determination*, page 521, using 300 mg of a sample previously dried and accurately weighed.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Specific Rotation, page 530 Determine in a solution containing 2 g of a previously dried sample in sufficient 1 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Decanoic Acid

Capric Acid

$$CH_3(CH_2)_8COOH$$

$C_{10}H_{20}O_2$ Mol wt 172.27

DESCRIPTION

White crystals having a characteristic, unpleasant, rancid odor. It is soluble in most organic solvents and practically insoluble in water.

REQUIREMENTS

Acid Value Between 320 and 329.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Not more than 0.6.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 320 and 331.
Titer (Solidification Point) Between 27° and 32°.
Unsaponifiable Matter Not more than 0.2%.
Water Not more than 0.2%.

TESTS

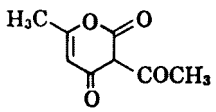
Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Residue on Ignition, page 533 Ignite 10 g as directed in the general method.
Saponification Value Determine as directed in the general method, page 509, using about 2 g, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.
Unsaponifiable Matter Determine as directed in the general method, page 509.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Component in the manufacture of other food-grade additives; defoaming agent.

Dehydroacetic Acid

3-Acetyl-6-methyl-1,2-pyran-2,4(3*H*)-dione;
Methylacetopyronone; DHA



$C_8H_8O_4$ Mol wt 168.15

DESCRIPTION

A white or nearly white crystalline powder. It is odorless or almost odorless, and has a faint, acid taste. It is soluble in aqueous solutions of fixed alkalies, and is very slightly soluble in water. One g dissolves in about 35 ml of alcohol, 5 ml of acetone, and 6 ml of benzene.

REQUIREMENTS

Identification

The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits maxima only at the same wavelengths as that of USP Dehydroacetic Acid Reference Standard.

Assay Not less than 98.0% of $C_8H_8O_4$, calculated on the anhydrous basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 1%.
Melting Range Between 109° and 111°.
Residue on Ignition Not more than 0.1%.

TESTS

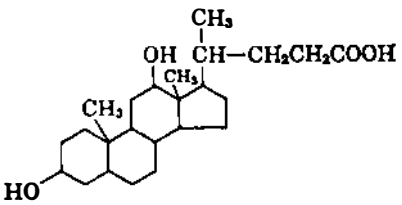
Assay Transfer about 500 mg, accurately weighed, to a 250-ml Erlenmeyer flask, dissolve it in 75 ml of neutral alcohol, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 16.82 mg of $C_8H_8O_4$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 80° for 4 h.
Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Preservative.

Desoxycholic Acid

Deoxycholic Acid; 13α,12α-Dihydroxycholanic Acid



$C_{24}H_{40}O_4$ Mol wt 392.58

DESCRIPTION

A white crystalline powder. It is practically insoluble in water, slightly soluble in chloroform and in ether, soluble in acetone and in solutions of alkali hydroxides and carbonates, and freely soluble in alcohol.

REQUIREMENTS

Identification

To about 10 mg of the sample add 2 drops of benzaldehyde and 3 drops of 75% sulfuric acid, heat at 50° for 5 min, and then add 10 ml of glacial acetic acid. A green color is produced. (Cholic acid produces a brown color.)

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{24}H_{40}O_4$, calculated on the anhydrous basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 1%.
Melting Range Between 172° and 175°.
Residue on Ignition Not more than 0.2%.

TESTS

Assay Transfer about 500 mg, accurately weighed, into a 250-ml Erlenmeyer flask, and add 20 ml of water and 40 ml of alcohol. Cover the flask with a watch glass, heat the mixture gently on a steam bath until the sample is dissolved, and allow to cool to room temperature. To the solution add a few drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 15 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 39.26 mg of $C_{24}H_{40}O_4$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 140° under a vacuum of not more than 5 mm of Hg for 4 h.
Melting Range Determine as directed in the general procedure, page 519.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in tight containers.
Functional Use in Foods Emulsifier.

Dexpanthenol

D(+)-Pantothenyl Alcohol; Panthenol

$HOCH_2C(CH_3)_2CH(OH)CONH(CH_2)_2CH_2OH$

$C_9H_{19}NO_4$ Mol wt 205.25

DESCRIPTION

The dextrorotatory isomer of the alcohol analogue of pantothenic acid. It occurs as a clear, viscous, somewhat hygroscopic liquid having a slight characteristic odor. Some crystallization may occur on standing. Its solutions are alkaline to litmus. It is freely soluble in water, in alcohol, in methanol, and in propylene glycol. It is soluble in chloroform and in ether, and is slightly soluble in glycerin. (NOTE: The physiological activity of 1.0 g of dexpanthenol is equivalent to 1.16 g of dextro-calcium pantothenate.)

REQUIREMENTS

Identification

A. To 1 ml of a 10% solution of the sample add 5 ml of sodium hydroxide TS and 1 drop of cupric sulfate TS, and shake vigorously. A deep blue color develops.
B. To 1 ml of a 1% solution of the sample add 1 ml of 1 *N* hydrochloric acid, and heat on a steam bath for about 30 min. Cool, add 100 mg of hydroxylamine hydrochloride, mix, and add 5 ml of sodium hydroxide TS. Allow to stand for 5 min, then adjust the pH to 2.5–3.0 with 1 *N* hydrochloric acid, and add 1 drop of ferric chloride TS. A purplish red color develops.

Assay Not less than 98.0% and not more than 102.0% of $C_9H_{19}NO_4$ (dexpanthenol), calculated on the anhydrous basis.

Aminopropanol Not more than 1%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Refractive Index Between 1.495 and 1.502 at 20°.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{25°}$: Between +29.0° and +31.5° on the anhydrous basis.
Water Not more than 1%.

TESTS

Assay Transfer about 400 mg, accurately weighed, into a 300-ml reflux flask fitted with a standard-taper glass joint, add 50.0 ml of 0.1 *N* perchloric acid in glacial acetic acid, and reflux for 5 h. Cool, covering the condenser with foil to prevent contamination by moisture, and rinse the condenser with glacial acetic acid. Add 5 drops of crystal violet TS, and titrate with 0.1 *N* potassium acid phthalate in glacial acetic acid to a blue green endpoint. Perform a blank determination and make any necessary correction (see page 2). Each ml of 0.1 *N* perchloric acid is equivalent to 20.53 mg of $C_9H_{19}NO_4$.
Aminopropanol Transfer about 5 g of the sample, accurately weighed, into a 50-ml flask, and dissolve in 10 ml of water. Add bromothymol blue TS, and titrate with 0.1 *N* sulfuric acid from a microburet to a yellow endpoint. Each ml of 0.1 *N* sulfuric acid is equivalent to 7.5 mg of aminopropanol.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Residue on Ignition Ignite a 1-g sample as directed in the general method, page 533.
Specific Rotation, page 530 Determine in a solution containing 500 mg, calculated on the anhydrous basis, in each 10 ml of water.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Dextrin

DESCRIPTION

Dextrin is partially hydrolyzed starch converted by heat alone, or by heating in the presence of suitable food-grade acids and buffers, from any of several grain- or root-based unmodified native starches (e.g., corn, waxy maize, high amylose, milo, waxy milo, potato, arrowroot, wheat, rice, tapioca, sago, etc.). The products thus obtained occur as free-flowing white, yellow, or brown powders and consist chiefly of polygonal, rounded, or oblong or truncated granules. They are partially to completely soluble in water.

REQUIREMENTS

Identification

Suspend about 1 g of the sample in 20 ml of water, and add a few drops of iodine TS. A dark blue to reddish brown color is produced.

Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.2%.
Crude Fat Not more than 1.0%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 5 ppm.
Loss on Drying Not more than 13%.
pH of Dispersions Between 2.0 and 9.0.
Protein Not more than 1.0%.
Reducing Sugars (dextrose equivalent) Not more than 18.0% (expressed as D-glucose), calculated on the dried basis.
Residue on Ignition Not more than 0.5%.

TESTS

Arsenic, Heavy Metals, Lead, Loss on Drying, pH of Dispersions, and Protein Determine as directed under *Food Starch, Modified*, page 126.
Chloride, page 471 Dissolve 1 g in 25 ml of boiling water, cool, dilute to 100 ml with water, and filter. To 1 ml of the filtrate add 24 ml of water, 2 ml of nitric acid, and 1 ml of silver nitrate TS. Any turbidity produced does not exceed that shown in a control containing 20 µg of chloride ion.
Crude Fat Determine as directed in the general method, page 543.
Reducing Sugars Transfer about 10 g of the sample, accurately weighed, into a 200-ml collecting flask, dilute to volume with water, shake for 30 min, and filter through Whatman No. 1 filter paper, or equivalent, collecting the filtrate in a clean, dry flask. Pipet 10 ml each of *Fehling's Solution A* and of *Fehling's Solution B* (see *Cupric Tartrate TS, Alkaline*, page 560) into a 250-ml Erlenmeyer flask, add 20.0 ml of the sample filtrate and 10 ml of water, and mix. Add two small glass beads, cover the mouth of the flask with a small glass funnel or glass bulb, and heat on a hot plate adjusted to bring the solution to a boil in 3 min. Continue boiling for exactly 2 min (total heating time, 5 min), and then quickly cool to room temperature in an ice bath or in a cold running-water bath. Add 10 ml each of 30% potassium iodide solution and of 28% sulfuric acid, and titrate immediately with 0.1 *N* sodium thiosulfate. Near the endpoint add 1 ml of starch TS, and continue titrating carefully, while agitating the solution continuously, until the blue color is discharged. Record the volume, in ml, of 0.1 *N* sodium thiosulfate required as *S*. Conduct two reagent blank determinations in the same manner, substituting water for the sample filtrate, and record the average volume, in ml, of the blanks as *B*. Obtain the *Titer Difference*, expressed as ml of 0.1 *N* sodium thiosulfate,

by subtracting *S* from *B*. Determine the weight, in mg, of reducing sugars, expressed as D-glucose (dextrose), by reference to the table entitled *Conversion of Titer Difference to Reducing Sugars Content*, as found on page 487 in the general tests in *Enzyme Preparations*, under the section on *Glucoamylase Activity*, and record this value as *R*. Calculate the percentage of reducing sugars, as D-glucose, on the dried basis, by the formula

$$(R \times 200 \times 100)/(W \times 20 \times 1000),$$

in which *W* is the weight of sample taken, in g, corrected for *Loss on Drying*.

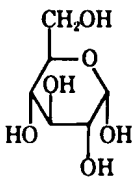
Residue on Ignition Ignite 5 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Thickener; colloidal stabilizer; binder; surface-finishing agent.

Dextrose

D-Glucose; Glucose; Corn Sugar; Grape Sugar



$C_6H_{12}O_6$ Mol wt 180.16

DESCRIPTION

Dextrose is purified and crystallized D-glucose. It is anhydrous or contains one molecule of water of crystallization. It occurs as white, odorless, crystalline granules or as a granular powder having a bland, sweet taste. It is freely soluble in water, very soluble in boiling water, and slightly soluble in alcohol.

REQUIREMENTS

Identification

Add a few drops of a 1 in 20 solution of the sample to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate of cuprous oxide is formed.

Assay Not less than 99.5% of reducing sugar content (dextrose equivalent), expressed as D-glucose, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.015%.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying *Anhydrous*: not more than 2%; *monohydrate*: not more than 10%.

Residue on Ignition Not more than 0.25%.

Specific Rotation $[\alpha]_D^{25}$: Between +52.7° and +53.3° after drying.

Starch Passes test.

Sulfur Dioxide Not more than 0.002%.

TESTS

Assay

Apparatus Mount a ring support on a ringstand 1 to 2 in. above a gas burner, and mount a second ring 6 to 7 in. above the first. Place a 6-in. open-wire gauze on the lower ring to support a 250-ml Erlenmeyer flask, and place a 4-in. watch glass with a center hole on the upper ring to deflect heat. Attach a 25-ml buret to the ringstand so that the tip just passes through the watch glass centered above the flask. Place an indirectly lighted white surface behind the assembly for observing the endpoint.

Standardized Fehling's Solution Measure a quantity of *Fehling's Solution A*, add an equal quantity of *Fehling's Solution B*, and mix (see *Cupric Tartrate TS, Alkaline*, page 560). Immediately prior to use, standardize as follows: Transfer 3.000 g of primary standard dextrose (NBS), previously dried in vacuum at 100° for 2 h, into a 500-ml volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25 ml of the mixed Fehling's solution into a 200-ml Erlenmeyer flask containing a few glass beads, and titrate with the standard dextrose solution as directed under *Procedure*. Adjust the concentration of *Fehling's Solution A* by dilution or addition of copper sulfate so that the titration requires 20.0 ml of the standard dextrose solution.

Procedure Transfer about 3 g of the sample, accurately weighed, into a 500-ml volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25.0 ml of *Standardized Fehling's Solution* into a 200-ml Erlenmeyer flask containing a few glass beads, and add the sample solution from a buret to within 0.5 ml of the anticipated endpoint (determined by preliminary titration). Immediately place the flask on the wire gauze of the *Apparatus*, and adjust the burner so that the boiling point will be reached in about 2 min. Bring to a boil, and boil gently for 2 min. As boiling continues, add 2 drops of a 1% aqueous solution of methylene blue, and complete the titration within 1 min by adding the sample solution dropwise or in small increments until the blue color disappears. Record the volume of sample solution required as *V*, in ml. Calculate the percentage of reducing sugars, as D-glucose, on the dried basis, by the formula

$$(500 \times 0.1200 \times 100)/(V \times W),$$

in which *W* is the weight of sample taken, in g, corrected for *Loss on Drying*.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 133-mg sample does not exceed that shown in a control containing 20 µg of chloride ion.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry 10 g of anhydrous dextrose, or

5 g of dextrose monohydrate, at 70° in a vacuum oven not exceeding 50 mm of Hg for 2 h, cool in a desiccator for 30 min, and weigh. Dry for successive 1-h intervals until the weight change is less than 2 mg.

Residue on Ignition Ignite 1 g as directed in the general method, page 530.

Specific Rotation, page 530 Determine in a solution containing 10 g of a previously dried sample and 0.2 ml of ammonia TS in sufficient water to make 100 ml.

Starch To 1 g dissolved in 10 ml of water add 1 drop of iodine TS. A yellow color indicates the absence of soluble starch.

Sulfur Dioxide Determine as directed in the general method, page 546, using a 100-g sample.

Packaging and Storage Store in tight containers in a dry place.

Functional Use in Foods Nutritive sweetener; humectant; texturizing agent; formulation and processing aid.

Diacetyl Tartaric Acid Esters of Mono- and Diglycerides

DESCRIPTION

The reaction product of partial glycerides of edible oils, fats, or fat-forming fatty acids with diacetyl tartaric anhydride. The esters range in appearance from sticky, viscous liquids through a fatlike consistency to a waxy solid, depending upon the iodine value of the oils or fats used in their manufacture. The diacetyl tartaroyl esters have a faint acid odor and are miscible in all proportions with oils and fats. They are soluble in most common fat solvents, in methanol, in acetone, and in ethyl acetate, but are insoluble in other alcohols, in acetic acid, and in water. They are dispersible in water and resistant to hydrolysis for moderate periods of time. The pH of a 3% dispersion in water is between 2 and 3.

REQUIREMENTS

Identification

To a solution of 500 mg in 10 ml of methanol add, dropwise, lead acetate TS. A white, flocculent, practically insoluble precipitate forms.

Assay for Tartaric Acid Between 17.0 and 20.0 g of tartaric acid ($C_4H_6O_6$) per 100 g after saponification.

Acetic Acid Between 14.0 and 17.0 g of CH_3COOH per 100 g after saponification.

Acid Value Between 62 and 76.

Arsenic (as As) Not more than 3 ppm.

Fatty Acids, Total Not less than 56.0 g of total fatty acids per 100 g after saponification.

Glycerin Not less than 12.0 g of $C_3H_8O_3$ per 100 g after saponification.

Heavy Metals (as Pb) Not more than 10 ppm.

Residue on Ignition Not more than 0.01%.

Saponification Value Between 380 and 425.

TESTS

Assay for Tartaric Acid

Standard Reference Curve Transfer 100 mg of reagent-grade tartaric acid, accurately weighed, into a 100-ml volumetric flask, dissolve it in about 90 ml of water, add water to volume, and mix well. Transfer 3.0-, 4.0-, 5.0-, and 6.0-ml portions into separate 19- × 150-mm matched cuvettes, and add sufficient water to make 10.0 ml. To each cuvette add 4.0 ml of a freshly prepared 1 in 20 solution of sodium metavanadate and 1.0 ml of acetic acid. (NOTE: Use these solutions within 10 min after color development.) Prepare a blank in the same manner, using 10 ml of water in place of the tartaric acid solutions. Set the instrument at zero with the blank, and then determine the absorbance of the four solutions of tartaric acid at 520 nm with a suitable spectrophotometer or a photoelectric colorimeter equipped with a 520-nm filter. From the data thus obtained, prepare a reference curve by plotting the absorbances on the ordinate against the corresponding quantities, in mg, of the tartaric acid on the abscissa.

Assay Preparation Transfer about 4 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, and add 80 ml of approximately 0.5 *N* potassium hydroxide and 0.5 ml of phenolphthalein TS. Connect an air condenser at least 65 cm in length to the flask, and heat the mixture on a hot plate for about 2.5 h. Add to the hot mixture approximately 10% phosphoric acid until it is definitely acid to congo red test paper. Reconnect the air condenser, and heat until the fatty acids are liquified and clear. Cool and then transfer the mixture into a 250-ml separator with the aid of small portions of water and chloroform. Extract the liberated fatty acids with three successive 25-ml portions of chloroform, and collect the extracts in a second separator. Wash the combined chloroform extracts with two 25-ml portions of water, and add the washings to the separator containing the water layer. Retain the combined chloroform extracts for the determination of *Total Fatty Acids.* Transfer the contents of the first separator to a 250-ml beaker, heat on a steam bath to remove traces of chloroform, filter through acid-washed, fine-texture filter paper into a 500-ml volumetric flask, and finally dilute to volume with water (*Solution I*). Pipet 25.0 ml of this solution into a 100-ml volumetric flask, and dilute to volume with water (*Solution II*). Retain the rest of *Solution I* for the determination of *Glycerin*.

Procedure Transfer 10.0 ml of *Solution II* prepared under *Assay Preparation* into a 19- × 150-mm cuvette, and continue as directed under *Standard Reference Curve*, beginning with " . . . add 4.0 ml of a freshly prepared 1 in 20 solution of sodium metavanadate. . . . " From the reference curve determine the weight, in mg, of tartaric acid in the final dilution, multiply this by 20, and divide the result by the weight of the original sample to obtain the percentage of tartaric acid.

Acetic Acid Determine as directed under *Volatile Acidity,*

page 510, using a 4-g sample, accurately weighed, and 30.03 as the equivalence factor (*e*).

Acid Value Transfer about 1 g, accurately weighed, into a 125-ml Erlenmeyer flask. Prepare a solvent by mixing 1 volume of benzene with 4 volumes of methanol, adding phenol red TS, and neutralizing, if necessary. Dissolve the sample in about 25 ml of this solvent by warming gently, if necessary. Titrate the solution with 0.1 *N* methanolic potassium hydroxide to a light red endpoint. Perform a blank determination on a 25-ml portion of the solvent, and make any necessary correction (see page 2). Calculate the acid value by the formula $56.1V \times N/W$, in which V is the volume, in ml, and N is the normality, respectively, of the methanolic potassium hydroxide, and W is the weight, in g, of the sample taken.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Fatty Acids, Total Dry the combined chloroform extracts of fatty acids obtained in the *Assay for Tartaric Acid* by shaking with a few g of anhydrous sodium sulfate. Filter the solution into a tared 250-ml beaker, evaporate the chloroform on a steam bath, cool, and weigh.

Glycerin Transfer 5.0 ml of *Solution I* prepared in the *Assay for Tartaric Acid* into a 250-ml glass-stoppered Erlenmeyer or iodine flask. Add to the flask 15 ml of glacial acetic acid and 25.0 ml of periodic acid solution, prepared by dissolving 2.7 g of periodic acid (H_5IO_6) in 50 ml of water, adding 950 ml of glacial acetic acid, and mixing thoroughly; protect this solution from light. Shake the mixture for 1 or 2 min, allow it to stand for 15 min, add 15 ml of potassium iodide solution (15 in 100) and 15 ml of water, swirl, let stand 1 min, and then titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a *Residual Blank Titration* (see page 2) using water in place of the sample. The corrected volume is the number of ml of 0.1 *N* sodium thiosulfate required for the glycerin and the tartaric acid in the sample represented by the 5 ml of *Solution I*. From the percentage determined in the *Assay for Tartaric Acid* calculate the volume of 0.1 *N* sodium thiosulfate required for the tartaric acid in the titration. The difference between the corrected volume and the calculated volume required for the tartaric acid is the number of ml of 0.1 *N* sodium thiosulfate consumed due to the glycerin in the sample. One ml of 0.1 *N* sodium thiosulfate is equivalent to 2.303 mg of glycerin and to 7.505 mg of tartaric acid.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Residue on Ignition Ignite 10 g as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509, using about 2 g, accurately weighed. Add 5 to 10 ml of water to samples and blanks before saponification; otherwise sufficient salts precipitate during saponification to cause serious bumping and spattering.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier.

Diatomaceous Earth

Diatomaceous Silica; Diatomite; D.E.

DESCRIPTION

A white to gray or buff-colored powder consisting of processed siliceous skeletons of diatoms. It is insoluble in water, in acids (except hydrofluoric), and in dilute alkalies. The *natural* powder (gray to off-white) is air dried and classified by particle size; the *calcined* powder (pink to buff-colored) is air dried, classified, calcined at a high temperature (1500° to 1800°F), and again classified; and the *flux-calcined* powder (white) is air dried, classified, calcined in the presence of a suitable flux (generally soda ash or other alkaline salt), and classified.

REQUIREMENTS

Identification

When examined with a 100- to 200-power microscope, typical diatom shapes are observed.

Arsenic (as As) Not more than 10 ppm.

Lead Not more than 10 ppm.

Loss on Drying *Natural powders:* not more than 10%; *calcined* and *flux-calcined powders:* not more than 3%.

Loss on Ignition *Natural powders:* not more than 7%, on the dried basis; *calcined* and *flux-calcined powders:* not more than 2%, on the dried basis.

Nonsiliceous Substances Not more than 25%, on the dried basis.

pH Passes test.

TESTS

Arsenic Transfer 10.0 g of the sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat at 70° for 15 min. Cool, and decant through a Whatman No. 3 filter paper into a 100-ml volumetric flask. Wash the slurry with three 10-ml portions of hot water and the filter paper with 15 ml of hot water, dilute to volume with water, and mix. A 3.0-ml portion of this solution meets the requirements of the *Arsenic Test*, page 464.

Lead A 10.0-ml portion of the solution prepared in the *Arsenic Test* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Loss on Ignition Weigh accurately about 1 g, and ignite to constant weight in a suitable tared crucible.

Nonsiliceous Substances Transfer about 200 mg, accurately weighed, into a tared platinum crucible, add 5 ml of hydrofluoric acid and 2 drops of sulfuric acid (1 in 2), and evaporate gently to dryness. Cool, add 5 ml of hydrofluoric acid, evaporate again to dryness, and then ignite to constant weight.

pH, page 531 Boil 10 g with 100 ml of water for 30 min, make up to 100 ml with water, and filter through a fine-porosity

sintered-glass funnel. The pH of the filtrate prepared with *natural* or *calcined* powders is between 5.0 and 10.0, and of that prepared with *flux-calcined* powders is between 8.0 and 11.0.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Filter aid in food processing.

Dilauryl Thiodipropionate

$(C_{12}H_{25}OOCCH_2CH_2)_2S$

$C_{30}H_{58}O_4S$ Mol wt 514.85

DESCRIPTION

White crystalline flakes having a characteristic sweetish, ester-like odor. It is insoluble in water, but is soluble in most organic solvents.

REQUIREMENTS

Identification

Dilauryl thiodipropionate may be identified by its solidification point (see below).

Assay Not less than 99.0% of $C_{30}H_{58}O_4S$.
Acidity (as thiodipropionic acid) Not more than 0.2% of $C_6H_{10}O_4S$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Solidification Point Not below 40°.

TESTS

Assay Transfer about 700 mg, accurately weighed, into a 250-ml Erlenmeyer flask, and add 100 ml of acetic acid and 50 ml of alcohol. Heat the mixture at a temperature of about 40° until the sample is completely dissolved, then add 3 ml of hydrochloric acid and 4 drops of *p*-ethoxychrysoidin TS, and immediately titrate the solution with 0.1 *N* bromine. When the endpoint is approached (pink color), add 4 more drops of the indicator solution and continue the titration, dropwise, to a color change from red to pale yellow. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* bromine is equivalent to 25.74 mg of $C_{30}H_{58}O_4S$. Multiply the percentage of thiodipropionic acid, determined in the *Acidity* test, by 2.89, and subtract this value from the percentage of dilauryl thiodipropionate calculated from the titration. The difference is the percentage purity of $C_{30}H_{58}O_4S$.
Acidity (as thiodipropionic acid) Transfer about 2 g, accurately weighed, into a 250-Erlenmeyer flask. Dissolve the sample in 50 ml of a mixture composed of 1 part of methyl alcohol and 3 parts of benzene, add 5 drops of phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium hydroxide. Each ml of 0.1 *N* alcoholic potassium hydroxide is equivalent to 8.91 mg of $C_6H_{10}O_4S$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Solidification Point Determine as directed in the general procedure, page 538.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Antioxidant.

Dill Seed Oil, European Type

DESCRIPTION

The volatile oil obtained by steam distillation from the crushed, dried fruit (or seeds) of *Anethum graveolens* L. (Fam. *Umbelliferae*). It is a slightly yellowish to light yellow liquid with a carawaylike odor and flavor. It is soluble in most fixed oils and in mineral oil. It is soluble, with slight opalescence, in propylene glycol, but it is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 593, using the same test conditions as specified therein.

Assay Not less than 42% and not more than 60%, by volume, of ketones as carvone.
Angular Rotation Between +70° and +82°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.483 and 1.490 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.890 and 0.915.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 80% alcohol, with slight opalescence that may not disappear on dilution to as much as 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Dill Seed Oil, Indian Type

Dill Seed Oil, Indian; Dill Oil, Indian Type

DESCRIPTION

The volatile oil obtained by steam distillation from the crushed mature fruit of Indian dill, *Anethum sowa* D.C. (Fam *Umbelliferae*). It is a light yellow to light brown liquid with a rather harsh carawaylike odor and flavor. It is soluble in most fixed oils and in mineral oil, occasionally with slight opalescence. It is sparingly soluble in propylene glycol and practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 609, using the same test conditions as specified therein.

Assay Not less than 20% and not more than 30%, by volume, of ketones as carvone.

Angular Rotation Between +40° and +58°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.486 and 1.495 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.925 and 0.980.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 90% alcohol and remains clear on dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or other suitably lined containers protected from light.

Functional Use in Foods Flavoring agent.

Dillweed Oil, American Type

Dill Oil; Dill Herb Oil, American Type

DESCRIPTION

The volatile oil obtained by steam distillation from the freshly cut stalks, leaves, and seeds of the plant *Anethum graveolens* L. It is a light yellow to yellow liquid. It is soluble in most fixed oils and in mineral oil. It is soluble, usually with opalescence or turbidity, in propylene glycol, but it is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 594, using the same test conditions as specified therein.

Assay Usually not less than 28% and not more than 45%, by volume, of ketones as carvone.

NOTE: Oil obtained from early season distillation may show a carvone content as low as 25.0% and a correspondingly lower specific gravity, lower refractive index, and higher angular rotation.

Angular Rotation Between +84° and +95°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.480 and 1.485 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.884 and 0.900.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 90% alcohol, frequently with opalescence that may not disappear on dilution to as much as 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Dimethylpolysiloxane

Dimethyl Silicone

DESCRIPTION

Dimethylpolysiloxane is a mixture of fully methylated linear siloxane polymers containing repeating units of the formula $[(CH_3)_2SiO]$ and stabilized with trimethylsiloxy end-blocking units of the formula $[(CH_3)_3SiO—]$. It occurs as a clear, colorless, viscous liquid that is soluble in most aliphatic and aromatic hydrocarbon solvents but insoluble in water. [NOTE: Dimethylpolysiloxane is frequently used in commerce as such, or as a liquid containing silica (usually 4% to 5%), which must be removed by high-speed centrifugation (about 20,000 rpm) before testing the dimethylpolysiloxane for *Identification, Refractive Index, Specific Gravity,* and *Viscosity.* This monograph does not apply to aqueous emulsions containing emulsifying agents and preservatives, in addition to silica.]

REQUIREMENTS

Identification

Moisten about 100 mg of the sample with a few drops of sulfuric acid and nitric acid in a platinum crucible, ignite at a red heat over a burner for about 10 min or until ashing is complete, and cool. Transfer the residue to a nickel crucible, fuse completely with 1 g of sodium hydroxide, and cool. Dissolve the residue in 50 ml of water, and filter. Place 1 drop of the filtrate on a sheet of filter paper, followed by 1 drop of ammonium molybdate TS and 1 drop of benzidine TS, and place the paper over ammonium hydroxide. A greenish blue spot is produced.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Heating Not more than 18%.

Refractive Index Between 1.400 and 1.404.

Specific Gravity Between 0.964 and 0.973.

Viscosity Between 300 and 600 centistokes.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*). (NOTE: If silica is present, it must be removed by filtration before the pH is adjusted.)

Loss on Heating Heat 15 g of the sample in an open tared aluminum cup, having an internal surface of about 30 cm^2, for 4 h at 200° in a circulating air oven, cool, and weigh.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

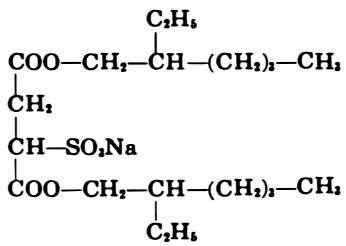
Viscosity Determine as directed in the general method, page 549.

Packaging and Storage Store in tight containers.

Functional Use in Foods Defoaming agent.

Dioctyl Sodium Sulfosuccinate

DSS



$C_{20}H_{37}NaO_7S$ Mol wt 444.56

DESCRIPTION

A white, waxlike, plastic solid having a characteristic odor suggestive of octyl alcohol. It is free from the odor of other solvents. One g dissolves slowly in about 70 ml of water. It is freely soluble in alcohol and in glycerin, and is very soluble in solvent hexane.

REQUIREMENTS

Identification

Dry 50 mg of the sample at 105° for 2 h, cool, and immediately dissolve in 2 ml of carbon tetrachloride. The infrared absorption spectrum of the solution, measured in 0.1-mm cells, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Dioctyl Sodium Sulfosuccinate Reference Standard, previously dried in the same manner.

Assay Not less than 98.5% of $C_{20}H_{37}NaO_7S$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Bis(2-ethylhexyl)maleate Not more than 0.4%.
Clarity of Solution Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 2%.
Residue on Ignition Between 15.5% and 16.2%.

TESTS

Assay

Sample Solution Transfer about 3.8 g of the sample, accurately weighed, into a 500-ml volumetric flask, dissolve in chloroform, dilute to volume with the same solvent, and mix.

Tetra-n-butylammonium Iodide Solution Transfer 1.250 g of tetra-*n*-butylammonium iodide to a 500-ml volumetric flask, dilute to volume with water, and mix.

Salt Solution Dissolve 100 g of anhydrous sodium sulfate and 10 g of sodium carbonate in sufficient water to make 1000.0 ml.

Procedure Pipet 10.0 ml of the *Sample Solution* into a 250-ml flask, and add 40 ml of chloroform, 50 ml of *Salt Solution*, and 10 drops of bromophenol blue TS. Titrate with *Tetra-n-butylammonium Iodide Solution* to the first appearance of a blue color in the chloroform layer after vigorous shaking. Calculate the percentage of $C_{20}H_{37}NaO_7S$ by the formula

$$(V \times 1.250 \times 444.6 \times 10)/(W \times 369.4),$$

in which V is the volume, in ml, of *Tetra-n-butylammonium Iodide Solution* required; 444.6 is the molecular weight of dioctyl sodium sulfosuccinate; W is the weight, in g, of the sample taken; and 369.4 is the molecular weight of tetra-*n*-butylammonium iodide.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Bis(2-ethylhexyl)maleate

Supporting Electrolyte Dissolve 21.2 g of anhydrous lithium perchlorate ($LiClO_4$) in 175 ml of water in a 250-ml beaker. Adjust the pH of this solution to 3.0 by the dropwise addition of glacial acetic acid (usually 1 or 2 drops is sufficient), using a suitable pH meter. Quantitatively transfer the solution into a 200-ml volumetric flask, dilute to volume with water, and mix.

Standard Solution Transfer 100 to 110 mg of bis(2-ethylhexyl)maleate,* accurately weighed, into a 100-ml volumetric flask. Record the exact weight, to the nearest 0.1 mg, as W_A. Add 60 to 70 ml of isopropyl alcohol, swirl to dissolve, then dilute to volume with water, and mix.

Sample Stock Solution Transfer 12.5 g of the sample, accurately weighed, into a 150-ml beaker. Record the exact weight, to the nearest 10 mg, as W_S. Add 80 to 90 ml of isopropyl alcohol, and stir with a glass stirring rod until the sample is dissolved. Quantitatively transfer this solution, with the aid of isopropyl alcohol, into a 250-ml volumetric flask, then dilute to volume with isopropyl alcohol, and mix.

Test Solution A Pipet 50.0 ml of the *Sample Stock Solution* and 20.0 ml of the *Supporting Electrolyte* into a 100-ml volumetric flask. Dilute to within 15 mm of the graduated volume line with isopropyl alcohol, stopper, shake to facilitate solution, and set aside for 2 min. Dilute to volume with isopropyl alcohol, and mix. A completely clear solution should be obtained.

Test Solution B Pipet 50.0 ml of the *Sample Stock Solution*, 10.0 ml of the *Standard Solution*, and 20.0 ml of the *Supporting Electrolyte* into a 100-ml volumetric flask, and complete the preparation as described for *Test Solution A*.

Blank Pipet 20.0 ml of the *Supporting Electrolyte* into a 100-ml volumetric flask, dilute to volume with isopropyl alcohol, and mix.

Procedure Rinse a polarographic H-cell several times with small portions of *Test Solution A*, then fill the cell half full with the solution, place a paper tissue in the top of the sample side of the cell, and pass a moderate stream of nitrogen through the solution for 15 min. (NOTE: The nitrogen should first be saturated by passing it through a suitable scrubber containing isopropyl alcohol.) After 15 min, divert the nitrogen stream over the surface of the solution, and remove the tissue from the cell.

Set the polarizing voltage of a suitable, previously calibrated polarograph (Metrohm Polarocord E-261 or equivalent) at −1.3 V, adjust the current sensitivity to the lowest range (most sensitive) at which the current oscillations will remain on scale, and record the polarogram, scanning a voltage range of −0.9 V to −1.5 V at this sensitivity and using a saturated calomel electrode as the reference electrode. Record the average oscillations, in mm, at −1.3 V as *A*, and those at −1.0 V as *B*. (NOTE: If a manual polarograph is used, record the average oscillations of the solutions at −1.3 V and −1.0 V, respectively.)

Repeat the entire procedure using *Test Solution B*, recording the average oscillations at −1.3 V as *D*, and those at −1.0 V as *E*. Similarly, repeat the entire procedure using the *Blank*, recording the average oscillations at −1.3 V as *G*, and those at −1.0 V as *H*.

Calculation Make the following preliminary calculations (in mA) to obtain *C* (net diffusion current of *Test Solution A*); *F* (net diffusion current of *Test Solution B*); *I* (net current introduced by the *Blank*); *J* (diffusion current due to added

*A suitable grade of bis(2-ethylhexyl)maleate is available as OT-35 from American Cyanamid Company, Fine Chemicals Department, Pearl River, N.Y. 10965.

maleate); and K (diffusion current due to originally present maleate):

$$C = (A - B) \times S_1;$$
$$F = (D - E) \times S_2;$$
$$I = (G - H) \times S_3;$$
$$J = F - C;$$
$$K = C - I;$$

in which S_1, S_2, and S_3 represent the current sensitivities used for *Test Solution A*, *Test Solution B*, and the *Blank*, respectively.

Finally, calculate the percentage of bis(2-ethylhexyl)maleate in the original sample taken by the formula

$$(K \times 50W_A)/(J \times W_S).$$

Clarity of Solution Dissolve 25 g in 94 ml of alcohol. The solution does not develop a haze within 24 h.

Heavy Metals Ignite 2 g in a platinum crucible until free from carbon, cool, moisten the residue with 1 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Add 2 ml of diluted acetic acid TS, digest on a steam bath for 5 min, filter into a 50-ml Nessler tube, and wash the residue with sufficient water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 2 h.

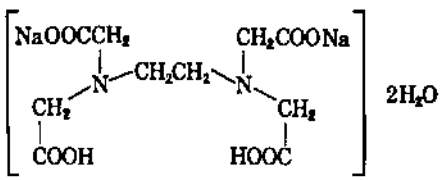
Residue on Ignition Ignite 1 g as directed in the general procedure, page 533.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; wetting agent.

Disodium EDTA

Disodium Ethylenediaminetetraacetate; Disodium (Ethylenedinitrilo)tetraacetate; Disodium Edetate



$C_{10}H_{14}N_2Na_2O_8.2H_2O$ Mol wt 372.24

DESCRIPTION

A white, crystalline powder. It is soluble in water.

REQUIREMENTS

Identification

A. A 1 in 20 solution responds to the flame test for *Sodium*, page 517.

B. To 5 ml of water in a test tube add 2 drops of ammonium thiocyanate TS and 2 drops of ferric chloride TS. To the deep red solution so obtained add about 50 mg of the sample, and mix. The deep red color disappears.

Assay Not less than 99.0% of $C_{10}H_{14}N_2Na_2O_8.2H_2O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Nitrilotriacetic Acid Passes test.

pH of a 1 in 100 Solution Between 4.3 and 4.7.

TESTS

Assay

Assay Preparation Transfer about 5 g of the sample, accurately weighed, into a 250-ml volumetric flask, dissolve in water, dilute to volume, and mix.

Procedure Place about 200 mg of chelometric standard calcium carbonate, accurately weighed, in a 400-ml beaker, add 10 ml of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 ml of diluted hydrochloric acid TS from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 ml with water. While stirring, preferably with a magnetic stirrer, add about 30 ml of the *Assay Preparation* from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Calculate the weight, in mg, of $C_{10}H_{14}N_2Na_2O_8.2H_2O$ in the sample taken by the formula $929.8(W/V)$, in which W is the weight, in mg, of calcium carbonate, and V is the volume, in ml, of the *Assay Preparation* consumed in the titration.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Nitrilotriacetic Acid

Stock Test Solution Transfer 10.0 g of the sample into a 100-ml volumetric flask, dissolve in 40 ml of potassium hydroxide solution (1 in 10), dilute to volume with water, and mix.

Diluted Stock Test Solution Pipet 10.0 ml of the *Stock Test Solution* into a 100-ml volumetric flask, dilute to volume with water, and mix.

Test Preparation Pipet 20.0 ml of the *Diluted Stock Test Solution* into a 150-ml beaker, add 1 ml of potassium hydroxide solution (1 in 10), 2 ml of ammonium nitrate solution (1 in 10), and about 50 mg of eriochrome black T indicator, and titrate with cadmium nitrate solution (3 in 100) to a red endpoint. Record the volume, in ml, of the titrant required as V, and discard the solution.

Pipet 20.0 ml of the *Diluted Stock Test Solution* into a 100-

ml volumetric flask, and add the volume, *V*, of cadmium nitrate solution (3 in 100) required in the initial titration, plus 0.05 ml in excess. Add 1.5 ml of potassium hydroxide solution (1 in 10), 10 ml of ammonium nitrate solution (1 in 10), and 0.5 ml of methyl red TS, then dilute to volume with water, and mix.

Stock Standard Solution Transfer 1.0 g of nitrilotriacetic acid into a 100-ml volumetric flask, dissolve in 10 ml of potassium hydroxide solution (1 in 10), dilute to volume with water, and mix.

Diluted Stock Standard Solution Pipet 1.0 ml of the *Stock Standard Solution* and 10.0 ml of the *Stock Test Solution* into a 100-ml volumetric flask, dilute to volume with water, and mix.

Standard Preparation Proceed as directed under *Test Preparation*, using *Diluted Stock Standard Solution* where *Diluted Stock Test Solution* is specified.

Procedure Rinse a polarographic cell with a portion of the *Standard Preparation*, then add a suitable volume to the cell, immerse it in a constant-temperature bath maintained at 25° ± 0.5°, and de-aerate by bubbling oxygen-free nitrogen through the solution for 10 min. Insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from −0.6 V to −1.2 V at a sensitivity of 0.006 mA per mm, using a saturated calomel electrode as the reference electrode. In the same manner, polarograph a portion of the *Test Preparation*. The diffusion current observed with the *Test Preparation* is not greater than 10% of the difference beween the diffusion currents observed with the *Standard Preparation* and the *Test Preparation*, respectively. (NOTE: An extra polarographic wave appearing ahead of the nitrilotriacetic acid–cadmium complex wave is probably due to uncomplexed cadmium. This wave should be ignored in measuring the diffusion current.)

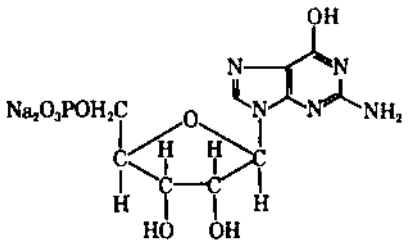
pH of a 1 in 100 Solution Determine by the *Potentiometric Method*, page 531.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Preservative; sequestrant; stabilizer.

Disodium Guanylate

Sodium 5′-Guanylate; Disodium Guanosine-5′-monophosphate



$C_{10}H_{12}N_5Na_2O_8P \cdot xH_2O$ Mol wt (anhydrous) 407.19

DESCRIPTION

Disodium guanylate contains approximately seven molecules of water of crystallization. It occurs as colorless or white crystals, or as a white, crystalline powder, having a characteristic taste. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.

REQUIREMENTS

Identification

A 1 in 50,000 solution of the sample in 0.01 *N* hydrochloric acid exhibits an absorbance maximum at 256 ± 2 nm, page 539.

Assay Not less than 97.0% and not more than the equivalent of 102.0% of $C_{10}H_{12}N_5Na_2O_8P$, calculated on the dried basis.

Amino Acids Passes test.

Ammonium Salts Passes test.

Arsenic (as As) Not more than 3 ppm.

Clarity and Color of Solution Passes test.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 25%.

Other Nucleotides Passes test.

pH of a 1 in 20 Solution Between 7.0 and 8.5.

TESTS

Assay Transfer about 500 mg of the sample, accurately weighed, into a 1000-ml volumetric flask, dissolve in 0.01 *N* hydrochloric acid, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Transfer 10.0 ml of this solution into a 250-ml volumetric flask, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Determine the absorbance of this solution and of a similar solution of FCC Disodium Guanylate Reference Standard, at a concentration of 20 μg per ml, in 1-cm cells, at the maximum at about 260 nm, with a suitable spectrophotometer, using 0.01 *N* hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{10}H_{12}N_5Na_2O_8P$ in the sample taken by the formula $25C \times A_U/A_S$, in which *C* is the exact concentration of the Reference Standard solution, in μg

per ml, A_U is the absorbance of the sample solution, and A_S is the absorbance of the Reference Standard solution.

Amino Acids To 5 ml of a 1 in 1000 solution of the sample add 1 ml of ninhydrin TS, and heat for 3 min. No color is produced.

Ammonium Salts Transfer about 100 mg of the sample into a small test tube, and add 50 mg of magnesium oxide and 1 ml of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min. The litmus paper does not change to blue.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Clarity and Color of Solution A 100-mg portion of the sample dissolved in 10 ml of water is colorless and shows no more than a trace of turbidity.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Other Nucleotides Prepare a strip of Whatman No. 2 or equivalent filter paper about 20 × 40 cm, and draw a line across the narrow dimension about 5 cm from one end. Using a micropipet, apply on the center of the line 10 µl of a 1 in 100 solution of the sample in water, and dry the paper in air. Fill the trough of an apparatus suitable for descending chromatography (see page 473) with a 160:3:40 mixture of saturated ammonium sulfate solution, *tert*-butyl alcohol, and 0.025 *N* ammonia, respectively, and suspend the strip in the chamber, placing the end of the strip in the trough at a distance about 1 cm from the pencil line. Seal the chamber, and allow the chromatogram to develop until the solvent front descends to a distance about 30 cm from the starting line. Remove the strip from the chamber, dry in air, and observe under shortwave (254 nm) ultraviolet light in the dark. Only one spot is visible.

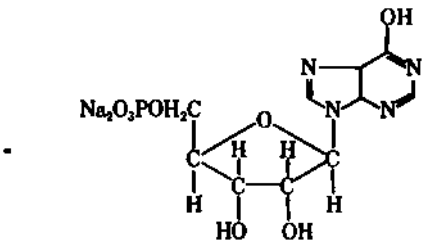
pH of a 1 in 20 Solution Determine by the *Potentiometric Method*, page 531.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Flavor enhancer.

Disodium Inosinate

Sodium 5′-Inosinate; Disodium Inosine-5′-monophosphate



$C_{10}H_{11}N_4Na_2O_8P.xH_2O$ Mol wt (anhydrous) 392.17

DESCRIPTION

Disodium inosinate contains approximately 7.5 molecules of water of crystallization. It occurs as colorless or white crystals, or as a white, crystalline powder, having a characteristic taste. It is soluble in water, sparingly soluble in alcohol, and practically insoluble in ether.

REQUIREMENTS

Identification

A 1 in 50,000 solution of the sample in 0.01 *N* hydrochloric acid exhibits an absorbance maximum at 250 ± 2 nm, page 539. The ratio A_{250}/A_{260} is between 1.55 and 1.65, and the ratio A_{280}/A_{260} is between 0.20 and 0.30.

Assay Not less than 97.0% and not more than the equivalent of 102.0% of $C_{10}H_{11}N_4Na_2O_8P$, calculated on the anhydrous basis.

Amino Acids Passes test.

Ammonium Salts Passes test.

Arsenic (as As) Not more than 3 ppm.

Barium Not more than 0.015%.

Clarity and Color of Solution Passes test.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Other Nucleotides Passes test.

pH of a 1 in 20 Solution Between 7.0 and 8.5.

Water Not more than 28.5%.

TESTS

Assay Transfer about 500 mg of the sample, accurately weighed, into a 1000-ml volumetric flask, dissolve in 0.01 *N* hydrochloric acid, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Transfer 10.0 ml of this solution into a 250-ml volumetric flask, dilute to volume with 0.01 *N* hydrochloric acid, and mix. Determine the absorbance of this solution and of a similar solution of FCC Disodium Inosinate Reference Standard, at a concentration of 20 µg per ml, in 1-cm cells, at the maximum at about 250 nm, with a suitable spectrophotometer, using 0.01 *N* hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_{10}H_{11}N_4Na_2O_8P$ in the

sample taken by the formula $25C \times A_U/A_S$, in which C is the exact concentration of the Reference Standard solution, in µg per ml, A_U is the absorbance of the sample solution, and A_S is the absorbance of the Reference Standard solution.

Amino Acids To 5 ml of a 1 in 1000 solution of the sample add 1 ml of ninhydrin TS. No color is produced.

Ammonium Salts Transfer about 100 mg of the sample into a small test tube, and add 50 mg of magnesium oxide and 1 ml of water. Moisten a piece of red litmus paper with water, suspend it in the tube, cover the mouth of the tube, and heat in a water bath for 5 min. The litmus paper does not change to blue.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Barium Dissolve 1 g of the sample in 100 ml of water, filter, and add 5 ml of diluted sulfuric acid TS to the filtrate. Any turbidity is not greater than that produced in a similar solution containing 1.5 ml of *Barium Standard Solution* (150 µg Ba).

Clarity and Color of Solution A 500-mg portion of the sample dissolved in 10 ml of water is colorless and shows no more than a trace of turbidity.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Other Nucleotides Prepare a strip of Whatman No. 2 or equivalent filter paper about 20 × 40 cm, and draw a line across the narrow dimension about 5 cm from one end. Using a micropipet, apply on the center of the line 10 µl of a 1 in 100 solution of the sample in water, and dry the paper in air. Fill the trough of an apparatus suitable for descending chromatography (see page 473) with a 160:3:40 mixture of saturated ammonium sulfate solution, *tert*-butyl alcohol, and 0.025 *N* ammonia, respectively, and suspend the strip in the chamber, placing the end of the strip in the trough at a distance about 1 cm from the pencil line. Seal the chamber, and allow the chromatogram to develop until the solvent front descends to a distance about 30 cm from the starting line. Remove the strip from the chamber, dry in air, and observe under shortwave (254 nm) ultraviolet light in the dark. Only one spot is visible.

pH of a 1 in 20 Solution Determine by the *Potentiometric Method*, page 531.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Flavor enhancer.

Enzyme Preparations

DESCRIPTION

Enzyme preparations used in food processing are derived from animal, plant, or microbial sources (see *Classification* below). They may consist of whole cells, parts of cells, or cell-free extracts of the source used, and they may contain one or more active components as well as diluents, preservatives, antioxidants, and other substances consistent with good manufacturing practice.

The individual preparations are usually named according to the substance to which they are applied, such as *Protease* or *Amylase*; such traditional names as *Malt*, *Pepsin*, and *Rennet* are also used, however.

The color of the preparations—which may be liquid, semiliquid, or dry—may vary from virtually colorless to dark brown. The active components consist of the biologically active proteins, which are sometimes conjugated with metals, carbohydrates, and/or lipids. Known molecular weights of the active components range from approximately 12,000 to several hundred thousand.

The activity of enzyme preparations is measured according to the reaction catalyzed by individual enzymes (see below) and is usually expressed in activity units per unit weight of the preparation. In commercial practice (but not for *Food Chemicals Codex* purposes), the activity of the product is sometimes also given as the quantity of the preparation to be added to a given quantity of food in order to achieve the desired effect.

Additional information relating to the nomenclature and the sources from which the active components are derived is provided in the *General Tests* section under *Enzyme Assays*, page 479.

CLASSIFICATION

Animal-Derived Preparations

Catalase (bovine liver) Partially purified liquid or powdered extracts from bovine liver. Major active principle: *catalase*. Typical application: manufacture of certain cheeses.

Lipase, Animal Obtained from two primary sources: (1) edible forestomach tissue of calves, kids, or lambs, and (2) animal pancreatic tissue. Produced as purified edible tissue preparations or as aqueous extracts. Dispersible in water; insoluble in alcohol. Major active principle: *lipase*. Typical applications: manufacture of cheese; modification of lipids.

Pepsin Obtained from the glandular layer of hog stomach. White to light tan water-soluble powders, amber pastes, or clear amber to brown aqueous liquids. Major active principle: *pepsin*. Typical applications: preparation of fish meal and other protein hydrolysates; clotting of milk in manufacture of cheese (in combination with rennet).

Rennet Aqueous extracts made from the fourth stomach of calves, kids, or lambs. Clear amber to dark brown liquid preparations, or white to tan powders. Major active principle: *protease* (rennin). Typical application: manufacture of cheese.

Rennet, Bovine Aqueous extracts made from the fourth stomach of bovine animals, sheep, and goats. Clear amber to dark brown liquids, or white to tan powders. Major active principle: *protease* (rennin). Typical application: manufacture of cheese.

Trypsin Obtained from purified extracts of porcine or bovine pancreas. White to tan amorphous powders, which are soluble in water but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *trypsin*. Typical applications: baking; meat tenderizing; production of protein hydrolysates.

Plant-Derived Preparations

Bromelain The purified proteolytic substance derived from the pineapples *Ananas comosus* and *Ananas bracteatus* L. White to light tan amorphous powder. Soluble in water (the solution being colorless to light yellow and somewhat opalescent) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *bromelain*. Typical applications: chillproofing of beer; meat tenderizing; preparation of precooked cereals; production of protein hydrolysates.

Ficin The purified proteolytic substance derived from the latex of *Ficus* sp., which include a variety of tropical fig trees. White to off-white powders, which are completely soluble in water. (Liquid fig latex concentrates are light brown to dark brown in color.) Major active principle: *ficin*. Typical applications: chillproofing of beer; meat tenderizing; dough conditioner in baking.

Malt The product of the controlled germination of barley. Clear amber to dark brown liquid preparations, or white to tan powders. Major active principles: (1) *α-amylase* and (2) *β-amylase. Typical applications: baking; manufacture of alcoholic beverages; manufacture of syrups.*

Papain The purified proteolytic substance derived from the fruit of the papaya *Carica papaya* L. (Fam. Caricaceae). Produced as white to light tan amorphous powders, or as liquids. Soluble in water (the solution being colorless or light yellow and somewhat opalescent) but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *papain* and (2) *chymopapain*. Typical applications: chillproofing of beer; meat tenderizing; preparation of precooked cereals; production of protein hydrolysates.

Microbially Derived Preparations

Carbohydrase (*Aspergillus niger* var.) Produced by the controlled fermentation of *Aspergillus niger* var. as off-white to tan amorphous powders, or as tan to dark brown liquids. Practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase*; (2) *pectinase* (usually a mixture of pectin methylesterase, polygalacturonase, and pectate lyase); (3) *cellulase*; (4) *glucoamylase* (amyloglucosidase); (5) *hemicellulase*; (6) *lactase*; and (7) *β-glucanase*. Typical applications: preparation of starch syrups, alcohol, beer, ale, fruit juices, chocolate syrup, bakery products, liquid coffee, wine, dextrose, and dairy products.

Carbohydrase (*Aspergillus oryzae* var.) Produced by the controlled fermentation of *Aspergillus oryzae* var. as off-white to tan amorphous powders, or as liquids. Soluble in water (the solutions being light yellow to dark brown in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase*, (2) *glucoamylase* (amyloglucosidase), and (3) *lactase*. Typical applications: preparation of starch syrups, alcohol, beer, ale, bakery products, and dairy products.

Carbohydrase (*Rhizopus oryzae* var.) A group of enzyme preparations produced by the controlled fermentation of *Rhizopus oryzae* var. as powders or liquids. Major active principles: (1) *α-amylase*; (2) *pectinase*; and (3) *glucoamylase* (amyloglucosidase). Typical applications: preparation of starch syrups and fruit juices; manufacture of cheese.

Carbohydrase (*Saccharomyces* species) The purified enzyme produced by the controlled fermentation of a number of species of *Saccharomyces* traditionally used in the manufacture of food. White to tan amorphous powders. Soluble in water (the solutions usually being light yellow in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *invertase* and (2) *lactase*. Typical applications: manufacture of candy and ice cream; modifications of dairy products.

Carbohydrase (*Trichoderma reesei* var.) Produced by the controlled fermentation of *Trichoderma reesei* var. as off-white to tan amorphous powders or liquids. Soluble in water (the solutions usually being tan to brown in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *cellulase*. Typical applications: preparation of fruit juices, wine, vegetable oils, and beer.

Carbohydrase and Protease, Mixed (*Bacillus licheniformis*) Produced by the controlled fermentation of *Bacillus licheniformis* var. as off-white to brown amorphous powders or as liquids. Soluble in water (the solution usually being light yellow to dark brown in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase* and (2) *protease*. Typical applications: preparation of starch syrups, alcohol, beer, dextrose, fish meal, protein hydrolysates.

Carbohydrase and Protease, Mixed (*Bacillus subtilis*) Produced by the controlled fermentation of *Bacillus subtilis* var. as off-white to tan amorphous powders, or as liquids. Soluble in water (the solutions usually being light yellow to dark brown in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *α-amylase* and *β-glucanase*, and (2) *protease*. Typical applications: preparation of starch syrups, alcohol, beer, dextrose, bakery products, fish meal; meat tenderizing; preparation of protein hydrolysates.

Catalase (*Aspergillus niger* var.) Produced by the controlled fermentation of *Aspergillus niger* var. as off-white to tan amorphous powders, or as liquids. Soluble in water (the solutions usually being tan to brown in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *catalase*. Typical applications: manufacture of cheese and egg products.

Catalase (*Micrococcus lysodeikticus*) Partially purified liquid or powdered extracts from submerged fermentations of

Micrococcus lysodeikticus. Major active principle: *catalase*. Typical application: manufacture of cheese.

Glucose Isomerase (*Actinoplanes missouriensis*, *Bacillus coagulans*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, or *Streptomyces rubiginosus*, var.) Produced by the controlled fermentation of any of the above organisms as off-white to tan or brown or pink amorphous powders, granules, or liquids. They are partially soluble in water, and are insoluble in alcohol, in chloroform, and in ether. Major active principle: *glucose* (or *xylose*) *isomerase*. Typical applications: manufacture of high-fructose corn syrup and other fructose starch syrups.

Glucose Oxidase (*Aspergillus niger* var.) Produced by the controlled fermentation of *Aspergillus niger* var. as yellow to brown solutions or as yellow to tan or off-white powders. Practically insoluble in alcohol, in chloroform, and in ether. Major active principles: (1) *glucose oxidase* and (2) *catalase*. Typical applications: removal of sugar from liquid eggs; deoxygenation of citrus beverages.

Lipase (*Aspergillus niger* var.) Produced by the controlled fermentation of *Aspergillus niger* var. as off-white to tan amorphous powders. Soluble in water (the solutions usually being light yellow in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical applications: hydrolysis of lipids (e.g., fish oil concentrates).

Lipase (*Aspergillus oryzae* var.) Produced by the controlled fermentation of *Aspergillus oryzae* var. as off-white to tan amorphous powders, or as liquids. Soluble in water (the solutions usually being light yellow in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *lipase*. Typical application: hydrolysis of lipids (e.g., fish oil concentrates).

Protease (*Aspergillus niger* var.) Produced by the controlled fermentation of species of *Aspergillus niger* var. The purified enzyme occurs as off-white to tan amorphous powders. Soluble in water (the solution usually being light yellow in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: production of protein hydrolysates.

Protease (*Aspergillus oryzae* var.) Produced by the controlled fermentation of species of *Aspergillus oryzae* var. The purified enzyme occurs as off-white to tan amorphous powders. Soluble in water (the solutions usually being light yellow in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical applications: chillproofing of beer; bakery products; meat tenderizing; production of protein hydrolysates.

Rennet, Microbial (*Endothia parasitica*) Produced by the controlled fermentation of nonpathogenic species of *Endothia parasitica* as an off-white to tan amorphous powder, or as a liquid. The powders are soluble in water (the solutions usually being tan to dark brown in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: manufacture of cheese.

Rennet, Microbial (*Mucor* species) Produced by the controlled fermentation of *Mucor miehei* or *M. pusillus* as white to tan amorphous powders. The powders are soluble in water (the solutions usually being light yellow in color) but practically insoluble in alcohol, in chloroform, and in ether. Major active principle: *protease*. Typical application: manufacture of cheese.

REACTIONS CATALYZED

NOTE: The reactions catalyzed by any given active component are essentially the same, regardless of the source from which that component is derived.

α-Amylase Hydrolysis of α-1,4-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding dextrins and oligo- and monosaccharides.

β-Amylase Hydrolysis of α-1,4-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding *beta* limit dextrins.

Bromelain Hydrolysis of polypeptides, amides, and esters (especially at bonds involving basic amino acids, or leucine or glycine), yielding peptides of lower molecular weight.

Catalase $2H_2O_2 \rightarrow O_2 + 2H_2O$.

Cellulase Hydrolysis of β-1,4-glucan bonds in such polysaccharides as cellulose, yielding β-dextrins.

Ficin Hydrolysis of polypeptides, amides, and esters (especially at bonds involving basic amino acids, or leucine or glycine), yielding peptides of lower molecular weight.

β-Glucanase Hydrolysis of β1,3- and β1,4-linkages in β-D-glucans, yielding oligosaccharides and glucose.

Glucoamylase (*Amyloglucosidase*) Hydrolysis of α-1,4- and α-1,6-glucan bonds in polysaccharides (starch, glycogen, etc.), yielding glucose (dextrose).

Glucose Isomerase Isomerization of glucose to fructose, and xylose to xylulose.

Glucose Oxidase β-D-glucose + $O_2 \rightarrow$ D-glucono-δ-lactone + H_2O_2.

Hemicellulase Hydrolysis of β-1,4-glucan bonds in such polysaccharides as locust (carob) bean and guar gums, yielding β-dextrins.

Invertase Hydrolysis of sucrose to a mixture of glucose and fructose (invert sugar).

Lactase Hydrolysis of lactose to a mixture of glucose and galactose.

Lipase Hydrolysis of triglycerides of simple fatty acid esters, yielding mono- and diglycerides, glycerol, and free fatty acids.

Pectinase

Pectin Methylesterase Demethylation of pectin.

Polygalacturonase Hydrolysis of α-1,4-galacturonide bonds in pectin.

Pepsin Hydrolysis of polypeptides, including those with bonds adjacent to aromatic or decarboxylic L-amino acid residues, yielding peptides of lower molecular weight.

Protease (general) Hydrolysis of polypeptides, yielding peptides of lower molecular weight.

Rennin Hydrolysis of polypeptides; specificity may be similar to pepsin.

Trypsin Hydrolysis of polypeptides, amides, and esters at bonds involving the carboxyl groups of L-arginine and L-lysine, yielding peptides of lower molecular weight.

GENERAL REQUIREMENTS

Enzyme preparations are produced in accordance with good manufacturing practices. Regardless of the source from which they are derived, they cause no increase in the total microbial count in the treated food over the level accepted for the respective food.

Animal tissues used for the production of enzymes must comply with the applicable federal meat inspection requirements and must be handled in accordance with good hygienic practices.

Plant material used in the production of enzymes, or culture media used for the growth of microorganisms, consists of components that leave no residues harmful to health in the finished food under normal conditions of use.

Preparations derived from microbial sources are produced by methods and under culture conditions that ensure a controlled fermentation, thus preventing the introduction of microorganisms that could be the source of toxic materials and other undesirable substances.

The carriers, diluents, and processing aids used in the production of the enzyme preparations shall be substances that are acceptable for general use in foods, including water and substances that are insoluble in foods but removed from the foods after processing.

Although tolerances have not been established for mycotoxins, appropriate measures should be taken to ensure that the products do not contain such contaminants.

ADDITIONAL REQUIREMENTS

Assay Not less than 85% and not more than 115% of the declared activity.
Arsenic (as As) Not more than 3 ppm.
Coliforms Not more than 30 per g.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Salmonella sp. Negative by test.

TESTS

Assay The following procedures, which are included in the *General Tests* section under *Enzyme Assays*, page 479, are provided for application as necessary in determining compliance with the declared representations for enzyme activity:* Alpha-Amylase Activity (Nonbacterial); Bacterial Alpha-Amylase Activity (BAU); Catalase Activity; Cellulase Activity; Diastase Activity (Diastatic Power, DP); β-Glucanase Activity; Glucoamylase Activity (Amyloglucosidase Activity); Glucose Isomerase Activity; Glucose Oxidase Activity; Hemicellulase Activity; Invertase Activity; Lactase (β-Galactosidase) Activity; Lipase Activity; Lipase/Esterase (Forestomach) Activity; Milk-Clotting Activity; Pepsin Activity; Plant Proteolytic Activity; Proteolytic Activity, Bacterial (PC); Proteolytic Activity, Fungal (HUT); Proteolytic Activity, Fungal (SAP); and Trypsin Activity.

*Because of the varied conditions under which pectinases are employed, and because laboratory hydrolysis of a purified pectin substrate does not correlate with results observed with the natural substrates under use conditions, it is recommended that pectinase suppliers and users develop their own assay procedures that would relate to the specific application under consideration.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Coliforms Determine as directed in Section 46.039, *Official Methods of Analysis of the AOAC*, Thirteenth Edition, 1980, page 825.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

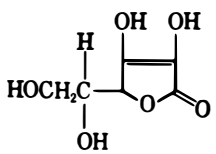
Salmonella sp. Determine as directed in Chapter VI, Procedure 7, *Bacteriological Analytical Manual*, Fifth Edition, Food and Drug Administration, 1978.

Packaging and Storage Store in tight containers in a cool, dry place.

Functional Use in Foods Enzyme (see discussion under *Classification* above).

Erythorbic Acid

D-Araboascorbic Acid



$C_6H_8O_6$ Mol wt 176.13

DESCRIPTION

White or slightly yellow crystals or powder. On exposure to light it gradually darkens. In the dry state it is reasonably stable in air, but in solution it rapidly deteriorates in the presence of air. It melts between 164° and 171° with decomposition. One g is soluble in about 2.5 ml of water and in about 20 ml of alcohol. It is slightly soluble in glycerin.

REQUIREMENTS

Identification

A. A 1 in 50 solution slowly reduces alkaline cupric tartrate TS at 25°, but more readily upon heating.
B. To 2 ml of a 1 in 50 solution add a few drops of sodium nitroferricyanide TS, followed by 1 ml of approximately 0.1 *N* sodium hydroxide. A transient blue color is produced immediately.
C. Dissolve about 15 mg in 15 ml of a trichloroacetic acid solution (1 in 20), add about 200 mg of activated charcoal,

and shake the mixture vigorously for 1 min. Filter through a small fluted filter, refiltering if necessary to obtain a clear filtrate. To 5 ml of the clear filtrate add 1 drop of pyrrole, agitate the mixture until the pyrrole is dissolved, then heat in a water bath at 50°. A blue color develops.

Assay Not less than 99.0% of $C_6H_8O_6$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Residue on Ignition Not more than 0.3%.
Specific Rotation $[\alpha]_D^{25}$: Between $-16.5°$ and $-18.0°$.

TESTS

Assay Dissolve about 400 mg, accurately weighed, in a mixture of 100 ml of water, recently boiled and cooled, and 25 ml of diluted sulfuric acid TS. Titrate the solution immediately with 0.1 *N* iodine, adding starch TS near the endpoint. Each ml of 0.1 *N* iodine is equivalent to 8.806 mg of $C_6H_8O_6$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation Transfer about 2.5 g, accurately weighed, into a 25-ml volumetric flask, dissolve it in about 20 ml of water, and dilute to volume. Determine the specific rotation as directed under *Optical Rotation*, page 530.

Packaging and Storage Store in tight, light-resistant containers.
Functional Use in Foods Preservative; antioxidant.

Ethoxylated Mono- and Diglycerides

Polyoxyethylene (20) Mono- and Diglycerides of Fatty Acids; Polyglycerate (60)

DESCRIPTION

A mixture of stearate, palmitate, and lesser amounts of myristate partial esters of glycerin condensed with approximately 20 moles of ethylene oxide per mole of alpha-monoglyceride reaction mixture, having an average molecular weight of 535 (± 10%). It occurs as a pale, slightly yellow colored, oily liquid or semigel having a faint, characteristic odor and a mildly bitter taste. It is soluble in water, in alcohol, and in xylene. It is partially soluble in mineral oil and in vegetable oils. It conforms to the regulations of the Federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources. [NOTE: If the product is manufactured by direct esterification of glycerin with a mixture of primary stearic, palmitic, and myristic acids, the intermediate product (before reaction with ethylene oxide) has an acid value of not greater than 0.3 and a water content not greater than 0.2%.]

IDENTIFICATION

A. To 5 ml of a 1 in 20 solution in water add 5 ml of sodium hydroxide TS, boil for a few min, cool, and acidify with hydrochloric acid TS. The solution is strongly opalescent.
B. A mixture of 46 volumes of the sample with 54 volumes of water at 40° or below yields a gelatinous mass.

Acid Value Not more than 2.
Arsenic (as As) Not more than 3 ppm.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Hydroxyl Value Between 65 and 80.
Oxyethylene Content (apparent) Not less than 60.5% and not more than 65.0%, calculated as ethylene oxide (C_2H_4O), on the anhydrous basis.
Saponification Value Between 65 and 75.
Stearic, Palmitic, and Myristic Acids Between 31 and 33 g per 100 g of sample.
Water Not more than 1%.

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 503.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Oxyethylene Content (apparent) Weigh accurately about 70 mg, and proceed as directed under *Oxyethylene Determination*, page 507.

Saponification Value Determine as directed in the general method, page 509, using about 6 g, accurately weighed.

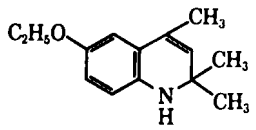
Stearic, Palmitic, and Myristic Acids Isolate the fatty acids as directed in the test for *Lauric Acid* under *Polysorbate 20*, page 234, and determine the weight of the acids. The product so obtained has an *Acid Value* between 199 and 211 (*Method I*, page 503) and a *Solidification Point*, page 538, not below 50°.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Dough conditioner; emulsifier.

Ethoxyquin

6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline



$C_{14}H_{19}NO$ Mol wt (monomer) 217.31

DESCRIPTION

Ethoxyquin is a mixture consisting predominantly of the monomer ($C_{14}H_{19}NO$). It occurs as a clear liquid that may darken with age without affecting its antioxidant activity. Its specific gravity is about 1.02, and its refractive index is about 1.57.

REQUIREMENTS

Identification

A solution of 1 mg of the sample in 10 ml of acetonitrile exhibits a strong fluorescence when viewed under short-wavelength ultraviolet light.

Assay Not less than 90.0% of $C_{14}H_{19}NO$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Transfer about 200 mg of the sample, accurately weighed, into a 150-ml beaker containing 50 ml of glacial acetic acid, and immediately titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically. Perform a blank determination and make any necessary correction (see page 2). Each ml of 0.1 *N* perchloric acid is equivalent to 21.73 mg of $C_{14}H_{19}NO$ (monomer).

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in tightly closed carbon steel or black iron (not rubber, neoprene, or nylon) containers in a cool, dark place. Prolonged exposure to sunlight causes polymerization.

Functional Use in Foods Antioxidant for apples and pears.

Ethyl Alcohol

Alcohol; Ethanol

C_2H_6O Mol wt 46.07

DESCRIPTION

A clear, colorless, mobile liquid having a slight, characteristic odor and a burning taste. It is miscible with water, with ether, and with chloroform. It boils at about 78° and is flammable. Its refractive index at 20° is about 1.364.

NOTE: This monograph applies only to undenatured alcohol.

REQUIREMENTS

Assay Not less than 94.9% by volume (92.3% by weight) of C_2H_6O.
Acidity (as acetic acid) Not more than 0.003%.
Alkalinity (as NH_3) Not more than 3 ppm.
Fusel Oil Passes test.
Heavy Metals (as Pb) Not more than 1 ppm.
Ketones, Isopropyl Alcohol Passes test.
Methanol Passes test.
Nonvolatile Residue Not more than 0.003%.
Solubility in Water Passes test.
Substances Darkened by Sulfuric Acid Passes test.
Substances Reducing Permanganate Passes test.

TESTS

Assay Its specific gravity, determined by any reliable method (see page 3), is not greater than 0.8096 at 25°/25° (equivalent to 0.8161 at 15.56°/15.56°).

Acidity Transfer 10 ml of the sample to a glass-stoppered flask containing 25 ml of water, add 0.5 ml of phenolphthalein TS, and add 0.02 *N* sodium hydroxide to the first appearance of a pink color that persists after shaking for 30 s. Add 25 ml (about 20 g) of the sample, mix, and titrate with 0.02 *N* sodium hydroxide until the pink color is restored. Not more than 0.5 ml is required.

Alkalinity Add 2 drops of methyl red TS to 25 ml of water, add 0.02 *N* sulfuric acid until a red color just appears, then add 25 ml (about 20 g) of the sample, and mix. Not more than 0.2 ml of 0.02 *N* sulfuric acid is required to restore the red color.

Fusel Oil Mix 10 ml of the sample with 1 ml of glycerin and 1 ml of water, and allow to evaporate from a piece of clean, odorless absorbent paper. No foreign odor is perceptible when the last traces of alcohol leave the paper.

Heavy Metals Evaporate 25 ml (about 20 g) of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. This solution

meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Ketones, Isopropyl Alcohol Transfer 1 ml. of the sample, 3 ml of water, and 10 ml of mercuric sulfate TS to a test tube, mix, and heat in a boiling water bath. No precipitate forms within 3 min.

Methanol Transfer 1 drop of the sample to a test tube, add 1 drop of dilute phosphoric acid (1 in 20) and 1 drop of potassium permanganate solution (1 in 20), mix, and allow to stand for 1 min. Add sodium bisulfite solution (1 in 10), dropwise, until the permanganate color is discharged. If a brown color remains, add 1 drop of the phosphoric acid solution. To the colorless solution add 5 ml of freshly prepared chromotropic acid TS, and heat on a water bath at 60° for 10 min. No violet color develops.

Nonvolatile Residue Evaporate 125 ml (about 100 g) of the sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Solubility in Water Transfer 50 ml of the sample to a 100-ml glass-stoppered graduate, dilute to 100 ml with water, and mix. Place the graduate in a water bath maintained at 10°, and allow to stand for 30 min. No haze or turbidity develops.

Substances Darkened by Sulfuric Acid Transfer 10 ml of sulfuric acid into a small Erlenmeyer flask, cool to 10°, and add 10 ml of the sample, dropwise, with constant agitation. The mixture is colorless or has no more color than either the acid or sample before mixing.

Substances Reducing Permanganate Transfer 20 ml of the sample, previously cooled to 15°, to a glass-stoppered cylinder, add 0.1 ml of 0.1 *N* potassium permanganate, mix, and allow to stand for 5 min. The pink color is not entirely discharged.

Packaging and Storage Store in tight containers, remote from fire.

Functional Use in Foods Extraction solvent; vehicle.

Ethyl Cellulose

DESCRIPTION

Ethyl cellulose is the ethyl ether of cellulose in the form of a free-flowing, white to light tan powder. It is heat-labile, and exposure to high temperatures (240°) causes color degradation and loss of properties. It is practically insoluble in water, in glycerin, and in propylene glycol, but is soluble in varying proportions in certain organic solvents, depending upon the ethoxyl content. Ethyl cellulose containing less than 46% to 48% of ethoxyl groups is freely soluble in tetrahydrofuran, in methyl acetate, in chloroform, and in aromatic hydrocarbon–alcohol mixtures. Ethyl cellulose containing 46% to 48% or more of ethoxyl groups is freely soluble in alcohol, in methanol, in toluene, in chloroform, and in ethyl acetate. A 1 in 20 aqueous suspension of the sample is neutral to litmus.

REQUIREMENTS

Identification

Dissolve 5 g of the sample in 95 g of an 80:20 (w/w) mixture of toluene-alcohol. A clear, stable, slightly yellow solution is formed. Pour a few ml of the solution onto a glass plate, and allow the solvent to evaporate. A thick, tough, continuous, clear film remains. The film is flammable.

Assay Not less than 44.0% and not more than 50.0% of ethoxyl groups (—OC_2H_5), after drying (equivalent to not more than 2.6 ethoxyl groups per anhydroglucose unit).

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 3%.

Residue on Ignition Not more than 0.4%.

Viscosity Not less than 90% and not more than 110% of that stated on the label for a labeled viscosity of 10 centipoises or more; not less than 80% and not more than 120% of that stated on the label for a labeled viscosity of less than 10 centipoises.

TESTS

Assay Place about 50 mg of the sample, previously dried at 105° for 2 h, in a tared gelatin capsule, weigh accurately, transfer the capsule and its contents into the boiling flask of a methoxyl determination apparatus, and proceed as directed under *Methoxyl Determination*, page 521. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 751 µg of ethoxyl groups (—OC_2H_5).

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Viscosity

Solvent Systems For ethyl cellulose containing less than 46% to 48% of ethoxyl groups, prepare a solvent consisting of a 60:40 (w/w) mixture of toluene-alcohol; for ethyl cellulose containing 46% to 48% or more of ethoxyl groups, prepare a solvent consisting of an 80:20 (w/w) mixture of toluene-alcohol.

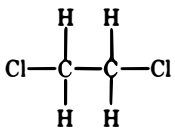
Procedure Transfer 5.0 g of the sample, previously dried at 105° for 2 h and accurately weighed, into a bottle containing 95 ± 0.5 g of the appropriate solvent system. Shake or tumble the bottle until the sample is completely dissolved, and then adjust the temperature of the solution to 25° ± 0.1°. Determine the viscosity as directed under

Viscosity of Methylcellulose, page 549, but make all determinations at 25° instead of 20° as directed therein.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Protective coating component for vitamin and mineral tablets; binder and filler in dry vitamin preparations.

Ethylene Dichloride

1,2-Dichloroethane



$C_2H_4Cl_2$ Mol wt 98.96

DESCRIPTION

A clear, colorless, flammable, oily liquid having a chloroform-like odor and a sweet taste. It is slightly soluble in water, and is soluble in alcohol, in ether, in acetone, and in carbon tetrachloride. Its refractive index at 20° is about 1.445.

REQUIREMENTS

Acidity (as HCl) Not more than 10 ppm.
Distillation Range Between 82° and 85°.
Free Halogens Passes test.
Heavy Metals (as Pb) Not more than 1 ppm.
Nonvolatile Residue Not more than 0.002%.
Specific Gravity Between 1.245 and 1.255.
Water Not more than 0.03%.

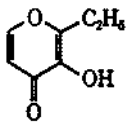
TESTS

Acidity Transfer 25 ml of alcohol to a 100-ml glass-stoppered flask, add 2 drops of phenolphthalein TS, and titrate with 0.01 *N* sodium hydroxide to the first appearance of a slight pink color. Add 25 ml (about 31 g) of the sample, mix, and titrate with 0.01 *N* sodium hydroxide until the faint pink color is restored. Not more than 0.85 ml is required.
Distillation Range Determine as directed in the general method, page 478.
Free Halogens Shake 10 ml of the sample vigorously for 2 min with 10 ml of 10% potassium iodide solution and 1 ml of starch TS. A blue color does not appear in the water layer.
Heavy Metals Evaporate 16 ml (about 20 g) of the sample to dryness (*Caution:* use hood) on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Filter if necessary through a small filter, wash the evaporating dish and the filter with about 10 ml of water, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Nonvolatile Residue Evaporate 80 ml (about 100 g) of the sample to dryness (*Caution:* use hood) in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.
Specific Gravity Determine by any reliable method (see page 3).
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Extraction solvent.

Ethyl Maltol

3-Hydroxy-2-ethyl-4-pyrone



$C_7H_8O_3$ Mol wt 140.14

DESCRIPTION

A white, crystalline powder having a characteristic odor and a sweet, fruitlike flavor in dilute solution. One g dissolves in about 55 ml of water, 10 ml of alcohol, 17 ml of propylene glycol, and 5 ml of chloroform. It melts at about 90°.

REQUIREMENTS

Identification

The infrared absorption spectrum of a 1 in 50 solution of ethyl maltol in chloroform, determined in a 0.1-mm cell, exhibits maxima only at the same wavelengths as that of FCC Ethyl Maltol Reference Standard, similarly measured.

Assay Not less than 99.0% of $C_7H_8O_3$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Residue on Ignition Not more than 0.2%.
Water Not more than 0.5%.

TESTS

Assay

Standard Solution Weigh accurately about 50 mg of FCC Ethyl Maltol Reference Standard, dissolve it in sufficient 0.1 *N* hydrochloric acid to make 250.0 ml, and mix. Transfer 5.0

ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Assay Solution Weigh accurately about 50 mg of the sample, dissolve it in sufficient 0.1 *N* hydrochloric acid to make 250.0 ml, and mix. Transfer 5.0 ml of this solution to a 100-ml volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Procedure Determine the absorbance of each solution in a 1-cm cell at the wavelength of maximum absorption at about 276 nm, with a suitable spectrophotometer, using 0.1 *N* hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_7H_8O_3$ in the sample taken by the formula $5C(A_U/A_S)$, in which C is the concentration, in µg per ml, of FCC Ethyl Maltol Reference Standard in the *Standard Solution*, A_U is the absorbance of the *Assay Solution*, and A_S is the absorbance of the *Standard Solution*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Residue on Ignition Ignite a 1-g sample as directed in the general method, page 533.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Flavoring agent.

Eucalyptus Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh leaves of *Eucalyptus globulus* Labillardiere and other species of *Eucalyptus* L'Heritier (Fam. *Myrtaceae*). It is a colorless or pale yellow liquid having a characteristic aromatic, somewhat camphoraceous odor and a pungent, spicy, cooling taste.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 594, using the same test conditions as specified therein.

Assay Not less than 70.0% of cineole ($C_{10}H_{18}O$).

Heavy Metals (as Pb) Passes test.

Phellandrene Passes test.

Refractive Index Between 1.458 and 1.470 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.905 and 0.925.

TESTS

Assay Transfer about 3 g, previously dried with anhydrous sodium sulfate and accurately weighed, into a 25- × 150-mm test tube. Add to the sample 2.100 g of melted *o*-cresol that is pure and dry, having a solidification point of 30° or higher. (NOTE: Moisture in the *o*-cresol may cause low results). Stir the mixture with the thermometer to induce crystallization, and note the highest temperature reading obtained. Warm the tube gently until the contents are completely melted, then insert the test tube into the apparatus assembled as directed under *Solidification Point*, page 538. Allow the mixture to cool slowly until crystallization starts, or until the temperature has fallen to the point previously noted. Stir the mixture vigorously with the thermometer, rubbing the sides of the test tube with an up and down motion to induce crystallization. Continue the stirring and rubbing so long as the temperature rises. Take the highest temperature obtained as the solidification point. Repeat the procedure until two results agreeing within 0.1° are obtained. Calculate the percentage of cineole from the *Percentage of Cineole* table, page 501.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Phellandrene Mix 2.5 ml of sample with 5 ml of solvent hexane, add 5 ml of a solution of sodium nitrite made by dissolving 5 g of sodium nitrite in 8 ml of water, then gradually add 5 ml of glacial acetic acid. No crystals form in the mixture within 10 min.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 5 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in well-filled, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Fennel Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried ripe fruit of *Foeniculum vulgare* Miller (Fam. *Umbelliferae*). It is a colorless or pale yellow liquid having the characteristic odor and taste of fennel.

NOTE: If solid material has separated, carefully warm the sample until it is completely liquefied, and mix it before using.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 594, using the same test conditions as specified therein.

Angular Rotation Between +12° and +24°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.528 and 1.538 at 20°.
Solidification Point Not lower than 3°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.953 and 0.973.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solidification Point Determine as directed in the general method, page 538.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 90% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Ferric Ammonium Citrate, Brown

Iron Ammonium Citrate

DESCRIPTION

A complex salt of undetermined structure, composed of iron, ammonia, and citric acid and occurring as thin, transparent brown, reddish brown, or garnet red scales or granules, or as a brownish yellow powder. It is odorless or has a slight ammoniacal odor and has a mild iron-metallic taste. It is very soluble in water but is insoluble in alcohol. The pH of a 1 in 20 solution is about 5.0 to 8.0. It is deliquescent in air and is affected by light.

REQUIREMENTS

Identification

A. A 500-mg sample, when ignited, chars and leaves a residue of iron oxide.
B. To 5 ml of a 1 in 10 solution of the sample add 0.3 ml of potassium permanganate TS and 4 ml of mercuric sulfate TS, and then heat the mixture to boiling. A white precipitate forms.
C. Dissolve about 500 mg of the sample in 5 ml of water, and add 5 ml of sodium hydroxide TS. A reddish brown precipitate forms, and ammonia is evolved when the mixture is heated.

Assay Not less than 16.5% and not more than 18.5% of iron (Fe).
Arsenic (as As) Not more than 3 ppm.
Ferric Citrate Passes test.
Lead Not more than 10 ppm.
Mercury Not more than 1 ppm.
Oxalate Passes test.
Sulfate Not more than 0.3%.

TESTS

Assay Transfer about 1 g, accurately weighed, into a 250-ml glass-stoppered Erlenmeyer flask, and dissolve in 25 ml of water and 5 ml of hydrochloric acid. Add 4 g of potassium iodide, stopper, and allow to stand protected from light for 15 min. Add 100 ml of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of iron (Fe).
Arsenic Determine as directed in the test for *Arsenic* under *Ferric Phosphate*, page 118.
Ferric Citrate Add potassium ferrocyanide TS to a 1 in 100 solution of the sample. No blue precipitate forms.
Lead (NOTE: The following method has been found to be satisfactory when the particular atomic absorption spectrophotometer specified is used. The method may be modified as necessary for use with other suitable atomic absorption spectrophotometers capable of determining lead in the sample at the limit specified.)

Standard Preparation Transfer 10.0 ml of *Lead Nitrate Stock Solution* (see page 512) into a 500-ml volumetric flask, dilute to volume with water, and mix. This solution should be prepared on the day of use. Each ml contains the equivalent of 2 µg of lead ion (Pb).

Sample Preparation Transfer about 15 g of the sample, accurately weighed, into a 100-ml volumetric flask (previously rinsed with nitric acid and water), dissolve in a mixture of 50 ml of water and 1 ml of nitric acid, dilute to volume with water, and mix.

Procedure Using a Perkin-Elmer 403 atomic absorption

spectrophotometer equipped with a deuterium arc background corrector, digital readout device, and a burner head capable of handling 15% solids content, blank the instrument with water following the manufacturer's operating instructions. Aspirate a portion of the *Standard Preparation*, and record the absorbance as A_S; then aspirate a portion of the *Sample Preparation*, and record the absorbance as A_U. Calculate the lead content, in ppm, of the sample taken by the formula

$$100 \times (C/W) \times (A_U/A_S),$$

in which C is the concentration of Pb in the *Standard Preparation*, in μg per ml, and W is the weight of the sample taken, in g.

Mercury

Standard Preparations Prepare a solution containing 1 μg of mercury (Hg) per ml as directed for *Standard Preparation* under *Mercury Limit Test*, page 520. Pipet 0.25, 0.50, 1.0, and 3.5 ml of this solution into each of four glass-stoppered bottles of about 300-ml capacity, such as BOD (biological oxygen demand) bottles. Dilute the contents of each bottle to 100 ml with water, and mix. These solutions contain the equivalent of 0.25, 0.50, 1.0, and 3.5 ppm of Hg, respectively.

Sample Preparation Transfer 1.000 g of the sample into a 200-ml screw-cap centrifuge bottle, and add 5 ml of nitric acid and 5 ml of hydrochloric acid. Close the bottle tightly with a Teflon-lined screw-cap, digest on a steam bath for 1 h, and cool. Quantitatively transfer into a suitable glass-stoppered bottle (see *Standard Preparations*), dilute to 100 ml with water, and bubble air through the sample for 2 min. Prepare a reagent blank in the same manner.

Apparatus Use a suitable atomic absorption spectrophotometer assembly designed for mercury analysis, such as the Coleman MAS-50 Mercury Analyzer. (NOTE: The *Apparatus* and *Procedure* described under *Mercury Limit Test*, page 520, may be suitably modified for this determination.)

Procedure Add 5 ml of a 10% stannous chloride solution (prepared fresh each week by dissolving 20 g of $SnCl_2.2H_2O$ in 40 ml of warm hydrochloric acid and diluting with 160 ml of water) to the solution to be tested, and immediately insert the bubbler of the mercury analysis apparatus. Obtain the absorbance reading by following the instrument manufacturer's operating instructions. Correct the sample readings for the reagent blank, and determine the mercury concentration of the *Sample Preparation* from a standard curve prepared by plotting the readings obtained with the *Standard Preparations* against mercury concentration, in ppm.

Oxalate Transfer 1 g of the sample into a 125-ml separator, dissolve in 10 ml of water, add 2 ml of hydrochloric acid, and extract successively with one 50-ml portion and one 20-ml portion of ether. Transfer the combined ether extracts to a 150-ml beaker, add 10 ml of water, and remove the ether by evaporation on a steam bath. Add 1 drop of glacial acetic acid and 1 ml of calcium acetate solution (1 in 20) to the residual aqueous solution. No turbidity is produced within 5 min.

Sulfate Dissolve a 100-mg sample of 1 ml in diluted hydrochloric acid TS, and dilute to 30 to 40 ml with water. Proceed as directed in the *Sulfate Limit Test*, page 471, beginning with the addition of 3 ml of barium chloride TS. Any turbidity produced does not exceed that shown in a control containing 300 μg of sulfate (SO_4).

Packaging and Storage Store in tight, light-resistant containers in a cool place.

Functional Use in Foods Nutrient; dietary supplement.

Ferric Ammonium Citrate, Green

Iron Ammonium Citrate

DESCRIPTION

A complex salt of undetermined structure, composed of iron, ammonia, and citric acid and occurring as thin, transparent green scales, as granules, as a powder, or as transparent green crystals. It is odorless and has a mild iron-metallic taste. It is very soluble in water but is insoluble in alcohol. Its solutions are acid to litmus. It may deliquesce in air and is affected by light.

REQUIREMENTS

Identification

It responds to the *Identification Tests* under *Ferric Ammonium Citrate, Brown*, page 116.

Assay Not less than 14.5% and not more than 16.0% of iron (Fe).

Arsenic (as As) Not more than 3 ppm.

Ferric Citrate Passes test.

Lead Not more than 10 ppm.

Mercury Not more than 1 ppm.

Oxalate Passes test.

Sulfate Not more than 0.3%.

TESTS

Determine as directed for the respective TESTS under *Ferric Ammonium Citrate, Brown*, page 116.

Packaging and Storage Store in tight, light-resistant containers in a cool place.

Functional Use in Foods Nutrient; dietary supplement; anticaking agent for sodium chloride.

Ferric Phosphate

Iron Phosphate; Ferric Orthophosphate

$FePO_4 \cdot xH_2O$ Mol wt (anhydrous) 150.82

DESCRIPTION

Ferric phosphate contains from one to four molecules of water of hydration. It occurs as an odorless, yellowish white to buff-colored powder. It is insoluble in water and in acetic acid, but is soluble in mineral acids.

REQUIREMENTS

Identification

Dissolve 1 g in 5 ml of dilute hydrochloric acid (1 in 2), and add an excess of sodium hydroxide TS. A reddish brown precipitate forms. Boil the mixture, filter to remove the iron, and strongly acidify a portion of the filtrate with hydrochloric acid. Cool, mix with an equal volume of magnesia mixture TS, and treat with a slight excess of ammonia TS. An abundant white precipitate forms. This precipitate, after being washed, turns greenish yellow when treated with a few drops of silver nitrate TS.

Assay Not less than 26.0% and not more than 32.0% of Fe.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Lead Not more than 10 ppm.
Loss on Ignition Not more than 32.5%.
Mercury Not more than 3 ppm.

TESTS

Assay Dissolve about 3.5 g of the sample, accurately weighed, in 75 ml of dilute hydrochloric acid (1 in 2), heat to boiling, and boil for about 5 min. Cool, transfer into a 100-ml volumetric flask, dilute to volume with the dilute hydrochloric acid, and mix. To 25.0 ml of this solution add 100 ml of the dilute hydrochloric acid, boil again for 5 min, and to the boiling solution add stannous chloride TS, dropwise, with stirring, until the iron is just reduced as indicated by the disappearance of the yellow color. Add 2 drops in excess (but no more) of the stannous chloride TS, dilute with about 50 ml of water, and cool to room temperature. While stirring vigorously, add 15 ml of a saturated solution of mercuric chloride, and then allow to stand for 5 min. Add 15 ml of a sulfuric acid–phosphoric acid mixture, prepared by slowly adding 75 ml of sulfuric acid to 300 ml of water, cooling, adding 75 ml of phosphoric acid, and then diluting to 500 ml with water, and mix. Add 0.5 ml of barium diphenylamine sulfonate TS, and titrate with 0.1 *N* potassium dichromate to a reddish violet endpoint. Each ml of 0.1 *N* potassium dichromate is equivalent to 5.585 mg of Fe.

Arsenic Assemble the special distillation apparatus as shown in Fig. 4 on page 465 of the general test. Transfer 2 g of the sample, 50 ml of hydrochloric acid, and 5 g of cuprous chloride into the distilling flask (*B*). Reassemble the distillation apparatus and apply gentle suction to flask *F* to produce a continuous stream of bubbles. Heat the solution in flask *B* to boiling and distil until between 30 and 35 ml of distillate has been collected in flask *D*. Quantitatively transfer the distillate to a 100-ml volumetric flask with the aid of water, dilute to volume with water, and mix (*Sample Solution*). Prepare *Standard* and *Blank Solutions* in the same manner, using 6.0 ml of *Standard Arsenic Solution* (page 464) in place of the sample in the *Standard Solution*, and 6.0 ml of water in the *Blank Solution*. Transfer 50.0 ml of the *Sample Solution* into the generator flask (Fig. 4, page 465), add 2 ml of potassium iodide solution (15 in 100), and continue as directed in the *Procedure* under *Arsenic Test*, page 465, beginning with "[add] 0.5 ml of *Stannous Chloride Solution*, and mix. . . . " Modify the *Procedure* by using 5.0 g of Devarda's metal in place of the 3.0 g of 20-mesh granular zinc, and maintain the temperature of the reaction mixture in the generator flask between 25° and 27°. Treat 50.0 ml each of the *Standard Solution* and of the *Blank Solution* in the same manner and under the same conditions. Determine the absorbance at 525 nm produced by each solution as directed under *Procedure*. Calculate the arsenic content (in ppm) of the sample by the formula

$$3 \times (A_U - A_B)/(A_S - A_B),$$

in which A_U is the absorbance produced by the *Sample Solution*, A_S is the absorbance produced by the *Standard Solution*, and A_B is the absorbance produced by the *Blank Solution*. (NOTE: If A_B exceeds 0.300, different samples of reagent-grade cuprous chloride and Devarda's metal should be tested for arsenic content by the procedure described herein, and lots of these reagents should be selected that will give blank readings that do not exceed 0.300.)

Fluoride Weigh accurately 1.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Lead

Citrate-Cyanide Wash Solution To 50 ml of water add 50 ml of *Ammonium Citrate Solution* (page 518) and 4 ml of *Potassium Cyanide Solution* (page 518), mix, and adjust the pH, if necessary, with stronger ammonia TS to 9.0.

pH 2.5 Buffer Solution To 25.0 ml of 0.2 *M* potassium biphthalate add 37.0 ml of 0.1 *N* hydrochloric acid, and dilute to 100.0 ml.

Dithizone-Carbon Tetrachloride Solution Dissolve 10 mg of dithizone in 1000 ml of carbon tetrachloride. Prepare this solution fresh for each determination.

pH 2.5 Wash Solution To 500 ml of dilute nitric acid (1 in 100) add ammonia TS until the pH of the mixture is 2.5, then add 10 ml of *pH 2.5 Buffer Solution*, and mix.

Ammonia-Cyanide Wash Solution To 35 ml of *pH 2.5 Wash Solution* add 4 ml of *Ammonia-Cyanide Solution*, and mix.

NOTE: Other solutions required are described under the *Lead Limit Test*, page 518.

Procedure Dissolve 200 mg of the sample, accurately weighed, in 10 ml of dilute hydrochloric acid (1 in 2), prepare

a control containing 2.0 ml of *Diluted Standard Lead Solution* and 10 ml of the dilute hydrochloric acid, and carry both solutions through the following procedure:

Add 25 ml of *Ammonium Citrate Solution*, heat on a steam bath for a few minutes, add 7 ml of stronger ammonia TS, and cool. Adjust the pH, if necessary, to 9.0, using the appropriate volumes of either stronger ammonia TS or hydrochloric acid, and transfer to a separator. Extract with 5-ml portions of *Dithizone Extraction Solution* until the extraction solution retains its original color, and combine the extracts in a second separator. Wash the combined extracts by shaking for 30 s with 10 ml of *Citrate-Cyanide Wash Solution*, then wash the wash solution with 3 ml of *Dithizone Extraction Solution*. Combine the chloroform layers, add 20 ml of dilute nitric acid (1 in 100), and shake for 30 s. Separate the layers, and shake the chloroform layer with an additional 5 ml of the dilute nitric acid. Combine the acid washes in a small beaker, and adjust the pH with ammonia TS to 2.5 ± 0.2 (by means of a glass electrode). Transfer the solution to a separator, add 2 ml of *pH 2.5 Buffer Solution*, and shake the solution for 30 s with 30 ml of *Dithizone-Carbon Tetrachloride Solution*. Wash the combined carbon tetrachloride layers with 10 ml of *pH 2.5 Wash Solution*, and combine the aqueous layers. Dislodge any drops of carbon tetrachloride remaining on the surface of the aqueous layer, and draw off and discard the carbon tetrachloride layer. (NOTE: Avoid any delay in completing the test after beginning the following extraction, since the color fades. Have the spectrophotometer ready to use, and carry the samples through the remainder of the procedure one at a time.) Add 4 ml of *Ammonia-Cyanide Solution*, mix, and extract at once with 5-ml portions of *Dithizone-Carbon Tetrachloride Solution* until the carbon tetrachloride shows no further pink color. Wash the combined extracts with 4 ml of *Ammonia-Cyanide Wash Solution*, dry the stem of the separator, and drain the carbon tetrachloride through a plug of cotton to remove the last trace of water. Determine the absorbances of both solutions in 1-cm cells at 520 nm with a suitable spectrophotometer, using carbon tetrachloride as the blank. The absorbance of the sample solution does not exceed that of the control solution.

Loss on Ignition Ignite at 800° for 1 h.

Mercury

Standard Preparations Dissolve 338.5 mg of mercuric chloride, $HgCl_2$, in about 200 ml of water in a 250-ml volumetric flask, add 14 ml of dilute sulfuric acid (1 in 2), dilute to volume with water, and mix. Pipet 10.0 ml of this solution into a 1000-ml volumetric flask containing about 800 ml of water and 56 ml of dilute sulfuric acid (1 in 2), dilute to volume with water, and mix. Pipet 10.0 ml of the second solution into a second 1000-ml volumetric flask containing 800 ml of water and 56 ml of dilute sulfuric acid (1 in 2), dilute to volume with water, and mix. Each ml of this diluted stock solution contains 0.1 μg of Hg. Pipet 1.25, 2.50, 5.00, 7.50, and 10.00 of the last solution (equivalent to 0.125, 0.250, 0.500, 0.750, and 1.00 μg of Hg, respectively) into each of five 150-ml beakers. To each add 25 ml of aqua regia, cover with watch glasses, heat just to boiling, simmer for about 5 min, and cool to room temperature. Transfer the solutions into separate 250-ml volumetric flasks, dilute to volume with water, and mix. Transfer a 50.0-ml aliquot from each solution into respective 150-ml beakers, and to each add 1.0 ml of dilute sulfuric acid (1 in 5) and 1.0 ml of a filtered solution of potassium permanganate (1 in 25). Heat the solutions just to boiling, simmer for about 5 min, and cool.

Sample Preparation Transfer 5.00 g of the sample into a 150-ml beaker, add 25 ml of aqua regia, cover with a watch glass, and allow to stand at room temperature for about 5 min. Heat just to boiling, allow to simmer for about 5 min, and cool. Transfer the solution into a 250-ml volumetric flask, dilute to volume with water, and mix. (NOTE: Disregard any undissolved material that may be present.) Transfer a 50.0-ml aliquot of this solution into a 150-ml beaker, and add 1.0 ml of dilute sulfuric acid (1 in 5) and 1.0 ml of a filtered solution of potassium permanganate (1 in 25). Heat the solution just to boiling, simmer for about 5 min, and cool. Prepare a reagent blank in the same manner.

Apparatus Use a *Mercury Detection Instrument* and *Aeration Apparatus* as described under the *Mercury Limit Test*, page 520. For the purposes of the test described herein, the Techtron AA-1000 atomic absorption spectrophotometer, equipped with a 10-cm silica absorption cell (Beckman Part No. 75144 or equivalent) and coupled with a strip chart recorder (Varian Series A-25 or equivalent), is satisfactory.

Procedure Assemble the *Aerating Apparatus* as shown in Fig. 15, page 520. Use magnesium perchlorate as the absorbent in the absorption cell (*e*), fill gas washing bottle *c* with 60 ml of water, and place stopcock *b* in the bypass position. Connect the assembly to the 10-cm absorption cell (analogous to *f* in Fig. 15) of the spectrophotometer, and adjust the air or nitrogen flow rate so that, in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.7 nm by following the manufacturer's instructions for operating the particular *Mercury Detection Instrument* in use. Using the Techtron AA-1000 spectrophotometer, the following conditions are suitable: *slit width*, 2 Å; *lamp current*, 3 mA; and *scale expansion*, ×1. With the Varian A-25 recorder, set the *chart speed* at 25 in./h and the *span* at 2 mV. Precondition the apparatus by an appropriate modification of the procedures described below for treatment of the test solutions. (NOTE: The fritted bubbler in gas washing bottle *c* should be kept immersed in water between determinations. After each determination, wash the bubbler with a stream of water.)

Treat the blank, each of the *Standard Preparations*, and the *Sample Preparation* as follows: Transfer the solution to be tested to a 125-ml gas washing bottle, using a few drops of hydroxylamine hydrochloride solution (1 in 10) to remove any manganese hydroxide from the beaker. Dilute to about 55 ml with water, and add a magnetic stirring bar. Discharge the permanganate color by the dropwise addition of the hydroxylamine hydrochloride solution, swirling after each drop is added. Add 15.0 ml of 10% stannous chloride solution (prepared by dissolving 20 g of $SnCl_2 2H_2O$ in 40 ml of warm hydrochloric acid and diluting with 160 ml of water), and immediately connect the gas washing bottle to the *Aeration Assembly*. Switch on the magnetic stirrer, turn

stopcock *b* from the bypass to the aerating position, and obtain the absorbance reading. Disconnect bottle *c* from the *Aerating Apparatus*, discard the solution just tested, wash bottle *c* with water, wash the fritted bubbler with water, and repeat the foregoing procedure with the remaining solutions. Correct the sample readings for the reagent blank, and determine the mercury concentration of the *Sample Preparation* from a standard curve prepared by plotting the readings obtained with the *Standard Preparations* against mercury concentration, in ppm, suitable adjustments being made for dilution factors.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Ferric Pyrophosphate

Iron Pyrophosphate

$Fe_4(P_2O_7)_3 \cdot xH_2O$ Mol wt (anhydrous) 745.22

DESCRIPTION

A tan or yellowish white, odorless powder. It is insoluble in water but is soluble in mineral acids.

REQUIREMENTS

Identification

Dissolve 500 mg in 5 ml of dilute hydrochloric acid (1 in 2), and add an excess of sodium hydroxide TS. A reddish brown precipitate forms. Allow the solution to stand for several min, and then filter, discarding the first few ml. To 5 ml of the clear filtrate add 1 drop of bromophenol blue TS, and titrate with 1 *N* hydrochloric acid to a green color. Add 10 ml of a 1 in 8 solution of zinc sulfate, and readjust the pH to 3.8 (green color). A white precipitate forms (distinction from *orthophosphates*).

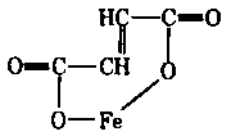
Assay Not less than 24.0% and not more than 26.0% of Fe.
Arsenic (as As) Not more than 3 ppm.
Lead Not more than 10 ppm.
Loss on Ignition Not more than 20%.
Mercury Not more than 3 ppm.

TESTS

Assay Proceed as directed in the *Assay* under *Ferric Phosphate*, page 118.
Arsenic Prepare and test a 2-g sample as directed in the test for *Arsenic* under *Ferric Phosphate*, page 118.
Lead Proceed as directed in the test for *Lead* under *Ferric Phosphate*, page 118.
Loss on Ignition Ignite at 800° for 1 h.
Mercury Determine as directed in the test for *Mercury* under *Ferric Phosphate*, page 118.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Ferrous Fumarate



$C_4H_2FeO_4$ Mol wt 169.90

DESCRIPTION

An odorless, reddish orange to red brown powder. It may contain soft lumps that produce a yellow streak when crushed.

REQUIREMENTS

Identification

A. To about 1.5 g add 25 ml of dilute hydrochloric acid (1 in 2), and dilute to 50 ml with water. Heat to effect complete solution, then cool, filter on a fine-porosity sintered-glass crucible, wash the precipitate with dilute hydrochloric acid (3 in 100), saving the filtrate for *Identification Test B*, and dry the precipitate at 105°. To 400 mg of the dried precipitate add 3 ml of water and 7 ml of 1 *N* sodium hydroxide, and stir until solution is complete. Add diluted hydrochloric acid TS, dropwise, until the solution is just acid to litmus, add 1 g of *p*-nitrobenzyl bromide and 10 ml of alcohol, and reflux the mixture for 2 h. Cool, filter, and wash the precipitate with two small portions of a mixture of 2 parts of alcohol and 1 part of water, followed by two small portions of water. The precipitate, recrystallized from hot alcohol and dried at 105°, melts at about 152° (see page 519).

B. A portion of the filtrate obtained in the preceding test gives positive tests for *Iron*, page 516.

Assay Not less than 96.5% and not more than the equivalent of 101.0% of $C_4H_2FeO_4$.
Arsenic (as As) Not more than 3 ppm.
Ferric Iron Not more than 2%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 1%.
Mercury Not more than 3 ppm.
Sulfate Not more than 0.2%.

TESTS

Assay Transfer about 500 mg, accurately weighed, into a 500-ml Erlenmeyer flask, add 25 ml of dilute hydrochloric acid (2 in 5), and heat to boiling. Add, dropwise, a solution of 5.6 g of stannous chloride in 50 ml of dilute hydrochloric acid (3 in 10) until the yellow color disappears, and then add 2 drops in

excess. Cool the solution in an ice bath to room temperature, add 8 ml of mercuric chloride TS, and allow to stand for 5 min. Add 200 ml of water, 25 ml of dilute sulfuric acid (1 in 2), and 4 ml of phosphoric acid, then add orthophenanthroline TS, and titrate wth 0.1 *N* ceric sulfate. Each ml of 0.1 *N* ceric sulfate is equivalent to 16.99 mg of $C_4H_2FeO_4$.

Arsenic Transfer 2 g of the sample into a beaker and add 10 ml of water and 10 ml of sulfuric acid. Warm to precipitate the fumaric acid completely, cool, dilute with 30 ml of water, and filter into a 100-ml volumetric flask. Wash the precipitate with water, adding the washings to the flask, cool, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into an arsine generator, and proceed as directed under *Procedure* in the *Arsenic Test*, page 464, omitting the addition of dilute sulfuric acid (1 in 5).

Ferric Iron Transfer 2 g of the sample into a 250-ml glass-stoppered Erlenmeyer flask, add 25 ml of water and 4 ml of hydrochloric acid, and heat on a hot plate until solution is complete. Stopper the flask, and cool to room temperature. Add 3 g of potassium iodide, stopper, swirl to mix, and allow to stand in the dark for 5 min. Remove the stopper, add 75 ml of water, and titrate with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Not more than 7.16 ml of 0.1 *N* sodium thiosulfate is consumed.

Lead

NOTE: See page 518 for the preparation of other special solutions not described below.

Citrate-Cyanide Wash Solution To 50 ml of water add 50 ml of *Ammonium Citrate Solution* and 4 ml of *Potassium Cyanide Solution*, mix, and adjust the pH, if necessary, with stronger ammonia TS to 9.0.

pH 2.5 Buffer Solution To 25.0 ml of 0.2 *M* potassium biphthalate add 37.0 ml of 0.1 *N* hydrochloric acid, and dilute to 100.0 ml with water.

Dithizone–Carbon Tetrachloride Solution Dissolve 10 mg of dithizone in 1000 ml of carbon tetrachloride. Prepare this solution fresh for each determination.

pH 2.5 Wash Solution To 500 ml of dilute nitric acid (1 in 100) add ammonia TS until the pH of the mixture is 2.5, then add 10 ml of *pH 2.5 Buffer Solution*, and mix.

Ammonia-Cyanide Wash Solution To 35 ml of *pH 2.5 Wash Solution* add 4 ml of *Ammonia-Cyanide Solution*, and mix.

Procedure Transfer 500 mg of the sample, accurately weighed, into a 150-ml beaker, and add 3 ml of nitric acid and 5 ml of perchloric acid. Prepare a control containing 5.0 ml of *Diluted Standard Lead Solution*, 3 ml of nitric acid, and 5 ml of perchloric acid, and carry both sample and control solutions through the following procedure: Evaporate to dryness, cool, add 10 ml of dilute hydrochloric acid (1 in 2), and heat on a steam bath until the residue is completely dissolved. Add 25 ml of *Ammonium Citrate Solution*, heat on the steam bath for an additional few min, then add 7 ml of stronger ammonia TS, and cool. Adjust the pH, if necessary, to 9.0 (by means of a glass electrode or pH indicator paper), using either stronger ammonia TS or hydrochloric acid as necessary, and transfer into a separator. Extract with 5-ml portions of *Dithizone Extraction Solution* until the extraction solution retains its original color, and combine the extracts in a second separator. Wash the combined extracts by shaking for 30 s with 10 ml of *Citrate-Cyanide Wash Solution*, and then wash the wash solution with 3 ml of *Dithizone Extraction Solution*. Combine the chloroform layers, add 20 ml of dilute nitric acid (1 in 100), and shake for 30 s. Separate the layers, and shake the chloroform layer with an additional 5 ml of the dilute nitric acid. Combine the acid washes in a small beaker, and adjust the pH with diluted ammonia TS to 2.5 ± 0.2 (by means of a glass electrode). Transfer the solution into a separator, add 2 ml of *pH 2.5 Buffer Solution*, and shake the solution for 30 s with 30 ml of *Dithizone–Carbon Tetrachloride Solution*. Wash the combined carbon tetrachloride layers with 10 ml of *pH 2.5 Wash Solution*, and combine the aqueous layers. Dislodge any drops of carbon tetrachloride remaining on the surface of the aqueous layer, and draw off and discard the carbon tetrachloride layer. (NOTE: Avoid any delay in completing the test after beginning the following extraction, since the color fades. Have the spectrophotometer ready to use, and carry the samples through the remainder of the procedure one at a time.) Add 4 ml of the *Ammonia-Cyanide Solution*, mix, and then extract at once with 5-ml portions of *Dithizone–Carbon Tetrachloride Solution* until the carbon tetrachloride shows no further pink color. Wash the combined extracts with 4 ml of *Ammonia-Cyanide Wash Solution*, dry the stem of the separator, and drain the carbon tetrachloride through a plug of cotton to remove the last trace of water. Determine the absorbances of both solutions in 1-cm cells at 520 nm with a suitable spectrophotometer, using carbon tetrachloride as the blank. The absorbance of the sample solution does not exceed that of the control solution.

Loss on Drying, page 518 Dry at 105° for 16 h.

Mercury

Dithizone Extraction Solution Dissolve 30 mg of dithizone in 1000 ml of chloroform, add 5 ml of alcohol, and mix. Store in a refrigerator. Before use, shake a suitable volume with about half its volume of dilute nitric acid (1 in 100), discarding the nitric acid. Do not use if more than a month old.

Diluted Dithizone Extraction Solution Just prior to use, dilute 5 ml of *Dithizone Extraction Solution* with 25 ml of chloroform.

Hydroxylamine Hydrochloride Solution Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 ml, transfer the solution into a separator, add a few drops of thymol blue TS, and then add stronger ammonia TS until the solution assumes a yellow color. Add 10 ml of sodium diethyldithiocarbamate solution (1 in 25), mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-ml portions of chloroform until a 5-ml test portion of the chloroform extract does not assume a yellow color when shaken with a dilute cupric sulfate solution. Add diluted hydrochloric acid TS until the extracted solution is pink, adding 1 or 2 drops more of thymol blue TS, if necessary, then dilute to 100 ml with water, and mix.

Mercury Stock Solution Transfer 135.4 mg, accurately weighed, of mercuric chloride into a 100-ml volumetric flask,

dissolve in 1 *N* sulfuric acid, dilute to volume with the acid, and mix. Dilute 5.0 ml of this solution to 500.0 ml with 1 *N* sulfuric acid. Each ml contains the equivalent of 10 μg of Hg.

Diluted Standard Mercury Solution On the day of use, transfer 10.0 ml of *Mercury Stock Solution* into a 100-ml volumetric flask, dilute to volume with 1 *N* sulfuric acid, and mix. Each ml contains the equivalent of 1 μg of Hg.

Sodium Citrate Solution Dissolve 250 g of sodium citrate dihydrate in 1000 ml of water.

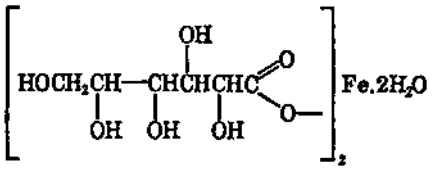
Sample Solution Dissolve 1 g of the sample in 30 ml of diluted nitric acid TS by heating on a steam bath. Cool to room temperature in an ice bath, stir, and filter through S and S No. 589, or equivalent, filter paper that has been previously washed with diluted nitric acid TS, followed by water. To the filtrate add 20 ml of *Sodium Citrate Solution* and 1 ml of *Hydroxylamine Hydrochloride Solution.*

Procedure [Because mercuric dithizonate is light-sensitive, this procedure should be performed in subdued light.] Prepare a control containing 3.0 ml of *Diluted Standard Mercury Solution* (3 μg Hg), 30 ml of diluted nitric acid TS, 5 ml of *Sodium Citrate Solution*, and 1 ml of *Hydroxylamine Hydrochloride Solution.* Treat the control and the *Sample Solution* as follows: Adjust the pH of each solution to 1.8 with stronger ammonia TS, using a pH meter, and transfer the solutions into different separators. Extract with two 5-ml portions of *Dithizone Extraction Solution*, and then extract again with 5 ml of chloroform, discarding the aqueous solutions. Transfer the combined extracts from each separator into different separators, add 10 ml of dilute hydrochloric acid (1 in 2) to each, shake well, and discard the chloroform layers. Extract the acid solutions with about 3 ml of chloroform, shake well, and discard the chloroform layers. Add to each separator 0.1 ml of 0.05 *M* disodium EDTA and 2 ml of 6 *N* acetic acid, mix, and then slowly add 5 ml of stronger ammonia TS. Stopper the separators, and cool under the stream of cold water. Dry the outside of the separators, pour the solutions (through the top of the separators) carefully, to avoid loss, into separate beakers, and adjust the pH of both solutions to 1.8 with ammonia TS, using a pH meter. Return the sample and control solutions to their original separators, add 5.0 ml of *Diluted Dithizone Extraction Solution*, and shake vigorously. Any color developed in the *Sample Solution* does not exceed that in the control.

Sulfate Mix 1 g of the sample with 100 ml of water in a 250-ml beaker, and heat on a steam bath, adding hydrochloric acid, dropwise, until complete solution is effected (about 2 ml of the acid will be required). Filter the solution, if necessary, and dilute the clear solution or filtrate to 100 ml with water. Heat to boiling, add 10 ml of barium chloride TS, warm on a steam bath for 2 h, cover, and allow to stand overnight. If crystals of ferrous fumarate form, warm on a steam bath to dissolve them, then filter through paper, wash the residue with hot water, and transfer the paper containing the residue to a tared crucible. Char the paper, without burning, and ignite the crucible and its contents at 600° to constant weight. Each mg of the residue is equivalent to 0.412 mg (412 μg) of SO_4.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Ferrous Gluconate



$C_{12}H_{22}FeO_{14}\cdot 2H_2O$ Mol wt 482.17

DESCRIPTION

Fine yellowish gray or pale greenish yellow powder or granules having a slight odor resembling that of burned sugar. One g dissolves in about 10 ml of water with slight heating. It is practically insoluble in alcohol. A 1 in 20 solution is acid to litmus.

REQUIREMENTS

Identification

A. To 5 ml of a warm 1 in 10 solution of the sample add 0.65 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, and heat the mixture on a steam bath for 30 min. Cool, and scratch the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

B. A 1 in 20 solution gives positive tests for *Ferrous Salts*, page 516.

Assay Not less than 95.0% of $C_{12}H_{22}FeO_{14}$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.07%.
Ferric Iron Not more than 2%.
Lead Not more than 10 ppm.
Loss on Drying Between 6.5% and 10%.
Mercury Not more than 3 ppm.
Oxalic Acid Passes test.
Reducing Sugars Passes test.
Sulfate Not more than 0.1%.

TESTS

Assay Dissolve about 1.5 g, accurately weighed, in a mixture of 75 ml of water and 15 ml of diluted sulfuric acid TS in a 300-ml Erlenmeyer flask, and add 250 mg of zinc dust. Close the flask with a stopper containing a Bunsen valve, allow to stand at room temperature for 20 min, then filter through a Gooch crucible containing an asbestos mat coated with a thin layer of zinc dust, and wash the crucible and contents with 10 ml of diluted sulfuric acid TS, followed by 10 ml of water. Add orthophenanthroline TS, and titrate the filtrate in the suction flask immediately with 0.1 *N* ceric sulfate. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* ceric sulfate is equivalent to 44.62 mg of $C_{12}H_{22}FeO_{14}$.

Arsenic Place 2 g of the sample in a 100-ml round bottom flask fitted with a 24/40 standard taper joint. Add 40 ml of dilute sulfuric acid (1 in 4) and 2 ml of 30% potassium bromide solution, and connect immediately to a modified Bethge distillation apparatus (see Fig. 3, page 464), or other suitable apparatus having a reservoir with a water jacket that is cooled with ice water. Heat the flask over an Argand burner until the sample dissolves, and collect 25 ml of distillate. Transfer the distillate to an arsine generator flask, and wash the condenser and reservoir several times with small portions of water. Add bromine TS until the distillate is slightly yellow, dilute to 35 ml with water, and continue as directed in the *Procedure* under *Arsenic Test*, page 464, using 6.0 ml of *Standard Arsenic Solution* in the preparation of the standard.

Chloride, page 471 Dissolve 1 g in 100 ml of water. Any turbidity produced by a 10-ml portion of this solution does not exceed that shown in a control containing 70 µg of chloride ion (Cl).

Ferric Iron Dissolve about 5 g, accurately weighed, in a mixture of 100 ml of water and 10 ml of hydrochloric acid in a 250-ml glass-stoppered flask, add 3 g of potassium iodide, shake well, and allow to stand in the dark for 5 min. Titrate any liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 5.585 mg of ferric iron.

Lead Determine as directed in the test for *Lead* under *Ferrous Fumarate*, page 121.

Loss on Drying, page 518 Dry at 105° for 4 h.

Mercury Determine as directed in the test for *Mercury* under *Ferrous Fumarate*, page 121.

Oxalic Acid Dissolve 1 g in 10 ml of water, add 2 ml of hydrochloric acid, transfer to a separator, and extract successively with 50 and 20 ml of ether. Combine the ether extracts, add 10 ml of water, and evaporate the ether on a steam bath. Add 1 drop of acetic acid (36%) and 1 ml of calcium acetate solution (1 in 20). No turbidity is produced within 5 min.

Reducing Sugars Dissolve 500 mg in 10 ml of water, warm, and make the solution alkaline with 1 ml of ammonia TS. Pass hydrogen sulfide gas into the solution to precipitate the iron, and allow the mixture to stand for 30 min to coagulate the precipitate. Filter, and wash the precipitate with two successive 5-ml portions of water. Acidify the combined filtrate and washings with hydrochloric acid, and add 2 ml of diluted hydrochloric acid TS in excess. Boil the solution until the vapors no longer darken lead acetate paper, and continue to boil, if necessary, until it has been concentrated to about 10 ml. Cool, add 5 ml of sodium carbonate TS and 20 ml of water, filter, and adjust the volume of the filtrate to 100 ml. To 5 ml of the filtrate add 2 ml of alkaline cupric tartrate TS, and boil for 1 min. No red precipitate is formed within 1 min.

Sulfate, page 471 Any turbidity produced by a 200-mg sample does not exceed that shown in a control containing 200 µg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement; color adjunct.

Ferrous Sulfate

$FeSO_4.7H_2O$ Mol wt 278.01

DESCRIPTION

Pale, bluish green crystals or granules. It is odorless, has a saline, styptic taste, and is efflorescent in dry air. In moist air it oxidizes readily to form brownish yellow basic ferric sulfate. Its 1 in 10 solution is acid to litmus, having a pH of about 3.7. One g dissolves in 1.5 ml of water at 25° and in 0.5 ml of boiling water. It is insoluble in alcohol.

REQUIREMENTS

Identification

It gives positive tests for *Ferrous Salts*, page 516, and for *Sulfate*, page 517.

Assay Not less than 99.5% and not more than the equivalent of 104.5% of $FeSO_4.7H_2O$.

Arsenic (as As) Not more than 3 ppm.

Lead Not more than 10 ppm.

Mercury Not more than 3 ppm.

TESTS

Assay Dissolve about 1 g, accurately weighed, in a mixture of 25 ml of diluted sulfuric acid TS and 25 ml of recently boiled and cooled water, add orthophenanthroline TS, and immediately titrate with 0.1 *N* ceric sulfate. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* ceric sulfate is equivalent to 27.80 mg of $FeSO_4.7H_2O$.

Arsenic Place 2 g of the sample in a 100-ml round bottom flask fitted with a 24/40 standard taper joint. Add 40 ml of dilute sulfuric acid (1 in 4) and 2 ml of 30% potassium bromide solution, and connect immediately to a modified Bethge distillation apparatus (see Fig. 3, page 464), or other suitable apparatus having a reservoir with a water jacket that is cooled with ice water. Heat the flask over an Argand burner until the sample dissolves, and collect 25 ml of distillate. Transfer the distillate to an arsine generator flask, and wash the condenser and reservoir several times with small portions of water. Add bromine TS until the distillate is slightly yellow, dilute to 35 ml with water, and continue as directed in the *Procedure* under *Arsenic Test*, page 465, using 6.0 ml of *Standard Arsenic Solution* in the preparation of the standard.

Lead Determine as directed in the test for *Lead* under *Ferrous Fumarate*, page 121.

Mercury Determine as directed in the test for *Mercury* under *Ferrous Fumarate*, page 121.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement.

Ferrous Sulfate, Dried

$FeSO_4.xH_2O$ Mol wt (anhydrous) 151.90

DESCRIPTION

A grayish white to buff-colored powder consisting primarily of $FeSO_4.H_2O$, with varying amounts of $FeSO_4.4H_2O$. It dissolves slowly in water but is insoluble in alcohol.

REQUIREMENTS

Identification

It gives positive tests for *Ferrous Salts*, page 516, and for *Sulfate*, page 517.

Assay Not less than 86.0% and not more than 89.0% of $FeSO_4$.
Arsenic (as As) Not more than 3 ppm.
Insoluble Substances Not more than 0.05%.
Lead Not more than 10 ppm.
Mercury Not more than 3 ppm.

TESTS

Assay Proceed as directed in the *Assay* under *Ferrous Sulfate*, page 123. Each ml of 0.1 *N* ceric sulfate is equivalent to 15.19 mg of $FeSO_4$.

Arsenic Place 2 g of the sample in a 100-ml round bottom flask fitted with a 24/40 standard taper joint. Add 40 ml of dilute sulfuric acid (1 in 4) and 2 ml of 30% potassium bromide solution, and connect immediately to a modified Bethge distillation apparatus (see Fig. 3, page 464), or other suitable apparatus having a reservoir with a water jacket that is cooled with ice water. Heat the flask over an Argand burner until the sample dissolves, and collect 25 ml of distillate. Transfer the distillate to an arsine generator flask, and wash the condenser and reservoir several times with small portions of water. Add bromine TS until the distillate is slightly yellow, dilute to 35 ml with water, and continue as directed in the *Procedure* under *Arsenic Test*, page 464, using 6.0 ml of *Standard Arsenic Solution* in the preparation of the standard.

Insoluble Residue Dissolve 2 g in 20 ml of freshly boiled dilute sulfuric acid (1 in 100), heat to boiling, and then digest in a covered beaker on a steam bath for 1 h. Filter through a tared filtering crucible, wash thoroughly, and dry at 105°. The weight of the insoluble residue does not exceed 1 mg.

Lead Determine as directed in the test for *Lead* under *Ferrous Fumarate*, page 121.

Mercury Determine as directed in the test for *Mercury* under *Ferrous Fumarate*, page 121.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Fir Needle Oil, Canadian Type

Balsam Fir Oil

DESCRIPTION

The volatile oil obtained by steam distillation from needles and twigs of *Abies balsamea* L., Mill (Fam. *Pinaceae*). It is a colorless to faintly yellow liquid having a pleasant balsamlike odor. It is soluble in most fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 594, using the same test conditions as specified therein.

Assay Not less than 8.0% and not more than 16.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −19° and −24°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.473 and 1.476 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.872 and 0.878.

TESTS

Assay Weigh accurately about 5 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 90% alcohol, occasionally with haziness.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitable containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Fir Needle Oil, Siberian Type

Pine Needle Oil

DESCRIPTION

The volatile oil obtained by steam distillation from needles and twigs of *Abies sibirica* Lebed. (Fam. *Pinaceae*). It is an almost colorless or faintly yellow liquid. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 595, using the same test conditions as specified therein.

Assay Not less than 32.0% and not more than 44.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −33° and −45°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.468 and 1.473 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.898 and 0.912.

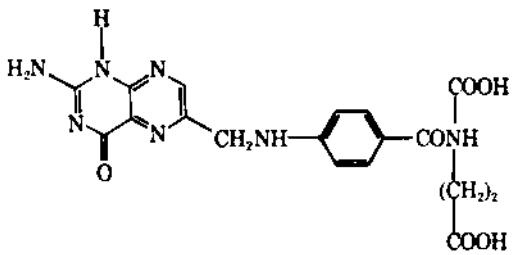
TESTS

Assay Weigh accurately about 2 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 90% alcohol. Occasionally the solution may become hazy upon further dilution.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Folic Acid

N-[4-[[(2-Amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl] amino]benzoyl]-L-glutamic Acid;
N-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino] benzoyl]glutamic Acid;
Pteroylglutamic Acid; Folacin



$C_{19}H_{19}N_7O_6$ Mol wt 441.40

DESCRIPTION

Yellow or yellowish orange, odorless crystals or crystalline powder. About 1.6 mg dissolves in 1 ml of water. It is insoluble in acetone, in alcohol, in chloroform, and in ether, but dissolves in solutions of alkali hydroxides and carbonates. The pH of a suspension of 1 g in 10 ml of water is between about 4.0 and 4.8.

REQUIREMENTS

Identification

The ultraviolet absorption spectrum of a 1 in 100,000 solution of the sample in sodium hydroxide solution (1 in 250) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Folic Acid Reference Standard, concomitantly measured. The ratio A_{256}/A_{365} is between 2.80 and 3.00.

Assay Not less than 95.0% and not more than 102.0% of $C_{19}H_{19}N_7O_6$, calculated on the anhydrous basis.
Residue on Ignition Not more than 0.3%.
Water Not more than 8.5%.

TESTS

Assay

Standard Solution Weigh accurately about 30 mg of USP Folic Acid Reference Standard, corrected for water content, and dissolve in an aqueous solvent containing 2 ml of ammonium hydroxide and 1 g of sodium perchlorate per 100 ml. Using the same solvent, adjust the volume quantitatively, according to the injection size to be used in the *Procedure*, so that between 5 and 20 µg of folic acid is chromatographed.

Sample Solution Prepare as directed for the *Standard Solution*, using an accurately weighed quantity of the sample, and adjust to the same volume as the *Standard Solution*.

Mobile Phase Transfer 35.1 g of sodium perchlorate, 1.40 g of monobasic potassium phosphate, 7.0 ml of 1 *N* potassium

hydroxide, and 40 ml of methanol to a 1000-ml volumetric flask, dilute with water to volume, and mix. Adjust the pH to 7.2 with 1 *N* potassium hydroxide. (NOTE: The methanol concentration may be varied to meet system suitability requirements and to provide a suitable elution time for folic acid.)

Chromatographic System Typically, a high-pressure liquid chromatograph, operated at room temperature, is fitted with a 25- to 30-cm × 4-mm stainless steel column packed with octadecyl silane chemically bonded to porous silica or ceramic microparticles 5 to 10 μm in diameter. The mobile phase is maintained at a pressure and flow rate capable of giving the required resolution (see *System Suitability Test*) and a suitable elution time. An ultraviolet detector that monitors absorption at 254 nm is used.

System Suitability Solution Prepare a solution containing about 1 mg per ml each of USP Folic Acid Reference Standard and USP Calcium Formyltetrahydrofolate Authentic Substance in an aqueous solvent containing 2 ml of ammonium hydroxide and 1 g of sodium perchlorate per 100 ml. Filter the solution before use through a membrane filter of 1-μm porosity or finer.

System Suitability Test Chromatograph five injections of equal volume, up to 25 μl, of the *Standard Solution*, and measure the peak response as directed in the *Procedure*. The relative standard deviation, calculated by the formula 100 × (standard deviation/mean peak response), for the peak response does not exceed 2%. Inject a volume, up to 25 μl, of the *System Suitability Solution* in a similar manner. The resolution factor between calcium formyltetrahydrofolate and folic acid, calculated by the formula given for *R* on page 471 of the general tests in *Chromatography*, is not less than 3.6. (NOTE: For a particular column, resolution may be increased by decreasing the amount of methanol in the mobile phase.)

Procedure Introduce equal volumes, up to 25 μl, of the *Sample Solution* and *Standard Solution* into the chromatograph by means of a suitable sampling valve or high-pressure microsyringe. Measure the responses for the major peaks obtained with the *Sample Solution* and the *Standard Solution*. Calculate the quantity, in mg, of $C_{19}H_{19}N_7O_6$ in the sample taken by the formula $VC \times (P_U/P_S)$, in which V is the volume of the *Sample Solution*, in ml; C is the concentration of USP Folic Acid Reference in the *Standard Solution*, in mg per ml; and P_U and P_S are the peak responses of the solutions from the *Sample Solution* and the *Standard Solution*, respectively.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552, using a 200-mg sample.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Food Starch, Modified

Modified Food Starch; Food Starch-Modified

DESCRIPTION

Modified food starches are products of the treatment of any of several grain- or root-based native starches (e.g., corn, sorghum, wheat, potato, tapioca, sago, etc.) with small amounts of certain chemical agents, which modify the physical characteristics of the native starches to produce desirable properties.

Starch molecules are polymers of anhydroglucose and occur in both linear and branched form. The degree of polymerization and, accordingly, the molecular weight of the naturally occurring starch molecules vary radically. Furthermore, they vary in the ratio of branched chain polymers (amylopectin) to linear chain polymers (amylose), both within a given type of starch and from one type to another. These factors, in addition to any type of chemical modification used, affect the viscosity, texture, and stability of the starch sols significantly.

Starch is chemically modified by mild degradation reactions or by reactions between the hydroxyl groups of the native starch and the reactant selected. One or more of the following processes are used: mild oxidation (bleaching), moderate oxidation, acid depolymerization, monofunctional esterification, polyfunctional esterification (cross-linking), monofunctional etherification, polyfunctional etherification (cross-linking), alkaline gelatinization, and certain combinations of these treatments. These methods of preparation can be used as a basis for classifying the starches thus produced (see *Additional Requirements* below). Generally, however, the products are called modified food starch, or food starch-modified.

Modified food starches are usually produced as white or nearly white, tasteless, odorless powders, as intact granules, and, if pregelatinized (i.e., subjected to heat treatment in the presence of water), as flakes, amorphous powders, or coarse particles. Modified food starches are insoluble in alcohol, in ether, and in chloroform. If not pregelatinized, they are practically insoluble in cold water. Upon heating in water, the granules usually begin to swell at temperatures between 45° and 80°, depending upon the botanical origin and the degree of modification. They gelatinize completely at higher temperatures. Pregelatinized starches hydrate in cold water.

REQUIREMENTS

Identification

A. Suspend about 1 g of the sample in 20 ml of water, and add a few drops of iodine TS. A dark blue to red color is produced.

B. Place about 2.5 g of the sample in a boiling flask, add 10 ml of dilute hydrochloric acid (3%) and 70 ml of water, mix, reflux for about 3 h, and cool. Add 0.5 ml of the resulting solution to 5 ml of hot alkaline cupric tartrate TS. A copious red precipitate is produced.

C. Examine a portion of the sample with a polarizing microscope in polarized light under crossed Nicol prisms. The

typical polarization cross is usually observed, except in the case of pregelatinized starches.

Arsenic (as As) Not more than 3 ppm.
Crude Fat Not more than 0.15%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 5 ppm.
Loss on Drying *Cereal starch:* not more than 15%; *potato starch:* not more than 21%; *sago and tapioca starch:* not more than 18%.
pH of Dispersions Between 3.0 and 9.0.
Protein Not more than 0.5%, except not more than 1% in modified high-amylose starches.
Sulfur Dioxide Not more than 0.008%.

Additional Requirements

The modified food starches listed below according to method of preparation must meet all of the above *Requirements* in addition to the specified methods of *Treatment* (the reagent for which, if not specifically limited, should not exceed the amount reasonably required to accomplish the intended modification) and any requirements for *Residuals Limitation*.

Acid Depolymerization (Thin-Boiling, or Acid-Modified Starch) This treatment results in partial depolymerization, causing a reduction in viscosity. The treatment may be used in combination with the other treatments that follow.

Treatment to Produce Thin-Boiling Starch	*Residuals Limitation*
Hydrochloric acid and/or sulfuric acid	—

Alkaline Gelatinization (Gelatinized Starch)

Treatment to Produce Gelatinized Starch	*Residuals Limitation*
Sodium hydroxide, not to exceed 1%	—

Etherification and Esterification (Starch Ether-Esters) The starch ether-esters are named individually, depending upon their method of preparation.

Treatment to Produce Acetylated Distarch Glycerol	*Residuals Limitation*
Acrolein, not to exceed 0.6%, and vinyl acetate, not to exceed 7.5%	Not more than 2.5% of acetyl groups introduced into finished product
Epichlorohydrin, not to exceed 0.3%, and acetic anhydride	Not more than 2.5% of acetyl groups introduced into finished product
Epichlorohydrin, not to exceed 0.3%, and succinic anhydride, not to exceed 4%	—

Treatment to Produce Hydroxypropyl Distarch Phosphate	*Residuals Limitation*
Phosphorus oxychloride, not to exceed 0.1%, and propylene oxide, not to exceed 10%	Not more than 5 ppm of residual propylene chlorohydrin

Etherification with Oxidation (Oxidized Starch Ethers)

Treatment to Produce Oxidized Hydroxypropyl Starch	*Residuals Limitation*
Chlorine, as sodium hypochlorite, not to exceed 0.055 lb (25 g) of chlorine per lb (454 g) of dry starch; active oxygen obtained from hydrogen peroxide, not to exceed 0.45%; and propylene oxide, not to exceed 25%	Not more than 5 ppm of residual propylene chlorohydrin

Mild Oxidation (Bleached Starch) The starches resulting from mild oxidation are not altered chemically; in all cases, extraneous color bodies are oxidized, solubilized, and removed by washing and filtration. These treatments may be used in combination with the other forms of treatment listed herein.

Treatment to Produce Bleached Starch	*Residuals Limitation*
Active oxygen obtained from hydrogen peroxide, and/or peracetic acid, not to exceed 0.45% of active oxygen	—
Ammonium persulfate, not to exceed 0.075%, and sulfur dioxide, not to exceed 0.05%	—
Chlorine, as sodium hypochlorite, not to exceed 0.0082 lb (3.72 g) of chlorine per lb (454 g) of dry starch	—
Potassium permanganate, not to exceed 0.2%	Not more than 0.005% of residual manganese (as Mn)
Sodium chlorite, not to exceed 0.5%	—

Moderate Oxidation (Oxidized Starch) The maximum specified treatment introduces about 1 carboxyl group per 28 anhydroglucose units. The starch is whitened, and its molecular weight and viscosity are reduced.

Treatment to Produce Oxidized Starch	*Residual Limitation*
Chlorine, as sodium hypochlorite, not to exceed 0.055 lb (25 g) of chlorine per lb (454 g) of dry starch	—

Monofunctional and/or Polyfunctional Esterification (Starch Esters) The starch esters are named individually, depending upon the method of preparation.

Treatment to Produce Starch Acetate	*Residuals Limitation*
Acetic anhydride or vinyl acetate	Not more than 2.5% of acetyl groups introduced into finished product

Treatment to Produce Acetylated Distarch Adipate	*Residuals Limitation*
Adipic anhydride, not to exceed 0.12%, and acetic anhydride	Not more than 2.5% of acetyl groups introduced into finished product

Treatment to Produce Starch Phosphate	*Residuals Limitation*
Monosodium orthophosphate	Not more than 0.4% of residual phosphate (calculated as P)

Treatment to Produce Starch Sodium Octenyl Succinate	*Residuals Limitation*
1-Octenyl succinic anhydride, not to exceed 3%	—

Treatment to Produce Starch Aluminum Octenyl Succinate	*Residuals Limitation*
1-Octenyl succinic anhydride, not to exceed 2%, and aluminum sulfate, not to exceed 2%	—

Treatment to Produce Distarch Phosphate	*Residuals Limitation*
Phosphorus oxychloride, not to exceed 0.1%	—
Sodium trimetaphosphate	Not more than 0.04% of residual phosphate (calculated as P)

Treatment to Produce Phosphated Distarch Phosphate	*Residuals Limitation*
Sodium tripolyphosphate and sodium trimetaphosphate	Not more than 0.4% of residual phosphate (calculated as P)

Treatment to Produce Acetylated Distarch Phosphate	*Residuals Limitation*
Phosphorus oxychloride, not to exceed 0.1%, followed by either acetic anhydride, not to exceed 8%, or vinyl acetate, not to exceed 7.5%	Not more than 2.5% of acetyl groups introduced into finished product

Treatment to Produce Starch Sodium Succinate	*Residuals Limitation*
Succinic anhydride, not to exceed 4%	—

Monofunctional and/or Polyfunctional Etherification (Starch Ethers-Hemiacetals, or Ethers) The starch ethers are named individually, depending upon the method of preparation.

Treatment to Produce Distarch-oxy Propanol	*Residuals Limitation*
Acrolein, not to exceed 0.6%	—

Treatment to Produce Distarch Glycerol	*Residuals Limitation*
Epichlorohydrin, not to exceed 0.3%	—

Treatment to Produce Hydroxypropyl Distarch Glycerol	*Residuals Limitation*
Epichlorohydrin, not to exceed 0.1%, and propylene oxide, not to exceed 10%, added in combination or in any sequence	Not more than 5 ppm of residual propylene chlorohydrin

Treatment to Produce Hydroxypropyl Starch	*Residuals Limitation*
Propylene oxide, not to exceed 25%	Not more than 5 ppm of residual propylene chlorohydrin

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Crude Fat Determine as directed in the general method, page 543.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*) and 500° as the ignition temperature.

Lead Transfer 2.0 g of the sample to an evaporating dish, add 1 ml of sulfuric acid solution (1 in 4), distributing it evenly through the sample, and evaporate most of the water on a steam bath. Char and dehydrate the sample by heating on a hot plate, while heating at the same time with an infrared lamp from above, and then heat in a muffle furnace at 500°

until the residue is free from carbon. Remove the dish from the furnace, cool, and cautiously wash down the inside of the dish with water. Add 1 ml of 1 *N* hydrochloric acid, evaporate to dryness on a steam bath, then add 2 ml of 1 *N* hydrochloric acid, and heat briefly, while stirring, on a steam bath. Quantitatively transfer the solution into a separator with the aid of small quantities of water, and neutralize with 1 *N* ammonium hydroxide. This *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry a 5-g sample in a vacuum oven, not exceeding 100 mm of Hg, at 120° for 4 h.

pH of Dispersions Mix 20 g of the sample with 80 ml of water, and agitate continuously at a moderate rate for 5 min. (In the case of pregelatinized starches, 3 g should be suspended in 97 ml of water.) Determine the pH of the resulting suspension by the *Potentiometric Method*, page 531. (NOTE: The distilled water used for sample dispersion should require not more than 0.05 ml of 0.1 *N* acid or alkali per 200 ml to obtain the methyl red or phenolphthalein endpoint, respectively.)

Protein Transfer about 10 g of the sample, accurately weighed, into an 800-ml Kjeldahl flask, and add 10 g of anhydrous potassium or sodium sulfate, 300 mg of copper selenite or mercuric oxide, and 60 ml of sulfuric acid. Gently heat the mixture, keeping the flask inclined at about a 45° angle, and after frothing has ceased, boil briskly until the solution has remained clear for about 1 h. Cool, add 30 ml of water, mix, and cool again. Cautiously pour about 75 ml (or enough to make the mixture strongly alkaline) of sodium hydroxide solution (2 in 5) down the inside of the flask so that it forms a layer under the acid solution, and then add a few pieces of granular zinc. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of an accurately measured excess of 0.1 *N* sulfuric acid contained in a 50-ml flask. Gently rotate the contents of the Kjeldahl flask to mix, and distil until all ammonia has passed into the absorbing acid solution (about 250 ml of distillate). To the receiving flask add 0.25 ml of methyl red–methylene blue TS, and titrate the excess acid with 0.1 *N* sodium hydroxide. (NOTE: Titrate with 0.1 *N* sulfuric acid if boric acid was used to absorb the ammonia.) Perform a blank determination, substituting pure sucrose or dextrose for the sample, and make any necessary correction (see page 2). Each ml of 0.1 *N* sulfuric acid consumed is equivalent to 1.401 mg of nitrogen (N). Calculate the percent N in the sample, and then calculate the percent protein by multiplying the percent N by 6.25, in the case of starches obtained from corn, or by 5.7, in the case of starches obtained from wheat. Other factors may be applied as necessary for starches obtained from other sources.

Sulfur Dioxide Determine as directed in the general method, page 546.

TESTS (Additional Requirements)

Acetyl Groups Determine the content of acetyl groups in starch acetate, acetylated distarch adipate, acetylated distarch phosphate, and acetylated distarch glycerol as directed in the general method, page 543.

Manganese Determine the residual manganese in bleached starch prepared with potassium permanganate as directed in the general method, page 543.

Phosphate Determine the residual phosphate (calculated as P) in starch phosphate, distarch phosphate, and phosphated distarch phosphate as directed in the general method, page 544.

Propylene Chlorohydrin Determine the residual propylene chlorohydrin in hydroxypropyl distarch glycerol, hydroxypropyl starch, hydroxypropyl starch phosphate, and oxidized hydroxypropyl starch as directed in the general method, page 544.

Packaging and Storage Store in well-closed containers.

Functional Use of Foods Thickener; colloidal stabilizer; binder.

Formic Acid

HCOOH

CH_2O_2 Mol wt 46.03

DESCRIPTION

A colorless, *highly corrosive* liquid having a characteristic pungent odor. It is miscible with water, with alcohol, with glycerin, and with ether. Its specific gravity is about 1.20.

REQUIREMENTS

Identification

To 5 ml add 2 ml of mercuric chloride TS, and warm the mixture. A white precipitate of mercurous chloride forms.

Assay Not less than 85.0% of CH_2O_2.

Acetic Acid Not more than 0.4%.

Arsenic (as As) Not more than 3 ppm.

Dilution Test Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.

Sulfate Not more than 0.004%.

TESTS

Assay Tare a small glass-stoppered Erlenmeyer flask containing about 15 ml of water. Transfer about 1.5 ml of the sample into the flask and weigh. Dilute the solution of the sample to 50 ml, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 46.03 mg of CH_2O_2.

Acetic Acid Dilute 1 ml (1.2 g) to 100 ml with water, transfer 50 ml of this solution into a 250-ml boiling flask, and add 5 g of yellow mercuric oxide. Boil the mixture under a reflux condenser with continuous stirring for 2 h, cool, filter, and

wash the residue with about 25 ml of water. To the combined filtrate and washings add phenolphthalein TS and titrate with 0.02 *N* sodium hydroxide. Not more than 2.0 ml of 0.02 *N* sodium hydroxide is required to produce a pink color.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Dilution Test Dilute 1 volume of the sample with 3 volumes of water. No turbidity is observed within 1 h.

Heavy Metals To 1.7 ml (2 g) in a beaker add about 10 mg of sodium carbonate, evaporate to dryness on a steam bath, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

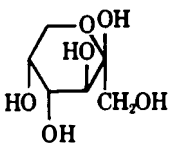
Sulfate, page 471 To 2.1 ml (2.5 g) in a beaker add about 10 mg of sodium carbonate and evaporate to dryness on a steam bath. Any turbidity produced by the residue does not exceed that shown in a control containing 100 µg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Functional Use in Foods Flavoring adjunct.

Fructose

d-Fructose; Levulose; Fruit Sugar



$C_6H_{12}O_6$ Mol wt 180.16

DESCRIPTION

White, hygroscopic, odorless crystals or crystalline powder having a sweet taste. Its density is about 1.6. It is soluble in methanol and ethanol, and is freely soluble in water.

REQUIREMENTS

Identification

A. To 5 ml of hot alkaline cupric tartrate TS add a few drops of a 1 in 10 solution of the sample. A copious red precipitate of cuprous oxide is formed.

B. Heat to about 60° a mixture of 1 g of phenylhydrazine hydrochloride and 1.5 g of sodium acetate with 10 ml of water, cool, and filter. To 5 ml of this solution in a test tube add 1.5 ml of a 1 in 10 solution of the sample, heat in a boiling water bath for 5 min, then cool, and filter. Wash the precipitate with three 10-ml portions of water followed by single washings of 5 ml each of alcohol and of ether, and dry the residue at 105° for 30 min. The yellow osazone so obtained melts between 205° and 210° with decomposition.

Assay Not less than 98.0% and not more than 102.0% of fructose ($C_6H_{12}O_6$).

Arsenic (as As) Not more than 1 ppm.

Chloride Not more than 0.018%.

Glucose Not more than 0.5%.

Heavy Metals (as Pb) Not more than 10 ppm.

Hydroxymethylfurfural Passes test.

Loss on Drying Not more than 0.5%.

Residue on Ignition Not more than 0.5%.

Sulfate Not more than 0.025%.

TESTS

Assay Transfer about 10 g of the sample, previously dried in vacuum at 70° for 4 h and accurately weighed, into a 100-ml volumetric flask, dissolve in 50 ml of water, add 0.2 ml of stronger ammonia TS, dilute to volume with water, and mix. After 30 min, determine the angular rotation (see page 530) in a 100-mm (or 200-mm) tube at 25° with the sodium D line. The observed rotation, in degrees (absolute value), multiplied by 1.110 (or 0.562 for the 200-mm tube), represents the weight, in g, of fructose ($C_6H_{12}O_6$) in the sample taken.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464, using 1 ml of *Standard Arsenic Solution* (1 µg As).

Chloride, page 471 Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 0.5 ml of 0.02 *N* hydrochloric acid.

Glucose

Reagent Solution Dissolve 40 mg of *o*-dianisidine dihydrochloride, 40 mg of horseradish peroxidase (Worthington Biochemical Co., Freehold, N.J., or equivalent), and 0.4 ml of purified glucose oxidase (1000 glucose oxidase units per ml, Miles Laboratories, Inc., or equivalent) in 0.1 *M* acetate buffer (prepared as follows: dissolve 13.608 g of sodium acetate trihydrate in sufficient water to make 1000 ml, add 2.7 ml of acetic acid, and adjust the pH to 5.5 with acetic acid or sodium acetate), and dilute to 100 ml with the buffer solution. (NOTE: Commercially available preparations containing the reagents in the proper proportions may also be used.)

Glucose Standard Solution Transfer 300 mg of USP Dextrose Reference Standard, previously dried in vacuum at 70° for 4 h and accurately weighed, into a 1000-ml volumetric flask, dissolve in and dilute to volume with water, and mix. Allow to stand for 2 h to allow mutarotation to occur, then transfer 20.0 ml to a 100-ml volumetric flask, dilute to volume with water, and mix. Prepare fresh on the day of use.

Sample Preparation Transfer 14 g of sample, accurately weighed, into a 100-ml volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 20.0 ml into a second 100-ml volumetric flask, dilute to volume with water, and mix.

Procedure Pipet 2 ml each of the *Sample Preparation*, the *Glucose Standard Solution*, and water into separate 18- × 150-mm test tubes. Heat the tubes in a water bath maintained at 30° for 5 min, and at zero time start the reaction by adding 1.0 ml of the *Reagent Solution* to the first tube. Allow 30- and 60-s intervals between enzyme addition to each tube, and add 1.0 ml of the *Reagent Solution* to each of the remaining tubes. Mix the content of the tubes, and allow them to react for

exactly 30 min from zero time. Immediately stop the reaction in the first tube by adding 10.0 ml of 25% sulfuric acid. Similarly, add 10.0 ml of 25% sulfuric acid to the remaining tubes after they have reacted for exactly 30 min. Mix the contents of the tubes, and cool them to room temperature. Using a suitable spectrophotometer, determine the absorbances of the sample and standard mixtures at 540 nm against the reagent mixture in the reference cell. Calculate the percentage of glucose in the sample by the formula $(50C/W) \times A_U/A_S$, in which C is the exact concentration of the *Glucose Standard Solution*, in mg per ml, W is the weight of sample taken, in g, A_U is the absorbance of the mixture obtained from the *Sample Preparation*, and A_S is the absorbance of the mixture obtained from the *Glucose Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxymethylfurfural The absorbance of a 1 in 20 solution of the sample, determined with a suitable spectrophotometer at 284 nm in a 1-cm cell, is not more than 0.32.

Loss on Drying, page 518 Dry in vacuum at 70° for 4 h.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Sulfate, page 471 Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 0.5 ml of 0.02 *N* sulfuric acid.

Packaging and Storage Store in tight containers protected from humidity.

Functional Use in Foods Nutritive sweetener; processing aid; formulation aid.

Fumaric Acid

trans-Butenedioic Acid; *trans*-1,2-Ethylenedicarboxylic Acid

$C_4H_4O_4$ Mol wt 116.07

DESCRIPTION

White, odorless granules or crystalline powder. It is soluble in alcohol, slightly soluble in water and in ether, and very slightly soluble in chloroform.

REQUIREMENTS

Assay Not less than 99.5% of $C_4H_4O_4$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Maleic Acid Not more than 0.1%.

Residue on Ignition Not more than 0.1%.

Water Not more than 0.5%.

TESTS

Assay Transfer about 1 g, accurately weighed, into a 250-ml Erlenmeyer flask, add 50 ml of methanol, and dissolve the sample by warming gently on a steam bath. Cool, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a pink color that persists for at least 30 s. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.5 *N* sodium hydroxide is equivalent to 29.02 mg of $C_4H_4O_4$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 2 g in a mixture of 10 ml of water and 15 ml of ammonia TS. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Maleic Acid

Buffer Solution Dissolve 53.5 g of ammonium chloride in about 900 ml of water, adjust the pH to 8.2 with approximately 0.3 *N* ammonium hydroxide, and dilute with water to 1000 ml.

Standard Solution Transfer to a 100-ml volumetric flask about 100 mg, accurately weighed, of maleic acid of the highest purity available, dissolve in about 10 ml of water, then dilute to volume with water, and mix.

Sample Solution Transfer about 50 g of the sample, accurately weighed, into a 250-ml beaker, add 80 ml of water, and stir for 10 min with a mechanical stirrer. Filter, using suction, and wash with about 40 ml of water. Transfer the combined filtrate and washings to a 250-ml beaker, add an additional 50-g sample, accurately weighed, to the beaker, and repeat the stirring, filtration, and washing procedure. Transfer the combined filtrate and washings to a 250-ml volumetric flask, add 2 drops of phenolphthalein TS, then add sodium hydroxide TS, with stirring, until a light pink color persists for at least 30 s, and dilute to volume with water.

Procedure Transfer 10.0 ml of the *Sample Solution* into a 100-ml volumetric flask, add 20 ml of *Buffer Solution*, dilute to volume with water, and mix (*Solution A*). Rinse a polarographic cell with a portion of the solution, then add a suitable volume of the solution to the cell, immerse it in a water bath regulated at 24.5° to 25.5°, and deaerate by bubbling purified nitrogen through the solution for at least 6 min. Insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from −1 to −2 V, using a saturated calomel electrode as the reference electrode. Determine the height of the wave occurring at the half-wave potential near −1.36 V. In the same manner polarograph a solution prepared by adding 10.0 ml of the *Sample Solution*, 20 ml of the *Buffer Solution*, and 2.0 ml of the *Standard Solution* to a 100-ml volumetric flask and diluting to volume with water (*Solution B*). Calculate the weight, in mg, of maleic acid in the total weight of sample taken by the formula

$$2500C \times A/(B - A),$$

in which *A* is the wave height of *Solution A*, *B* is the wave height of *Solution B*, and *C* is the concentration, in mg per ml, of added maleic acid in *Solution B*.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Acidifier; flavoring agent.

Garlic Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the crushed bulbs or cloves of the common garlic plant, *Allium sativum* L. (Fam. *Liliaceae*). It is a clear yellow to reddish orange liquid having a strong pungent odor and a flavor characteristic of garlic. It is soluble in most fixed oils and in mineral oil. It may be incompletely soluble in alcohol. It is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 595, using the same test conditions as specified therein.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.559 and 1.579 at 20°.

Specific Gravity Between 1.040 and 1.090.

TESTS

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Geranium Oil, Algerian Type

Rose Geranium Oil, Algerian Type

DESCRIPTION

The oil obtained by steam distillation from the leaves of *Pelargonium graveolens* L'Her. (Fam. *Geraniaceae*). It is a light yellow to deep yellow liquid having a characteristic odor resembling rose and geraniol. It is soluble in most fixed oils, and it is soluble, usually with opalescence, in mineral oil and in propylene glycol. It is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 595, using the same test conditions as specified therein.

Assay Not less than 13.0% and not more than 29.5% of esters, calculated as geranyl tiglate ($C_{15}H_{24}O_2$).

Acid Value Between 1.5 and 9.5.

Angular Rotation Between −7° and −13°.

Ester Value after Acetylation Between 203 and 234.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.464 and 1.472 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.886 and 0.898.

TESTS

Assay Proceed as directed under *Ester Value*, page 501, using about 6 g, accurately weighed. The ester value multiplied by 0.422 equals the percentage of geranyl tiglate ($C_{15}H_{24}O_2$).

Acid Value Determine as directed in the general method, page 499, using about 5 g, accurately weighed, and modifying the procedure by using 15 ml of water, instead of alcohol, as diluent and by agitating the mixture thoroughly during the titration to keep the oil in suspension.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value after Acetylation Proceed as directed under *Total Alcohols*, page 499, using about 1.9 g of the acetylated oil, accurately weighed, for saponification. Calculate the ester value after acetylation by the formula $A \times 28.05/B$, in which *A* is the number of ml of 0.5 *N* alcoholic potassium hydroxide consumed in the saponification, and *B* is the weight of acetylated oil, in g.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

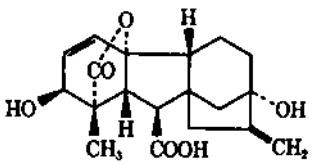
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 70% alcohol, but on further dilution with the alcohol opalescence may occur, sometimes followed by separation of paraffin particles.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Gibberellic Acid



$C_{19}H_{22}O_6$ Mol wt 346.37

DESCRIPTION

A white to pale yellow, odorless or practically odorless, crystalline powder. It is slightly soluble in water and is soluble in alcohol and in acetone. It melts at about 234°.

REQUIREMENTS

Identification

Dissolve a few mg of the sample in 2 ml of sulfuric acid. A reddish solution having a green fluorescence is formed.

Assay Not less than 90.0% of $C_{19}H_{22}O_6$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 3%.
Specific Rotation $[\alpha]_D^{20}$: Between +75.0° and +90.0°.

TESTS

Assay

Standard Preparation Transfer an accurately weighed quantity of FCC Gibberellic Acid Reference Standard, equivalent to about 25 mg of pure gibberellic acid (corrected for phase purity and volatiles content), into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Transfer 10.0 ml of this solution into a second 50-ml volumetric flask, dilute to volume with methanol, and mix.

Assay Preparation Transfer about 40 mg of the sample, accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Transfer 10.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with methanol, and mix.

Procedure Transfer 5.0 ml of the *Assay Preparation* into a 25- × 200-mm glass-stoppered tube, and transfer 4.0-ml and 5.0-ml portions of the *Standard Preparation* into separate similar tubes. Place the tubes in a boiling water bath, evaporate to dryness, and then dry in an oven at 90° for 10 min. Remove the tubes from the oven, stopper, and allow to cool to room temperature. Dissolve the residue in each tube in 10.0 ml of dilute sulfuric acid (8 in 10), heat in a boiling water bath for 10 min, and then cool in a 10° water bath for 5 min. Determine the absorbance of the solutions in 1-cm cells at 535 nm with a suitable spectrophotometer, using the dilute sulfuric acid as the blank. Record the absorbance of the solution from the *Assay Preparation* as A_U. Note the absorbance of the two solutions prepared from the 4.0-ml and 5.0-ml aliquots of the *Standard Preparation*, and record the absorbance of the final solution giving the value nearest to that of the sample as A_S; record as V the volume of the aliquot used in preparing this solution. Calculate the quantity, in mg, of $C_{19}H_{22}O_6$ in the sample taken by the formula

$$500C \times (V/5) \times (A_U/A_S),$$

in which C is the exact concentration, in mg per ml, of the *Standard Preparation*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 100° in vacuum for 7 h.

Specific Rotation, page 530 Determine in a solution in alcohol containing 100 mg in each ml. Avoid the use of heat in preparing the solution.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Enzyme activator.

Ginger Oil

DESCRIPTION

The volatile oil obtained by steam distillation of the dried ground rhizome of *Zingiber officianale*, Roscoe (Fam. *Zingiberaceae*). It is a light yellow to yellow liquid having the aromatic, characteristic odor of ginger. It is soluble in most fixed oils and in mineral oil. It is soluble, usually with turbidity, in alcohol, but it is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 595, using the same test conditions as specified therein.

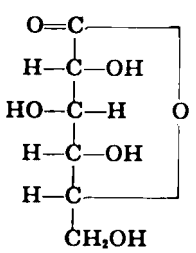
Angular Rotation Between −28° and −47°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.488 and 1.494 at 20°.
Saponification Value Not more than 20.
Specific Gravity Between 0.870 and 0.882.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass, aluminum, or tin-lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Glucono Delta-Lactone



$C_6H_{10}O_6$ Mol wt 178.14

DESCRIPTION

A fine, white, practically odorless, crystalline powder. It is freely soluble in water and is sparingly soluble in alcohol. It decomposes at about 153°.

REQUIREMENTS

Identification

Dissolve about 500 mg in 5 ml of warm water in a test tube, add 1 ml of freshly distilled phenylhydrazine, heat on a steam bath for 30 min, and allow to cool. Induce crystallization, if necessary, by scratching the inner surface of the tube with a glass rod. Crystals of gluconic acid phenylhydrazide form.

Assay Not less than 99.0% of $C_6H_{10}O_6$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Reducing Substances (as D-glucose) Not more than 0.5%.

TESTS

Assay Dissolve about 6 g, accurately weighed, in 100 ml of water in a 300-ml Erlenmeyer flask, add 50.0 ml of 1 *N* sodium hydroxide, and allow to stand for 15 min. Add phenolphthalein TS, and titrate the excess alkali with 1 *N* hydrochloric acid. Perform a blank determination (see page 2). Each ml of 1 *N* hydrochloric acid is equivalent to 178.1 mg of $C_6H_{10}O_6$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Reducing Substances Weigh accurately 10.0 g into a 400-ml beaker, dissolve the sample in 40 ml of water, add phenolphthalein TS, and neutralize with sodium hydroxide solution (1 in 2). Dilute to 50.0 ml with water, and add 50 ml of alkaline cupric tartrate TS. Heat the mixture on an asbestos gauze over a Bunsen burner, regulating the flame so that boiling begins in 4 min, and continue the boiling for exactly 2 min. Filter through a Gooch crucible, wash the filter with 3 or more small portions of water, and place the crucible in an upright position in the original beaker. Add 5 ml of water and 3 ml of nitric acid to the crucible, mix with a stirring rod to ensure complete solution of the cuprous oxide, and wash the solution into a beaker with several ml of water. To the beaker add sufficient bromine TS (5 to 10 ml) until the color becomes yellow, and dilute with water to about 75 ml. Add a few glass beads, boil over a Bunsen burner until the bromine is completely removed, and cool. Slowly add ammonium hydroxide until a deep blue color appears, then adjust the pH to approximately 4 with glacial acetic acid, and dilute to about 100 ml with water. Add 4 g of potassium iodide, and titrate with 0.1 *N* sodium thiosulfate, adding starch TS just before the endpoint is reached. Not more than 16.1 ml is required.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Acid; leavening agent; sequestrant.

L-Glutamic Acid

Glutamic Acid; L-2-Aminopentanedioic Acid

$HOOCCH_2CH_2CH(NH_2)COOH$

$C_5H_9NO_4$ Mol wt 147.13

DESCRIPTION

A white, practically odorless, free-flowing, crystalline powder. It is slightly soluble in water, forming acidic solutions. The pH of a saturated solution is about 3.2.

REQUIREMENTS

Identification

A. Dissolve about 150 mg in a mixture of 4 ml of water and 1 ml of sodium hydroxide TS, add 1 ml of ninhydrin TS and 100 mg of sodium acetate, and heat in a boiling water bath for 10 min. An intense, violet blue color is formed.

B. The glutamic acid dissolves completely on stirring when either 5.6 ml of 1 *N* hydrochloric acid or 6.8 ml of 1 *N* sodium hydroxide is added to a suspension of 1 g of the sample in 9 ml of water.

Assay Not less than 99.0% of $C_5H_9NO_4$.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.2%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.1%.
Specific Rotation $[\alpha]^{25^\circ}_{546.1\ nm}$: Between + 37.7° and +38.5°; $[\alpha]^{20^\circ}_{D}$: between +31.5° and +32.2°.

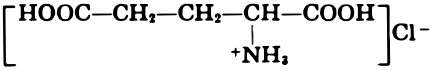
TESTS

Assay Dissolve about 500 mg, accurately weighed, in 250 ml of water, add bromothymol blue TS, and titrate with 0.1 *N* sodium hydroxide to a blue endpoint. Each ml of 0.1 *N* sodium hydroxide is equivalent to 14.71 mg of $C_5H_9NO_4$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Chloride, page 471 Any turbidity produced by a 10-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 85° for 3 h.
Specific Rotation, page 530 $[\alpha]^{25^\circ}_{546.1\ nm}$: Determine in a solution containing 11.8 g in sufficient 1.5 *N* hydrochloric acid to make 100 ml; $[\alpha]^{20^\circ}_{D}$: determine in a solution containing 10 g in sufficient 2 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Salt substitute; nutrient; dietary supplement.

L-Glutamic Acid Hydrochloride

2-Aminopentanedioic Acid Hydrochloride



$C_5H_9NO_4.HCl$ Mol wt 183.59

DESCRIPTION

A white, crystalline powder. One g dissolves in about 3 ml of water. It is almost insoluble in alcohol and in ether. Its solutions are acid to litmus.

REQUIREMENTS

Identification

A. To 1 ml of a 1 in 3 solution add 1 ml of barium hydroxide solution (1 in 50), filter, and add 10 ml of alcohol. A white crystalline precipitate of barium glutamate forms on standing.

B. To 1 ml of a 1 in 30 solution add 1 ml of ninhydrin TS and 100 mg of sodium acetate, and boil for 10 min. An intense violet blue color is produced.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $C_5H_9NO_4.HCl$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.25%.
Specific Rotation $[\alpha]^{25^\circ}_{546.1\ nm}$: Between +30.2° and +30.9°; $[\alpha]^{20^\circ}_{D}$: between +25.2° and +25.8°.

TESTS

Assay Dissolve about 300 mg, previously dried at 80° for 4 h and accurately weighed, in 50 ml of water, add bromothymol blue TS, and titrate with 0.1 *N* sodium hydroxide. Each ml of 0.1 *N* sodium hydroxide is equivalent to 9.180 mg of $C_5H_9NO_4.HCl$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 80° for 4 h.

Readily Carbonizable Substances Dissolve 500 mg of the sample in 5 ml of sulfuric acid TS. The solution is colorless.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Specific Rotation, page 530 $[\alpha]^{25°}_{546.1\ nm}$: Determine in a solution containing 14.7 g in sufficient 0.7 *N* hydrochloric acid to make 100 ml; $[\alpha]^{20°}_{D}$: determine in a solution containing 10 g in sufficient 2 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Salt substitute; flavoring agent; nutrient; dietary supplement.

L-Glutamine

L-2-Aminoglutaramic Acid

$$H_2NOCCH_2CH_2CH(NH_2)COOH$$

$C_5H_{10}N_2O_3$ Mol wt 146.15

DESCRIPTION

White, odorless crystals or crystalline powder having a slightly sweet taste. It is soluble in water and practically insoluble in alcohol and in ether. Its solutions are acid to litmus. It melts with decomposition at about 185°.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A purple color appears.

Assay Not less than 98.0% and not more than 101.0% of $C_5H_{10}N_2O_3$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.2%.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]^{20°}_{D}$: Between +6.3° and +7.3° after drying.

TESTS

Assay Dissolve about 150 mg of the sample, previously dried at 80° for 3 h and accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 14.62 mg of $C_5H_{10}N_2O_3$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 80° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in solution containing 4 g of a previously dried sample in sufficient water to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Glycerin

Glycerol

$$CH_2OH.CHOH.CH_2OH$$

$C_3H_8O_3$ Mol wt 92.10

DESCRIPTION

A clear, colorless, syrupy liquid having a sweet taste. It has not more than a slight characteristic odor, which is neither harsh nor disagreeable. It is hygroscopic and its solutions are neutral. Glycerin is miscible with water and with alcohol. It is insoluble in chloroform, in ether, and in fixed and volatile oils.

REQUIREMENTS

Identification

Heat a few drops of glycerin in a test tube with about 500 mg of potassium bisulfate. The characteristic, pungent vapors of acrolein are evolved.

Assay Not less than 95% of $C_3H_8O_3$.

Acrolein, Glucose, and Ammonium Compounds Passes test.

Arsenic (as As) Not more than 3 ppm.

Chlorinated Compounds (as Cl) Not more than 0.003%.

Color Passes test.

Fatty Acids and Esters Passes test (limit about 0.1%, calculated as butyric acid).

Heavy Metals (as Pb) Not more than 5 ppm.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.01%.

Specific Gravity Not less than 1.249.

TESTS

Assay

Sodium Periodate Solution Dissolve 60 g of sodium metaperiodate ($NaIO_4$) in sufficient water containing 120 ml of 0.1 *N* sulfuric acid to make 1000 ml. Do not heat to dissolve the periodate. If the solution is not clear, filter through a sintered-glass filter. Store the solution in a glass-stoppered, light-resistant container. Test the suitability of this solution as follows: Pipet 10 ml into a 250-ml volumetric flask, dilute to volume with water, and mix. To about 550 mg of glycerin dissolved in 50 ml of water add 50 ml of the diluted periodate solution with a pipet. For a blank, pipet 50 ml of the solution into a flask containing 50 ml of water. Allow the solutions to stand for 30 min, then add to each 5 ml of hydrochloric acid and 10 ml of potassium iodide TS, and rotate to mix. Allow to stand for 5 min, add 100 ml of water, and titrate with 0.1 *N* sodium thiosulfate, shaking continuously and adding starch TS near the endpoint. The ratio of the volume of 0.1 *N* sodium thiosulfate required for the glycerin-periodate mixture to that required for the blank should be between 0.750 and 0.765.

Procedure Transfer about 400 mg of the sample, accurately weighed, into a 600-ml beaker, dilute with 50 ml of water, add bromothymol blue TS, and acidify with 0.2 *N* H_2SO_4 to a definite green or greenish yellow color. Neutralize with 0.05 *N* sodium hydroxide to a definite blue endpoint, free of green color. Prepare a blank containing 50 ml of water, and neutralize in the same manner. Pipet 50 ml of the *Sodium Periodate Solution* into each beaker, mix by swirling gently, cover with a watch glass, and allow to stand for 30 min at room temperature (not above 35°) in the dark or in subdued light. Add 10 ml of a mixture consisting of equal volumes of ethylene glycol and water, and allow to stand for 20 min. Dilute each solution to about 300 ml with water, and titrate with 0.1 *N* sodium hydroxide to a pH of 8.1 ± 0.1 for the sample and 6.5 ± 0.1 for the blank, using a pH meter previously calibrated with pH 4.0 Acid Phthalate Standard Buffer Solution (see page 557). Each ml of 0.1 *N* sodium hydroxide, after correction for the blank, is equivalent to 9.210 mg of glycerin ($C_3H_8O_3$).

Acrolein, Glucose, and Ammonium Compounds Heat a mixture of 5 ml of glycerin and 5 ml of potassium hydroxide solution (1 in 10) at 60° for 5 min. It neither becomes yellow nor emits an odor of ammonia.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chlorinated Compounds Transfer 5.0 g of glycerin into a dry 100-ml round bottom, ground joint flask and add to it 15 ml of morpholine. Connect the flask with a ground joint reflux condenser, and reflux the mixture gently for 3 h. Rinse the condenser with 10 ml of water, receiving the washing into the flask, and cautiously acidify with nitric acid. Transfer the solution to a suitable comparison tube, add 0.5 ml of silver nitrate TS, dilute with water to 50.0 ml, and mix thoroughly. Any turbidity does not exceed that produced by 150 µg of chloride (Cl) in an equal volume of solution containing the quantities of reagents used in the test, omitting the refluxing.

Color The color of glycerin, when viewed downward against a white surface in a 50-ml Nessler tube, is not darker than the color of a standard made by diluting 0.40 ml of ferric chloride CS with water to 50 ml and similarly viewed in a Nessler tube of approximately the same diameter and color as that containing the sample.

Fatty Acids and Esters Mix a 40.0-ml (50-g) sample with 50 ml of recently boiled water and 5.0 ml of 0.5 *N* sodium hydroxide. Boil the mixture for 5 min, cool, add phenolphthalein TS, and titrate the excess alkali with 0.5 *N* hydrochloric acid. Not more than 1 ml of 0.5 *N* sodium hydroxide is consumed.

Heavy Metals Mix a 4.0-ml (5-g) sample with 2 ml of 0.1 *N* hydrochloric acid, add water to make 25 ml, and proceed as directed under the *Heavy Metals Test*, page 512. Any color does not exceed that produced in a control (*Solution A*) containing 25 µg of lead ion (Pb).

Readily Carbonizable Substances, page 532 Rinse a glass-stoppered, 25-ml cylinder with sulfuric acid TS, and allow it to drain for 10 min. Add 5 ml of glycerin and 5 ml of sulfuric acid TS, shake vigorously for 1 min, and allow to stand for 1 h. The mixture is no darker than *Matching Fluid H*.

Residue on Ignition Heat 50 g in a tared, open dish, and ignite the vapors, allowing them to burn until the sample has been completely consumed. After cooling, moisten the residue with 0.5 ml of sulfuric acid, and complete the ignition by heating for 15-min periods at 800° ± 25° to constant weight.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in tight containers.

Functional Use in Foods Humectant; solvent; bodying agent; plasticizer.

Glycerol Ester of Partially Dimerized Rosin

DESCRIPTION

A hard, pale, amber-colored resin (color M or paler as determined by ASTM Designation D 509) produced by the esterification of partially dimerized rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify glycerol ester of partially dimerized rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 715. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 3 and 8.

Arsenic (as As) Not more than 3 ppm.

Drop Softening Point Between 109° and 119°.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.

TESTS

Acid Value Determine as directed in the general procedure, page 533.
Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Drop Softening Point Determine as directed in the general procedure, page 534, using a bath temperature of 125°.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Masticatory substance in chewing gum base.

Glycerol Ester of Partially Hydrogenated Wood Rosin

DESCRIPTION

A medium-hard, pale amber–colored resin (color N or paler as determined by ASTM Designation D 509) produced by the esterification of partially hydrogenated wood rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water and in alcohol.

REQUIREMENTS

Identification

Identify glycerol ester of partially hydrogenated wood rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 716. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 3 and 10.
Arsenic (as As) Not more than 3 ppm.
Drop Softening Point Between 79° and 88°.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.

TESTS

Acid Value Determine as directed in the general procedure, page 533.
Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Drop Softening Point Determine as directed in the general procedure, page 543, using a bath temperature of 100°.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Masticatory substance in chewing gum base.

Glycerol Ester of Polymerized Rosin

DESCRIPTION

A hard, pale amber–colored resin (color M or paler as determined by ASTM Designation D 509) produced by the esterification of polymerized rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water and in alcohol.

REQUIREMENTS

Identification

Identify glycerol ester of polymerized rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 716. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 3 and 12.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Ring-and-Ball Softening Point Between 80° and 126°.

TESTS

Acid Value Determine as directed in the general procedure, page 533.
Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution

meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Ring-and-Ball Softening Point Determine as directed in the general procedure, page 535.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Glycerol Ester of Tall Oil Rosin

DESCRIPTION

A pale amber–colored resin (color N or paler as determined by ASTM Designation D 509) produced by the esterification of tall oil rosin with food-grade glycerin and purified by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify glycerol ester of tall oil rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 716. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 2 and 12.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 3 ppm.

Ring-and-Ball Softening Point Between 80° and 88°.

TESTS

Acid Value Determine as directed in the general procedure, page 533.

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Ring-and-Ball Softening Point Determine as directed in the general procedure, page 535.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Glycerol Ester of Wood Rosin

DESCRIPTION

A hard, pale amber–colored resin (color N or paler as determined by ASTM Designation D 509) produced by the esterification of pale wood rosin with food-grade glycerin. When intended for use in chewing gum base, the product is usually purified by steam stripping, but when intended for use in adjusting the density of citrus oils for beverages, it is purified by countercurrent steam distillation. It is soluble in acetone and in benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify glycerol ester of wood rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 717. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 3 and 9.

Arsenic (as As) Not more than 3 ppm.

Drop Softening Point Between 88° and 96°.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 3 ppm.

TESTS

Acid Value Determine as directed in the general procedure, page 533.

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Drop Softening Point Determine as directed in the general procedure, page 534, using a bath temperature of 105°.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base; beverage stabilizer.

Glycine

Aminoacetic Acid; Glycocoll

H_2NCH_2COOH

$C_2H_5NO_2$ Mol wt 75.07

DESCRIPTION

A white, odorless, crystalline powder having a sweetish taste. Its solution is acid to litmus. One g dissolves in about 4 ml of water. It is very slightly soluble in alcohol and in ether.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 10 solution add 5 drops of diluted hydrochloric acid TS and 5 drops of a solution of sodium nitrite (1 in 2). A vigorous evolution of a colorless gas is produced.
B. Add 1 ml of ferric chloride TS to 2 ml of a 1 in 10 solution. A red color is produced that disappears upon the addition of an excess of diluted hydrochloric acid TS, and that reappears upon the addition of an excess of stronger ammonia TS.
C. To 2 ml of a 1 in 10 solution add 1 drop of liquefied phenol and 5 ml of sodium hypochlorite TS. A blue color is produced.

Assay Not less than 98.5% and not more than the equivalent of 101.5% of $C_2H_5NO_2$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 5 ppm.
Loss on Drying Not more than 0.2%.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 175 mg, previously dried at 105° for 2 h and accurately weighed, to a 250-ml flask. Dissolve the sample in 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 7.507 mg of $C_2H_5NO_2$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 2 h.
Readily Carbonizable Substances, page 532 Dissolve 500 mg in 5 ml of sulfuric acid TS. The solution is no darker than *Matching Fluid A*.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Grapefruit Oil, Coldpressed

Grapefruit Oil, Expressed; Oil of Shaddock

DESCRIPTION

The oil obtained by expression from the fresh peel of the grapefruit *Citrus paradisi* Macfayden (*Citrus decumana* L.). It is a yellow, sometimes reddish liquid, often showing a flocculent separation of waxy material. It is soluble in most fixed oils and in mineral oil, often with opalescence or cloudiness. It is slightly soluble in propylene glycol and insoluble in glycerin. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 596, using the same test conditions as specified therein.

Angular Rotation Between +91° and +96°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.475 and 1.478 at 20°.
Residue on Evaporation Between 5% and 10%.
Specific Gravity Between 0.848 and 0.856.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Residue on Evaporation Proceed as directed in the general method, page 533, heating for 5 h.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Guar Gum

DESCRIPTION

A gum obtained from the ground endosperms of *Cyamopsis tetragonolobus* (L.) Taub (Fam. *Leguminosae*). It consists chiefly of a high-molecular-weight hydrocolloidal polysaccharide, composed of galactose and mannose units combined through glycosidic linkages, which may be described chemically as a galactomannan. It is a white to yellowish white, nearly odorless powder. It is dispersible in either hot or cold water, forming a sol, having a pH between 5.4 and 7.0, that may be converted to a gel by the addition of small amounts of sodium borate.

REQUIREMENTS

Identification

A. Transfer a 2-g sample into a 400-ml beaker, moisten it thoroughly with about 4 ml of isopropyl alcohol, add with vigorous stirring 200 ml of cold water, and continue the stirring until the gum is completely and uniformly dispersed. An opalescent, viscous solution is formed.

B. Transfer 100 ml of the solution prepared in *Identification Test A* into another 400-ml beaker, heat the mixture in a boiling water bath for about 10 min, and then cool to room temperature. No appreciable increase in viscosity is produced.

Acid-Insoluble Matter Not more than 7%.
Arsenic (as As) Not more than 3 ppm.
Ash (Total) Not more than 1.5%.
Galactomannans Not less than 66.0%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 15%.
Protein Not more than 10%.
Starch Passes test.

TESTS

Acid-Insoluble Matter Transfer 1.5 g of the sample, accurately weighed, into a 250-ml beaker containing 150 ml of water and 1.5 ml of concentrated sulfuric acid. Cover the beaker with a watch glass and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. At the end of the 6-h heating period add about 500 mg of a suitable filter aid, accurately weighed, and filter through a tared Gooch crucible provided with an asbestos pad. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator, and weigh. The difference between the weight of the filter aid and that of the residue is the weight of *Acid-Insoluble Matter*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Total) Determine as directed in the general method, page 466.

Galactomannans The difference between the sum of the percentages of *Acid-Soluble Matter*, *Total Ash*, *Loss on Drying*, and *Protein* and 100 represents the percentage of *Galactomannans*.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 5 h.

Protein Transfer about 3.5 g, accurately weighed, into a 500-ml Kjeldahl flask and proceed as directed under *Nitrogen Determination*, page 521. The percentage of nitrogen determined, multiplied by 6.25, gives the percentage of protein in the sample.

Starch To a 1 in 10 solution of the gum add a few drops of iodine TS. No blue color is produced.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Stabilizer; thickener; emulsifier.

Gum Guaiac

Guaiac Resin

DESCRIPTION

The resin of the wood of *Guajacum officinale* L. or of *Guajacum sanctum* L. (Fam. *Zygophyllaceae*). It occurs as irregular masses enclosing fragments of vegetable tissues, or in large, nearly homogeneous masses, and occasionally in more or less rounded or ovoid tears; it is externally brownish black to dusky brown, acquiring a greenish color on long exposure, the fractured surface having a glassy luster, the thin pieces being transparent and varying in color from brown to yellowish orange. The powder is moderate yellow brown, becoming olive brown on exposure to the air. It has a balsamic odor and a slightly acrid taste. Gum guaiac dissolves incompletely but

readily in alcohol, in ether, in chloroform, and in solutions of alkalies. It is slightly soluble in carbon disulfide and in benzene.

REQUIREMENTS

Identification

A. Add 1 drop of ferric chloride TS to 5 ml of an alcoholic solution of the sample (1 in 100). A blue color is produced that gradually changes to green, finally becoming greenish yellow.

B. A mixture of 5 ml of an alcoholic solution of the sample (1 in 100) and 5 ml of water becomes blue upon shaking with 20 mg of lead peroxide. Filter the solution, and boil a portion of the filtrate. The color disappears but may be restored by the addition of lead peroxide and shaking. Add a few drops of diluted hydrochloric acid TS to a second portion of the filtrate. The color is immediately discharged.

Alcohol-Insoluble Residue Not more than 15%.
Arsenic (as As) Not more than 3 ppm.
Ash (Acid-Insoluble) Not more than 2%.
Ash (Total) Not more than 5%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Melting Range Between 85° and 90°.
Rosin Passes test.

TESTS

Alcohol-Insoluble Residue Place 2 g of the sample, finely powdered and accurately weighed, in a dry, tared extraction thimble, and extract it with alcohol in a suitable continuous extraction apparatus for 3 h or until completely extracted. Dry the insoluble residue in the thimble for 4 h at 105°, and weigh.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Acid-Insoluble) Determine as directed in the general method, page 466.

Ash (Total) Determine as directed in the general method, page 466.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

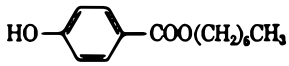
Melting Range Determine as directed in the general method, page 519.

Rosin A 1 in 10 solution of the sample in petroleum ether is colorless, and when shaken with an equal quantity of a fresh solution of cupric acetate (1 in 200) is not more green than a similar solution of cupric acetate in petroleum ether.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Preservative; antioxidant.

Heptylparaben

n-Heptyl-*p*-hydroxybenzoate



$C_{14}H_{20}O_3$ Mol wt 236.31

DESCRIPTION

Small, colorless crystals or a white crystalline powder. It is odorless or has a faint, characteristic odor and a slight burning taste. It is very slightly soluble in water but is freely soluble in alcohol and in ether.

REQUIREMENTS

Identification

Dissolve 500 mg in 10 ml of sodium hydroxide TS, boil for 30 min, allow the solution to evaporate to a volume of about 5 ml, and cool. Acidify the solution with diluted sulfuric acid TS, collect the crystals on a filter, wash several times with small portions of water, and dry in a desiccator over silica gel. The *p*-hydroxybenzoic acid so obtained melts between 212° and 217° (see page 519).

Assay Not less than 99.0% of $C_{14}H_{20}O_3$, calculated on the dried basis.
Acidity Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Melting Range Between 48° and 51°.
Residue on Ignition Not more than 0.05%.

TESTS

Assay Transfer into a flask about 3.5 g, accurately weighed, add 40.0 ml of 1 *N* sodium hydroxide, and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, cool, and titrate the excess sodium hydroxide with 1 *N* sulfuric acid to pH 6.5. Perform a blank determination with the same quantities of the same reagents in the same manner, and make any necessary correction (see page 2). Each ml of 1 *N* sodium hydroxide is equivalent to 236.3 mg of $C_{14}H_{20}O_3$, calculated on the dried basis.

Acidity Heat 750 mg with 15 ml of water at 80° for 1 min, cool, and filter. The filtrate is acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 *N* sodium hydroxide and 2 drops of methyl red TS. The solution is yellow, without even a light cast of pink.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals, page 512 Dissolve 2 g in 23 ml of acetone, and add 2 ml of diluted acetic acid TS, 2 ml of water, and 10 ml of

hydrogen sulfide TS. Any color does not exceed that produced in a control (*Solution A*) made with 23 ml of acetone, 2 ml of diluted acetic acid TS, 2 ml of *Standard Lead Solution* (20 µg Pb ion), and 10 ml of hydrogen sulfide TS.

Loss on Drying, page 518 Dry in a desiccator over silica gel for 5 h.

Melting Range Determine as directed in the general procedure, page 519.

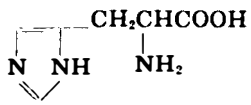
Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Packaging and Storage Store in tight containers.

Functional Use in Foods Preservative; antimicrobial agent.

L-Histidine

L-α-Amino-4(or 5)-imidazolepropionic Acid



$C_6H_9N_3O_2$ Mol wt 155.16

DESCRIPTION

White, odorless crystals or crystalline powder having a slightly bitter taste. It is soluble in water, very slightly soluble in alcohol, and insoluble in ether. It melts with decomposition between about 277° and 288°.

REQUIREMENTS

Identification

To 5 ml of a 1 in 100 solution of the sample add 2 ml of bromine TS. A yellow color is produced. When the solution is heated gently, it first becomes colorless, then reddish brown, and finally it forms a dark gray precipitate.

Assay Not less than 98.0% and not more than 101.0% of $C_6H_9N_3O_2$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.2%.

Residue on Ignition Not more than 0.2%.

Specific Rotation $[\alpha]_D^{20}$: Between +11.5° and +13.0° after drying.

TESTS

Assay Dissolve about 150 mg of the sample, previously dried at 105° for 3 h and accurately weighed, in 2 ml of formic acid and 50 ml of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 15.52 mg of $C_6H_9N_3O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

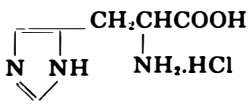
Specific Rotation, page 530 Determine in a solution containing 11 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Histidine Monohydrochloride

L-α-Amino-4(or 5)-imidazolepropionic Acid Monohydrochloride



$C_6H_9N_3O_2.HCl.H_2O$ Mol wt 209.63

DESCRIPTION

White, odorless crystals or crystalline powder having a slightly acid, bitter taste. It is soluble in water but insoluble in alcohol and in ether. It melts with decomposition at about 250° (after drying).

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS. A reddish purple color is produced.

B. A 1 in 1000 solution gives positive tests for *Chloride*, page 516.

Assay Not less than 98.0% and not more than 101.0% of $C_6H_9N_3O_2.HCl.H_2O$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20°}$: Between +8.5° and +10.5° after drying.

TESTS

Assay Dissolve about 100 mg of the sample, previously dried at 98° for 3 h and accurately weighed, in 1 ml of formic acid and 50 ml of glacial acetic acid. Add 6 ml of mercuric acetate TS, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 10.47 mg of $C_6H_9N_3O_2.HCl.H_2O$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 98° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 11 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Hops Oil

DESCRIPTION

The volatile oil obtained by steam distillation of the freshly dried membranous cones of the female plants of *Humulus lupulus* L. or *Humulus americanus* Nutt. (Fam. *Moraceae*). It is a light yellow to greenish yellow liquid having a characteristic aromatic odor. Age darkens the color, and the oil tends to become viscous. It is soluble in most fixed oils and, sometimes with opalescence, in mineral oil. It is practically insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 596, using the same test conditions as specified therein.

Acid Value Not more than 11.0.
Angular Rotation Between −2° and +2°5′.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.470 and 1.494 at 20°.
Saponification Value Between 14 and 69.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.825 and 0.926.

TESTS

Acid Value Determine as directed in the general method, page 499, using about 5 g, accurately weighed.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml usually is not soluble in 95% alcohol. Old oils are less soluble than fresh oils.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Hydrochloric Acid

HCl Mol wt 36.46

DESCRIPTION

A water solution of hydrogen chloride of varying concentrations. It is a clear, colorless or slightly yellowish, corrosive liquid having a pungent odor. It is miscible with water and with alcohol. Concentrations of hydrochloric acid commercially available are usually expressed in Baumé degrees (Be°) from which percentages of HCl and specific gravities can readily be derived. (See *Hydrochloric Acid Table*, page 514). The usually available concentrations are 18°, 20°, 22°, and 23° Be. Concentrations above 8.5° Be (12.5%) fume in moist air, lose hydrogen chloride, and create a corrosive atmosphere. Because of these characteristics, suitable precautions must be observed during sampling and analysis to prevent losses.

NOTE: Hydrochloric acid is produced directly or as a by-product during the manufacture of a variety of organic compounds. By-product hydrochloric acid usually con-

tains traces of organic contaminants, many of which may be toxic or hazardous (e.g., benzene, pesticides, herbicides, fungicides, insecticides, vinyl chloride). The manufacturer, vendor, or user is responsible in accordance with good manufacturing practice (see page 573) for identifying specific organic contaminants and establishing the suitability of the hydrochloric acid for its intended application in foods or in food processing (see also *Trace Impurities*, page 3). At the present time, no single analytical procedure applicable to the determination of organic contaminants in all types of by-product hydrochloric acid is available. The procedure for *Extractable Organic Substances* in the monograph on *Hydrochloric Acid* in *Reagent Chemicals: American Chemical Society Specifications*, Sixth Edition (1981), may be useful to investigators in adapting it for their own products, with suitable modifications being made (on the basis of known or expected impurities) in the preparation of the *Standard Solution* and in the interpretation of the results.

REQUIREMENTS

Identification

It gives positive tests for *Chloride*, page 516.

Assay Not less than the minimum or within the range of Baumé degrees claimed or implied by the vendor.
Arsenic (as As) Not more than 1 ppm.
Color Passes test.
Concentration of HCl Not less than the minimum or within the range specified or implied by the vendor.
Heavy Metals (as Pb) Not more than 5 ppm.
Iron Not more than 5 ppm.
Nonvolatile Residue Not more than 0.5%.
Oxidizing Substances (as Cl_2) Not more than 0.003%.
Reducing Substances (as SO_3) Not more than 0.007%.
Specific Gravity Not less than the minimum or within the range specified or implied by the vendor.
Sulfate Not more than 0.5%.

TESTS

Assay Transfer about 200 ml of the sample, previously cooled to a temperature below 15°, into a 250-ml hydrometer cylinder. Insert a suitable Baumé hydrometer graduated at 0.1° intervals, adjust the temperature to 15.6° (60°F), and note the reading at the bottom of the meniscus.

Arsenic A dilution of 3 g (2.6 ml) in 10 ml of water meets the requirements of the *Arsenic Test*, page 464, using as a control a mixture of 3 ml of *Standard Arsenic Solution* and 2.6 ml of ACS reagent-grade hydrochloric acid.

Color It shows no more color than *Matching Fluid A*, page 533.

Concentration of HCl Tare accurately a 125-ml glass-stoppered Erlenmeyer flask containing 35.0 ml of 1 *N* sodium hydroxide. Partially fill, without the use of vacuum, a 10-ml serological pipet from near the bottom of a representative sample, remove any acid adhering to the outside, and discard the first ml flowing from the pipet. Hold the tip of the pipet just above the surface of the sodium hydroxide solution, and transfer between 2.5 and 3 ml of the sample into the flask, leaving at least 1 ml in the pipet. Stopper the flask, mix the contents, and weigh accurately to obtain the weight of the sample. Add methyl orange TS, and titrate the excess sodium hydroxide with 1 *N* hydrochloric acid. Each ml of 1 *N* sodium hydroxide is equivalent to 36.46 mg of HCl. Alternatively, the concentration of the HCl in the sample may be obtained from the specific gravity by reference to the *Hydrochloric Acid Table*, page 514.

Heavy Metals Evaporate 4 g (3.5 ml) to dryness on a steam bath, dissolve the residue in 2 ml of diluted acetic acid TS, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron Dilute 4.3 ml (5 g) to a volume of 40 ml, and add about 40 mg of ammonium persulfate and 10 ml of ammonium thiocyanate TS. Any red color does not exceed that produced by 2.5 ml of *Iron Standard Solution* (25 µg Fe) in an equal volume of solution containing the same quantities of ACS reagent-grade hydrochloric acid and the reagents used in the test.

Nonvolatile Residue Transfer 1 g into a tared glass dish, evaporate to dryness on a steam bath, and then dry at 110° for 1 h, cool in a desiccator, and weigh. The weight of the residue does not exceed 5 mg.

Oxidizing Substances Transfer 1 ml into a 30-ml test tube, dilute to 20 ml with freshly boiled and cooled water, and add 1 ml of potassium iodide TS and 1 ml of starch TS. Stopper the test tube, and mix thoroughly. Any blue color does not exceed that produced in a control consisting of 1.0 ml of 0.001 *N* iodine in an equal volume of water containing the same quantities of the same reagents and 1 ml of ACS reagent-grade hydrochloric acid.

Reducing Substances Transfer 1 ml of ACS reagent-grade hydrochloric acid into a 30-ml test tube, dilute to 20 ml with recently boiled and cooled water, and add 1 ml of potassium iodide TS, 1 ml of starch TS, and 2.0 ml of 0.001 *N* iodine. Stopper the test tube, and mix thoroughly. The blue color produced is not discharged by 1 ml of the sample.

Specific Gravity Determine at 15.6° (60°F) with a hydrometer, calculate it from the Baumé degrees observed in the *Assay*, or obtain it by reference to the *Hydrochloric Acid Table*, page 514.

Sulfate, page 471 Dilute a 1-g sample to 100.0 ml with water, transfer 5.0 ml of this dilution into a 50-ml tall-form Nessler tube, and dilute to 20 ml with water. Add a drop of phenolphthalein TS, neutralize the solution with ammonia TS, and then add 1 ml of hydrochloric acid TS. To the clear solution, previously filtered, if necessary, add 3 ml of barium chloride TS, dilute to 50 ml with water, and mix. Prepare a control consisting of 1 ml of ACS reagent-grade hydrochloric acid and 250 µg of sulfate (SO_4) and the same quantities of the reagents used for the sample. Any turbidity shown in the sample does not exceed that of the control.

Packaging and Storage Store in tight containers.
Functional Use in Foods Acid.

Hydrogen Peroxide

H_2O_2 Mol wt 34.01

DESCRIPTION

A clear, colorless liquid having a slightly pungent odor. It is miscible with water. The grades of hydrogen peroxide suitable for food use usually have a concentration between 30% and 50%.

NOTE: Although hydrogen peroxide undergoes exothermic decomposition in the presence of dirt and other foreign materials, it is safe and stable under recommended conditions of handling and storage. Information on safe handling and use may be obtained from the supplier.

REQUIREMENTS

Identification

Shake 1 ml of the sample with 10 ml of water containing 1 drop of diluted sulfuric acid TS, and add 2 ml of ether. The subsequent addition of a drop of potassium dichromate TS produces an evanescent blue color in the water layer that upon agitation and standing passes into the ether layer.

Assay Not less than the labeled concentration or within the range stated on the label.
Acidity (as H_2SO_4) Not more than 0.03%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iron Not more than 0.5 ppm.
Phosphate Not more than 0.005%.
Residue on Evaporation Not more than 0.006%.
Tin Not more than 10 ppm.

TESTS

Assay Accurately weigh a volume of the sample equivalent to about 300 mg of H_2O_2 into a 100-ml volumetric flask, dilute to volume with water, and mix thoroughly. To a 20.0-ml portion of this solution add 25 ml of diluted sulfuric acid TS, and titrate with 0.1 *N* potassium permanganate. Each ml of 0.1 *N* potassium permanganate is equivalent to 1.701 mg of H_2O_2.

Acidity Dilute 9 ml (10 g) with 90 ml of carbon dioxide–free water, add methyl red TS, and titrate with 0.02 *N* sodium hydroxide. The volume of sodium hydroxide solution should not be more than 3 ml greater than the volume required for a blank test on 90 ml of the water used for dilution.

Arsenic Add 1 ml of ammonia TS to 1 ml of the sample, evaporate to dryness on a steam bath, and dissolve the residue in 35 ml of water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Evaporate 1.8 ml (2 g) to dryness on a steam bath with 10 mg of sodium chloride, and dissolve the residue in 25 ml of water. The solution so obtained meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron Evaporate 18 ml (20 g) to dryness on a steam bath with 10 mg of sodium chloride, dissolve the residue in 2 ml of hydrochloric acid, and dilute to 50 ml with water. Add about 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS, and mix. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 µg Fe) in an equal volume of solution containing the quantities of the reagents used in the test.

Phosphate Evaporate 400 mg to dryness on a steam bath. Dissolve the residue in 25 ml of approximately 0.5 *N* sulfuric acid, add 1 ml of ammonium molybdate solution (500 mg of $(NH_4)_6Mo_7O_{24}.4H_2O$ in each 10 ml of water) and 1 ml of *p*-methylaminophenol sulfate TS, and allow to stand for 2 h. Any blue color does not exceed that produced by 2.0 ml of *Phosphate Standard Solution* (20 µg PO_4) in an equal volume of solution containing the quantities of the reagents used in the test.

Residue on Evaporation Evaporate 25 g to dryness in a tared porcelain or silica dish on a steam bath, and dry to constant weight at 105°. The weight of the residue does not exceed 1.5 mg.

Tin

Aluminum Chloride Solution Dissolve 8.93 g of aluminum chloride, $AlCl_3.6H_2O$, in sufficient water to make 1000 ml.

Gelatin Solution On the day of use, dissolve 100 mg of gelatin in 50 ml of boiled water that has been cooled to between 50° and 60°.

Tin Stock Solution Dissolve 250.0 mg of lead-free tin foil in 10 to 15 ml of hydrochloric acid, and dilute to 250.0 ml with dilute hydrochloric acid (1 in 2).

Standard Solution On the day of use, transfer 5.0 ml of *Tin Stock Solution* into a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 2.0 ml of this solution (100 µg Sn) into a 250-ml Erlenmeyer flask, and add 15 ml of water, 5 ml of nitric acid, and 2 ml of sulfuric acid. Place a small stemless funnel in the mouth of the flask, and heat until strong fumes of sulfuric acid are evolved. Cool, add 5 ml of water, evaporate again to strong fumes, and cool. Repeat the addition of water and heating to strong fumes, then add 15 ml of water, heat to boiling, and cool. Dilute to about 35 ml with water, add 1 drop of methyl red TS and 2.0 ml of the *Aluminum Chloride Solution*, and mix. Make the solution just alkaline by the dropwise addition of stronger ammonia TS, stirring gently, and then add 0.1 ml in excess. (*Caution:* To avoid dissolving the aluminum hydroxide precipitate, do not add more than 0.1 ml in excess of the ammonia solution.) Centrifuge for about 15 min at 4000 rpm, and then decant the supernatant liquid as completely as possible without disturbing the precipitate. Dissolve the precipitate in 5 ml of dilute hydrochloric acid (1 in 2), add 1.0 ml of the *Gelatin Solution*, and dilute to 20.0 ml with a saturated solution of aluminum chloride.

Sample Solution Transfer 9 ml (10 g) of the sample into a 250-ml Erlenmeyer flask, and add 15 ml of water, 5 ml of nitric acid, and 2 ml of sulfuric acid. Mix, and heat gently on a hot plate to initiate and maintain a vigorous decomposition.

When decomposition is complete, place a small stemless funnel in the mouth of the flask, and continue as directed for the *Standard Solution*, beginning with " . . . and heat until strong fumes of sulfuric acid are evolved."

Procedure Rinse a polarographic cell or other vessel with a portion of the *Standard Solution*, then add a suitable volume to the cell, immerse it in a constant temperature bath maintained at 35° ± 0.2°, and deaerate by bubbling oxygen-free nitrogen or hydrogen through the solution for at least 10 min. Insert the dropping mercury electrode of a suitable polarograph, and record the polarogram from −0.2 to −0.7 V and at a sensitivity of 0.0003 μA per mm, using a saturated calomel reference electrode. In the same manner, polarograph a portion of the *Sample Solution* at the same current sensitivity. The height of the wave produced by the *Sample Solution* is not greater than that produced by the *Standard Solution* at the same half-wave potential.

Packaging and Storage Store in a cool place in containers with a vent in the stopper.

Functional Use in Foods Bleaching, oxidizing agent; starch modifier; preservative.

Hydroxylated Lecithin

DESCRIPTION

Hydroxylated lecithin is derived from a complex mixture of acetone-insoluble phosphatides from soybean and other plant lecithins, consisting chiefly of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol, as well as other minor phospholipids and glycolipids mixed with varying amounts of triglycerides, fatty acids, sterols, and carbohydrates. The mixture is treated with hydrogen peroxide, benzoyl peroxide, lactic acid, and sodium hydroxide, or with hydrogen peroxide, acetic acid, and sodium hydroxide, to produce a hydroxylated product having an iodine value approximately 10% lower than that of the starting material. Hydroxylated lecithin may vary in consistency from fluid to plastic, depending upon the content of free fatty acid and oil and whether or not it contains diluents. It is light yellow in color and has a characteristic "bleached" odor. It is partially soluble in water, but hydrates readily to form emulsions; it is more dispersible and hydrates more readily than crude lecithin.

REQUIREMENTS

Acetone-Insoluble Matter (phosphatides) Not less than 50%.
Acid Value Not more than 70.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Hexane-Insoluble Matter Not more than 0.3%.
Iodine Value Between 85 and 95.
Lead Not more than 10 ppm.
Peroxide Value Not more than 100.
Water Not more than 1.5%.

TESTS

Acetone-Insoluble Matter (phosphatides)

Purification of Phosphatides Dissolve 5 g of phosphatides from previous *Acetone-Insoluble Matter* determinations in 10 ml of petroleum ether, and add 25 ml of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-ml centrifuge tubes using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 ml with acetone, stir again, chill for 15 min in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, crush the solids with a stirring rod, refill the tube with acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the *Phosphatide-Acetone Solution*. Five g of the purified phosphatides are required to saturate about 16 L of acetone.

Phosphatide-Acetone Solution Add a quantity of purified phosphatides to sufficient acetone, previously cooled to a temperature of about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h, shaking it vigorously at 15-min intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

Procedure If it is plastic or semisolid, soften a portion of the sample by warming it in a water bath at a temperature not exceeding 60° and then mixing it thoroughly. Transfer 2 g of a well-mixed sample, accurately weighed, into a 40-ml centrifuge tube, previously tared with a glass stirring rod, and add 15 ml of *Phosphatide-Acetone Solution* from a buret. Warm the mixture in a water bath until the sample melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, and then transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about one half of the required volume of *Phosphatide-Acetone Solution*, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 ml with chilled *Phosphatide-Acetone Solution* (5°), again stir, and return the tube and contents to the ice bath for 15 min. At the end of the 15-min chilling period, stir again while still in the ice bath, remove the stirring rod, temporarily supporting it in a vertical upside-down position, and centrifuge the mixture immediately at about 2000 rpm for 5 min. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the same stirring rod previously used, and refill the tube to the 40-ml mark with chilled (5°) *Phosphatide-Acetone Solution*, and repeat the chilling, stirring, centrifugation, and decantation procedure previously followed. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the assigned stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min in a forced-draft oven, cool, and

weigh. Calculate the percentage of acetone-insoluble matter by the formula $(100R/S) - B$, in which R is the weight of residue, S is the weight of the sample, and B is the percentage of *Hexane-Insoluble Matter* determined as directed in this monograph.

Acid Value If it is plastic or semisolid, soften a portion of the sample by warming it in a water bath at a temperature not exceeding 60°, and then mix it thoroughly. Transfer about 2 g of a well-mixed sample into a 250-ml Erlenmeyer flask, and dissolve it in 50 ml of petroleum ether. To this solution add 50 ml of alcohol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide, and mix well. Add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 5 s. Calculate the number of mg of potassium hydroxide required to neutralize the acids in 1 g of the sample by multiplying the number of ml of 0.1 *N* sodium hydroxide consumed in the titration by 5.6 and dividing the result by the weight of the sample.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Hexane-Insoluble Matter If plastic or semisolid, soften a portion of the sample by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Weigh 10 g of a previously well-mixed sample into a 250-ml wide-mouth Erlenmeyer flask, add 100 ml of solvent hexane, and shake until the sample is dissolved. Filter the solution through a 30-ml Corning "C" porosity or equivalent filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 25-ml portions of solvent hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.

Iodine Value Determine by the *Wijs Method*, page 505.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Peroxide Value Weigh accurately about 10 g of the sample, add 30 ml of a 3:2 mixture of glacial acetic acid–chloroform, and mix. Add 1 ml of a saturated solution of potassium iodide, mix, and allow to stand for 10 min. Add 100 ml of water, begin titrating with 0.05 *N* sodium thiosulfate, adding starch TS as the endpoint is approached, and continue the titration until the blue starch color has just disappeared. Perform a blank determination (see page 2) and make any necessary correction. Calculate the peroxide value, as meq of peroxide per kg of sample, by the formula

$$S \times N \times 1000/W,$$

in which S is the net volume, in ml, of sodium thiosulfate solution required for the sample, N is the exact normality of the sodium thiosulfate solution, and W is the weight, in g, of the sample taken.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; clouding agent.

Hydroxypropyl Cellulose

DESCRIPTION

Hydroxypropyl cellulose is a cellulose ether containing hydroxypropyl substitution. It occurs as a white powder. It is soluble in water and in certain organic solvents. It may contain a suitable anticaking agent.

REQUIREMENTS

Identification

A. When a 0.1% solution of the sample is shaken, a layer of foam appears (distinction from sodium carboxymethylcellulose).

B. To 5 ml of a 0.5% solution of the sample add 5 ml of a 5% solution of copper sulfate (or aluminum sulfate). No precipitate appears (distinction from sodium carboxymethylcellulose).

Assay Not more than 80.5% of hydroxypropoxyl groups ($-OCH_2CHOHCH_3$) after drying, equivalent to not more than 4.6 hydroxypropyl groups per anhydroglucose unit.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 5%.

pH of a 1% Solution Between 5.0 and 8.0.

Residue on Ignition Not more than 0.5%.

Viscosity of a 10% Solution Not less than 145 centipoises.

TESTS

Assay Weigh accurately about 85 mg of the sample, previously dried at 105° for 3 h, and determine the hydroxypropoxyl content as directed under *Hydroxypropoxyl Determination*, page 514.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, adding 1 ml of hydroxylamine hydrochloride solution (1 in 5) to the solution of the residue. Any color does not exceed that produced in a control (*Solution A*) containing 20 μg of lead ion (Pb).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

pH of a 1% Solution Determine by the *Potentiometric Method*, page 531.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Viscosity of a 10% Solution Transfer an accurately weighed sample, equivalent to 40 g of hydroxypropyl cellulose on the dried basis, into a tared sample container, and proceed as directed under *Viscosity of Sodium Carboxymethylcellulose*, page 550.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; film former; protective colloid; stabilizer; suspending agent; thickener.

Hydroxypropyl Methylcellulose

Propylene Glycol Ether of Methylcellulose

DESCRIPTION

Hydroxypropyl methylcellulose is the propylene glycol ether of methylcellulose in which both the hydroxypropyl and methyl groups are attached to the anhydroglucose rings of cellulose by ether linkages. Several product types are available that are defined by varying combinations of methoxyl and hydroxypropoxyl content. It occurs as a white to off-white, fibrous powder or as granules. It is soluble in water and in certain organic solvent systems. Aqueous solutions are surface active, form films upon drying, and undergo a reversible transformation from sol to gel upon heating and cooling, respectively.

REQUIREMENTS

Identification

A. Add 1 g to 100 ml of water. It swells and disperses to form a clear to opalescent, mucilaginous solution, depending upon the intrinsic viscosity, which is stable in the presence of most electrolytes.

B. Add 1 g to 100 ml of boiling water and stir the mixture. A slurry is formed that, when cooled to 20°, dissolves to form a clear or opalescent mucilaginous solution.

C. Pour a few ml of the solution prepared for *Identification Test B* onto a glass plate, and allow the water to evaporate. A thin, self-sustaining film results.

Assay for Hydroxypropoxyl Groups Within the range claimed by the vendor for any product type between a minimum of 3.0% and a maximum of 12.0% of hydroxypropoxyl groups ($—OCH_2CHOHCH_2$).

Assay for Methoxyl Groups Within the range claimed by the vendor for any product type between a minimum of 19.0% and a maximum of 30.0% of methoxyl groups ($—OCH_3$).

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 5%.

Residue on Ignition Not more than 1.5% for products with viscosities of 50 centipoises or above; not more than 3.0% for products with viscosities below 50 centipoises.

Viscosity The viscosity of a solution containing 2 g in each 100 g of solution is not less than 80% and not more than 120% of that stated on the label for viscosity types of 100 centipoises or less, and not less than 75% and not more than 140% of that stated on the label for viscosity types higher than 100 centipoises.

TESTS

Assay

Caution: Perform all steps involving hydriodic acid carefully in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be extremely careful when handling the hot vials, since they are under pressure. In the event of hydriodic acid exposure, wash with copious amounts of water and seek medical attention at once.

Internal Standard Solution Transfer about 2.5 g of toluene, accurately weighed, into a 1000-ml volumetric flask containing 10 ml of *o*-xylene, dilute with *o*-xylene to volume, and mix.

Standard Preparation Transfer about 135 mg of adipic acid into a suitable serum vial, add 4.0 ml of hydriodic acid followed by 4.0 ml of the *Internal Standard Solution*, and close the vial securely with a septum stopper. Weigh the vial and its contents accurately, add 30 µl of isopropyl iodide with a syringe through the septum, reweigh, and calculate the weight of isopropyl iodide added. Similarly, add 90 µl of methyl iodide, and calculate the weight added. Shake well, and allow the layers to separate.

Assay Preparation Transfer about 0.065 g of the sample, accurately weighed, into a 5-ml vial equipped with a pressure-tight septum closure, add an amount of adipic acid equal to the weight of the sample, and pipet 2 ml of the *Internal Standard Solution* into the vial. Cautiously pipet 2 ml of hydriodic acid into the mixture, immediately secure the closure, and weigh accurately. Shake the vial for 30 s, heat at 150° for 20 min, remove from the heat, shake again, using extreme caution, and heat at 150° for 40 min. Allow the vial to cool for about 45 min, and then weigh. If the weight loss is greater than 10 mg, discard the mixture and prepare another *Assay Preparation*.

Chromatographic System Use a suitable gas chromatograph equipped with a thermal conductivity detector. Under typical conditions, the instrument contains a 1.8-m × 4-mm glass column packed with 10% methylsilicone oil (UCW 982 or equivalent) on 100/120-mesh flux-calcined chromatographic siliceous earth (Chromosorb WHP or equivalent). The column is maintained at 100°, and the injection port and detector at 200°, and helium is used as the carrier gas flowing at the rate of 20 ml per min.

Calibration Inject about 2 µl of the upper layer of the *Standard Preparation* into the chromatograph, and record the chromatogram. The retention times for methyl iodide, isopropyl iodide, toluene, and *o*-xylene are approximately 3, 5, 7, and 13 min, respectively. Calculate the relative response

factor, F, of equal weights of toluene and methyl iodide by the formula Q/A, in with Q is the quantity ratio of methyl iodide to toluene in the *Standard Preparation*, and A is the peak area ratio of methyl iodide to toluene obtained from the *Standard Preparation*. Similarly, calculate the relative response factor, F', of equal weights of toluene and isopropyl iodide by the formula Q'/A', in which Q' is the quantity ratio of isopropyl iodide to toluene in the *Standard Preparation*, and A' is the peak area ratio of isopropyl iodide to toluene obtained from the *Standard Preparation*.

Procedure Inject about 2 µl of the upper layer of the *Assay Preparation* into the chromatograph, and record the chromatogram. Calculate the percentage of methoxyl groups (—OCH_3) in the sample by the formula

$$2 \times (31/142) \times F \times a \times (W/w),$$

in which 31/142 is the ratio of the formula weights of methoxyl to methyl iodide; a is the ratio of the area of the methyl iodide peak to that of the toluene peak obtained from the *Assay Preparation*; W is the weight of toluene in the *Internal Standard Solution*, in g; and w is the weight of sample taken, in g. Similarly, calculate the percentage of hydroxypropoxyl groups (—$OCH_2CHOHCH_3$) in the sample by the formula

$$2 \times (75/170) \times F' \times a' \times (W/w),$$

in which a' is the ratio of the area of the isopropyl iodide peak to that of the toluene peak obtained from the *Assay Preparation*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, adding 1 ml of hydroxylamine hydrochloride solution (1 in 5) to the solution of the residue. Any color does not exceed that produced in a control (*Solution A*) containing 20 µg of lead ion (Pb).

Loss on Drying, page 518 Dry a 3-g sample at 105° for 2 h.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

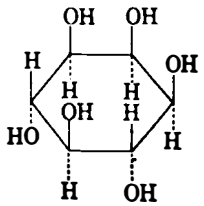
Viscosity Weigh accurately a sample, equivalent to 2 g of solids on the dried basis, transfer to a wide-mouth, 250-ml centrifuge bottle, and add 98 g of water previously heated to between 80° and 90°. Stir with a mechanical stirrer for 10 min, then place the bottle in an ice bath until solution is complete, adjust the weight of the solution to 100 g if necessary, and centrifuge it to expel any entrapped air. Adjust the temperature of the solution to 20° ± 0.1°, and determine the viscosity as directed under *Viscosity of Methylcellulose*, page 549.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Thickening agent; stabilizer; emulsifier.

Inositol

1,2,3,5/4,6-Cyclohexanehexol; *i*-Inositol; *meso*-Inositol



$C_6H_{12}O_6$ Mol wt 180.16

DESCRIPTION

It occurs as fine, white crystals or as a white crystalline powder. It is odorless, has a sweet taste, and is stable in air. Its solutions are neutral to litmus. It is optically inactive. One g is soluble in 6 ml of water. It is slightly soluble in alcohol, and is insoluble in ether and in chloroform.

REQUIREMENTS

Identification

A. To 1 ml of a 1 in 50 solution in a porcelain evaporating dish, add 6 ml of nitric acid, and evaporate to dryness on a water bath. Dissolve the residue in 1 ml of water, add 0.5 ml of a 1 in 10 solution of strontium acetate, and again evaporate to dryness on a steam bath. A violet color is produced.

B. The inositol hexaacetate obtained in the *Assay* melts between 212° and 216° (see page 519).

Assay Not less than 97.0% of $C_6H_{12}O_6$ after drying.

Arsenic (as As) Not more than 3 ppm.

Calcium Passes test.

Chloride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

Melting Range Between 224° and 227°.

Residue on Ignition Not more than 0.1%.

Sulfate Not more than 0.006%.

TESTS

Assay Transfer about 200 mg, previously dried at 105° for 4 h and accurately weighed, to a 250-ml beaker, add 5 ml of a mixture consisting of 1 part of diluted sulfuric acid TS in 50 parts of acetic anhydride, and cover the beaker with a watch glass. Heat on a steam bath for 20 min, then chill in an ice bath, and add 100 ml of water. Boil for 20 min, allow to cool, and transfer quantitatively, with the aid of a little water, to a 250-ml separator. Extract the solution with 6 successive 30-, 25-, 20-, 15-, 10-, and 10-ml portions of chloroform, using the solvent to rinse the original flask. Collect the chloroform extracts in a second 250-ml separator and wash the combined

extracts with 10 ml of water. Transfer the chloroform solution through a funnel containing a pledget of cotton into a 150-ml tared Soxhlet flask. Wash the separator and funnel with 10 ml of chloroform and add to the combined extracts. Evaporate to dryness on a steam bath, dry in an oven at 105° for 1 h, cool in a desiccator, and weigh. The weight of the inositol hexaacetate obtained, multiplied by 0.4167, represents the equivalent of $C_6H_{12}O_6$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Calcium To 10 ml of a 1 in 10 solution add 1 ml of ammonium oxalate TS. The solution remains clear for at least 1 min.

Chloride, page 471 Any turbidity produced by a 400-mg sample does not exceed that shown in a control containing 20 μg of chloride ion (Cl).

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Sulfate, page 471 Any turbidity produced by a 5-g sample does not exceed that shown in a control containing 300 μg of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Iron, Carbonyl

Fe At wt 55.85

DESCRIPTION

Carbonyl iron is elemental iron produced by the decomposition of iron pentacarbonyl as a dark gray powder. When viewed under a microscope having a magnifying power of 500 diameters or greater, it appears as spheres built up with concentric shells. It is stable in dry air.

REQUIREMENTS

Identification

It dissolves in dilute mineral acids with the evolution of hydrogen and the formation of solutions of the corresponding salts, which give positive tests for *Ferrous Salts*, page 516.

Assay Not less than 98.0% of Fe.
Acid-Insoluble Substances Not more than 0.2%.
Arsenic (as As) Not more than 4 ppm.
Lead Not more than 0.002%.
Mercury Not more than 2 ppm.
Sieve Analysis Not less than 100% passes through a 200-mesh sieve; not less than 95% passes through a 325-mesh sieve.

TESTS

Assay Determine as directed under *Iron, Reduced*, page 152.
Acid-Insoluble Substances Determine as directed under *Iron, Electrolytic*, page 152.
Arsenic Dissolve 1.5 g in 25 ml of diluted sulfuric acid TS, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.
Lead Determine as directed under *Iron, Reduced*, page 152, but prepare the control, as directed in the *Procedure*, with 4.0 ml of *Diluted Standard Lead Solution* (4 μg Pb).
Mercury Determine as directed under *Iron, Reduced*, page 153, but use 2 g of the sample and 40 ml of *Sodium Citrate Solution* in preparing the *Sample Solution*, and prepare the *Diluted Standard Mercury Solution* as follows: Transfer 4.0 ml of *Mercury Stock Solution* into a 250-ml volumetric flask, dilute to volume with 1 *N* hydrochloric acid, and mix (1 ml = 4 μg Hg). Modify the first sentence of the *Procedure* to read: "Prepare a control by treating 1.0 ml of *Diluted Standard Mercury Solution* (4 μg Hg) in the same manner. . . . "
Sieve Analysis Determine as directed under *Sieve Analysis of Granular Metal Powders*, page 537.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Iron, Electrolytic

Fe At wt 55.85

DESCRIPTION

Electrolytic iron is elemental iron obtained by electrodeposition in the form of an amorphous, lusterless, grayish black powder. It is stable in dry air.

REQUIREMENTS

Identification

It dissolves in dilute mineral acids with the evolution of hydrogen and the formation of solutions of the corresponding salts, which give positive tests for *Ferrous Salts*, page 516.

Assay Not less than 97.0% of Fe.
Acid-Insoluble Substances Not more than 0.2%.
Arsenic (as As) Not more than 4 ppm.

Lead Not more than 0.002%.
Mercury Not more than 2 ppm.
Sieve Analysis Not less than 100% passes through a 100-mesh sieve; not less than 95% passes through a 325-mesh sieve.

TESTS

Assay Determine as directed under *Iron, Reduced*, this page.
Acid-Insoluble Substances Dissolve 1 g in 25 ml of diluted sulfuric acid TS, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible, wash with water until free from sulfate, dry at 105° for 1 h, cool, and weigh.
Arsenic Dissolve 1 g in 25 ml of diluted sulfuric acid TS, heat on a steam bath until the evolution of hydrogen ceases, cool, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464, using 4.0 ml of *Standard Arsenic Solution* (4 µg As) in the control.
Lead Determine as directed under *Iron, Reduced*, this page, but prepare the control, as directed in the *Procedure*, with 4.0 ml of *Diluted Standard Lead Solution* (4 µg Pb).
Mercury Determine as directed under *Iron, Reduced*, page 153, but use 2 g of the sample and 40 ml of *Sodium Citrate Solution* in preparing the *Sample Solution*, and prepare the *Diluted Standard Mercury Solution* as follows: Transfer 4.0 ml of *Mercury Stock Solution* into a 250-ml volumetric flask, dilute to volume with 1 *N* hydrochloric acid, and mix (1 ml = 4 µg of Hg). Modify the first sentence of the *Procedure* to read: "Prepare a control by treating 1.0 ml of *Diluted Standard Mercury Solution* (4 µg Hg) in the same manner. . . . "
Sieve Analysis Determine as directed under *Sieve Analysis of Granular Metal Powders*, page 537.
Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Iron, Reduced

Fe At wt 55.85

DESCRIPTION

Reduced iron is elemental iron obtained by a chemical process in the form of a grayish black powder, all of which should pass through a 100-mesh sieve. It is lusterless or has not more than a slight luster. When viewed under a microscope having a magnifying power of 100 diameters, it appears as an amorphous powder, free from particles having a crystalline structure. It is stable in dry air.

REQUIREMENTS

Identification

It dissolves in dilute mineral acids with the evolution of hydrogen and the formation of solutions of the corresponding salts, which give positive tests for *Ferrous Salts*, page 516.

Assay Not less than 96.0% of Fe.
Acid-Insoluble Substances Not more than 1.25%.
Arsenic (as As) Not more than 8 ppm.
Lead Not more than 0.0025%.
Mercury Not more than 5 ppm.

TESTS

Assay Transfer about 200 mg, accurately weighed, into a 300-ml Erlenmeyer flask, add 50 ml of diluted sulfuric acid TS, and close the flask with a stopper containing a Bunsen valve, made by inserting a glass tube connected to a short piece of rubber tubing with a slit on the side and a glass rod inserted in the other end and arranged so that gases can escape but air cannot enter. Heat on a steam bath until the iron is dissolved, cool the solution, dilute it with 50 ml of recently boiled and cooled water, add 2 drops of orthophenanthroline TS, and titrate with 0.1 *N* ceric sulfate until the red color changes to a weak blue. Each ml of 0.1 *N* ceric sulfate is equivalent to 5.585 mg of Fe.
Acid-Insoluble Substances Dissolve 1 g in 25 ml of diluted sulfuric acid TS, and heat on a steam bath until the evolution of hydrogen ceases. Filter through a tared filter crucible, collecting the filtrate in a 100-ml volumetric flask, wash with water until free from sulfate, and dry at 105° for 1 h. The weight of the residue does not exceed 12.5 mg. Dilute the filtrate to volume with water for use in the test for *Arsenic*.
Arsenic Transfer 40 ml of the filtrate (equivalent to 400 mg of Fe) obtained in the test for *Acid-Insoluble Substances* into an arsine generator flask, and continue as directed under *Procedure* in the *Arsenic Test*, page 465, beginning with "Add 20 ml of dilute sulfuric acid (1 in 5). . . . "
Lead

Solutions Prepare as directed in the *Lead* test under *Ferrous Fumarate*, page 121.

Procedure Transfer 200 mg of the sample into a 150-ml beaker, and add 8 ml of hydrochloric acid and 2 ml of nitric acid. Prepare a control containing 5.0 ml of *Diluted Standard Lead Solution* (5 µg Pb), 8 ml of hydrochloric acid, and 2 ml of nitric acid, and carry the sample and the control solutions through the following procedure: After the initial reaction subsides, evaporate to dryness on a steam bath, cool, and dissolve in 10 ml of dilute hydrochloric acid (1 in 2), warming on the steam bath, if necessary, to effect solution. Add 25 ml of *Ammonium Citrate Solution*, heat on the steam bath for an additional few min, then add 7 ml of stronger ammonia TS, and cool. Adjust the pH, if necessary, to 9.0 (by means of a glass electrode or pH indicator paper), using either stronger ammonia TS or hydrochloric acid, and transfer to a separator. Extract with 5-ml portions of *Dithizone Extraction Solution* until the extraction solution retains its original color, and combine the extracts in a second separator. Wash the combined extracts by shaking for 30 s with 10 ml of *Citrate-Cyanide Wash Solution*, and then wash the wash solution with 3 ml of *Dithizone Extraction Solution*. Combine the chloroform layers, add 20 ml of dilute nitric acid (1 in 100),

and shake for 30 s. Separate the layers, and shake the chloroform layer with an additional 5 ml of the dilute nitric acid. Combine the acid washes in a small beaker, and adjust the pH with diluted ammonia TS to 2.5 ± 0.2 (by means of a glass electrode). Transfer the solution into a separator, add 2 ml of *pH 2.5 Buffer Solution*, and shake the solution for 30 s with 30 ml of *Dithizone–Carbon Tetrachloride Solutions*. Wash the carbon tetrachloride layer with 10 ml of *pH 2.5 Wash Solution*, discard the carbon tetrachloride, and combine the aqueous layers. Add 4 ml of *Ammonia-Cyanide Solution*, mix, and extract at once with 5-ml portions of *Dithizone–Carbon Tetrachloride Solution* until the carbon tetrachloride shows no further pink color. Wash the combined extracts with 4 ml of *Ammonia-Cyanide Wash Solution*, dry the stem of the separator, and drain the carbon tetrachloride through a plug of cotton to remove the last trace of water. Determine the absorbances of both solutions in 1-cm cells at 520 nm with a suitable spectrophotometer, using carbon tetrachloride as the blank. The absorbance of the sample solution should not exceed that of the control.

Mercury

Dithizone Stock Solution Dissolve 30 mg of dithizone in 1000 ml of chloroform, add 5 ml of alcohol, and mix. Store in a refrigerator in a dark bottle. Prepare fresh each month.

Dithizone Extraction Solution On the day of use, dilute 30 ml of *Dithizone Stock Solution* to 100 ml with chloroform.

Hydroxylamine Hydrochloride Solution Prepare as directed in the test for *Mercury* under *Ferrous Fumarate*, page 121.

Mercury Stock Solution Transfer 33.8 mg, accurately weighed, of mercuric chloride into a 100-ml volumetric flask, dissolve in 1 *N* hydrochloric acid, dilute to volume with the acid, and mix. This solution contains the equivalent of 250 µg of Hg in each ml.

Diluted Standard Mercury Solution Transfer 2.0 ml of *Mercury Stock Solution* into a 100-ml volumetric flask, dilute to volume with 1 *N* hydrochloric acid, and mix. Each ml contains the equivalent of 5 µg of Hg.

Sodium Citrate Solution Dissolve 250 g of sodium citrate dihydrate in 1000 ml of water.

Sample Solution Transfer 1 g of the sample into a 250-ml beaker, add 20 ml of dilute nitric acid (1 in 2), and digest on a steam bath for about 45 min. Add 5 ml of dilute hydrochloric acid (1 in 3), and continue heating on the steam bath until the sample is dissolved. Cool to room temperature, and filter, if necessary, through a medium-porosity filter paper. Wash with a few ml of water, add 20 ml of *Sodium Citrate Solution* and 1 ml of *Hydroxylamine Hydrochloride Solution* to the filtrate, and adjust the pH to 1.8 with stronger ammonia TS.

Procedure (Because mercuric dithizonate is light-sensitive, this procedure should be performed in subdued light.) Prepare a control by treating 1.0 ml of *Diluted Standard Mercury Solution* (5 µg Hg) in the same manner and with the same reagents as directed for the preparation of the *Sample Solution*. Transfer the control and the *Sample Solution* into separate 250-ml separators, and treat both solutions as follows: Extract with 5 ml of *Dithizone Extraction Solution*, shaking the mixtures vigorously for 1 min. Drain carefully, collecting the chloroform in another separator. If the chloroform does not show a pronounced green color due to excess reagent, add another 5 ml of the extraction solution, shake again, and drain into the separator. Continue the extraction with 5-ml portions, if necessary, collecting each successive extract in the second separator, until the final chloroform layer contains dithizone in marked excess. To the combined chloroform extracts add 15 ml of dilute hydrochloric acid (1 in 3), shake the mixture vigorously for 1 min, and discard the chloroform. Extract with 2 ml of chloroform, drain carefully, and discard the chloroform. Add 1 ml of 0.05 *M* disodium EDTA and 2 ml of 6 *N* acetic acid to the aqueous layer. Slowly add 5 ml of ammonia TS, and cool the separator. Transfer the solution into a 150-ml beaker, adjust the pH to 1.8 with ammonia TS or dilute nitric acid (1 in 10), using a pH meter, and return the solution to the separator. Add 5.0 ml of *Dithizone Extraction Solution*, and shake vigorously for 1 min. Allow the layers to separate, insert a plug of cotton into the stem of the separator, and collect the dithizone extract in a test tube. Determine the absorbance of each solution in 1-cm cells at 490 nm with a suitable spectrophotometer, using chloroform as the blank. The absorbance of the *Sample Solution* does not exceed that of the control.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Isobutylene-Isoprene Copolymer

Butyl Rubber

DESCRIPTION

A synthetic copolymer containing from 0.5 to 2.0 molar percent of isoprene, the remainder, respectively, consisting of isobutylene. It is prepared by copolymerization of isobutylene and isoprene in methyl chloride solution, using aluminum chloride as catalyst. After completion of polymerization, the rubber particles are treated with hot water containing a suitable food-grade deagglomerating agent, such as stearic acid. Finally, the coagulum is dried to remove residual volatiles.

REQUIREMENTS

Identification

Identify isobutylene-isoprene copolymer by comparing its infrared absorption spectrum with a typical spectrum as shown on page 717. Prepare the sample by dissolving it in hot toluene and evaporating on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Total Unsaturation Not less than 0.5% and not more than 2.0%, as isoprene.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

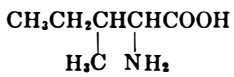
Total Unsaturation Determine as directed in the general method, page 470.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

DL-Isoleucine

DL-2-Amino-3-methylvaleric Acid



$C_6H_{13}NO_2$ Mol wt 131.17

DESCRIPTION

A white, odorless, crystalline powder having a slightly bitter taste. It is soluble in water, but is practically insoluble in alcohol and in ether. It melts with decomposition at about 292°. The pH of a 1 in 100 solution is between 5.5 and 7.0.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A bluish purple color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_6H_{13}NO_2$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure green color or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

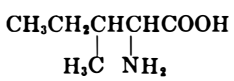
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Isoleucine

L-2-Amino-3-methylvaleric Acid



$C_6H_{13}NO_2$ Mol wt 131.18

DESCRIPTION

Crystalline leaflets or a white crystalline powder having a bitter taste. It is soluble in 25 parts of water, slightly soluble in hot alcohol, and soluble in diluted mineral acids and in alkaline solutions. It sublimes at between 168° and 170°, and melts with decomposition at about 284°. The pH of a 1 in 100 solution is between 5.5 and 7.0.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A reddish purple or bluish purple color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_6H_{13}NO_2$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Residue on Ignition Not more than 0.2%.

Specific Rotation $[\alpha]_D^{20°}$: Between +38.0° and +41.5°, on the dried basis.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure green color or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution containing 4 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Isopropyl Alcohol

2-Propanol; Isopropanol

$CH_3CHOHCH_3$

C_3H_8O Mol wt 60.10

DESCRIPTION

A clear, colorless, flammable liquid having a characteristic odor and a slightly bitter taste. It is miscible with water, with ethyl alcohol, with ether, and with many other organic solvents. Its refractive index at 20° is about 1.377.

REQUIREMENTS

Identification

Add 3 ml of water and 1 ml of mercuric sulfate TS to 2 ml of the sample contained in a test tube, and warm gently. A white or yellowish precipitate is formed.

Assay Not less than 99.7% of C_3H_8O, by weight.

Acidity (as acetic acid) Not more than 10 ppm.

Distillation Range Within a range of 1°, including 82.3°.

Heavy Metals (as Pb) Not more than 1 ppm.

Nonvolatile Residue Not more than 10 ppm.

Solubility in Water Passes test.

Substances Reducing Permanganate Passes test.

Water Not more than 0.2%.

TESTS

Assay Its specific gravity, determined by any reliable method (see page 3), is not greater than 0.7840 at 25°/25° (equivalent to 0.7870 at 20°/20°).

Acidity Add 2 drops of phenolphthalein TS to 100 ml of water, add 0.01 *N* sodium hydroxide to the first pink color that persists for at least 30 s, then add 50 ml (about 39 g) of the sample, and mix. Not more than 0.7 ml of 0.01 *N* sodium hydroxide is required to restore the pink color.

Distillation Range Proceed as directed in the general method, page 478.

Heavy Metals Evaporate 25 ml (about 20 g) of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Evaporate 125 ml (about 100 g) of the sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Solubility in Water Mix 10 ml of the sample with 40 ml of water. After 1 h, the solution is as clear as an equal volume of water.

Substances Reducing Permanganate Transfer 50 ml of the sample into a 50-ml glass-stoppered cylinder, add 0.25 ml of 0.1 *N* potassium permanganate, mix, and allow to stand for 10 min. The pink color is not entirely discharged.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers, remote from fire.

Functional Use in Foods Extraction solvent.

Juniper Berries Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried ripe fruit of the plant *Juniperus communis* L. var. *erecta* Pursh (Fam. *Cupressaceae*). It is a colorless, faintly greenish, or yellowish liquid with a characteristic odor and an aromatic bitter taste. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol. The oil tends to polymerize on long storage.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 596, using the same test conditions as specified therein.

Angular Rotation Between −15° and 0°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.474 and 1.484 at 20°.
Specific Gravity Between 0.854 and 0.879.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or galvanized containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Kaolin

China Clay

DESCRIPTION

A purified clay consisting mainly of alumina, silica, and water. It occurs as a fine, white to yellowish white or grayish powder having an earthy taste. It becomes darker and has a distinct claylike odor when moistened. It is insoluble in water, in alcohol, in dilute acids, and in alkali solutions.

REQUIREMENTS

Identification

Mix 1 g of the sample with 10 ml of water and 5 ml of sulfuric acid in a porcelain dish, and evaporate until the water is removed. Continue heating until dense white fumes of sulfur trioxide are evolved, then cool, and cautiously add 20 ml of water. Boil for a few min, and filter. A gray residue of silica remains on the filter. To a portion of the filtrate add ammonia TS. A gelatinous, white precipitate of aluminum hydroxide is produced that is insoluble in an excess of ammonia TS.

Acid-Soluble Substances Not more than 2%.
Arsenic (as As) Not more than 3 ppm.
Carbonate Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Iron Passes test.
Lead Not more than 10 ppm.
Loss on Ignition Not more than 15%.
Sulfide Passes test.

TESTS

Acid-Soluble Substances Digest a 1-g sample with 20 ml of diluted hydrochloric acid TS for 15 min, and filter. Evaporate 10 ml of the filtrate to dryness in a tared dish, ignite gently, cool, and weigh.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead Transfer 10.0 g of the sample into a 250-ml flask, and add 50 ml of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3 filter paper, or equivalent, into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Carbonate Mix a 1-g sample with 10 ml of water, cool, and keep cool while adding 5 ml of sulfuric acid. No effervescence occurs during the addition of the acid.

Heavy Metals A 5-ml portion of the *Sample Solution* diluted to 25 ml with water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron Mix a 2-g sample with 10 ml of water in a mortar, and add 500 mg of sodium salicylate. No more than a light reddish tint is produced.

Lead A 10-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Ignition Ignite a 2-g sample, accurately weighed, in a tared crucible at 575° ± 25° to constant weight, cool, and weigh.

Sulfide Add a 1-g sample to 25 ml of water in a 250-ml flask, then add 15 ml of dilute hydrochloric acid TS, and immediately cover the top of the flask with filter paper moistened with lead acetate TS. Heat to boiling, and boil for several min. The paper does not show any brown coloration.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Anticaking agent.

Karaya Gum

Sterculia Gum

DESCRIPTION

A dried gummy exudation from *Sterculia urens* Roxburgh and other species of *Sterculia* (Fam. *Sterculiaceae*), or from *Cochlospermum gossypium* A. P. De Condolle or other species of *Cochlospermum* Kunth (Fam. *Bixaceae*). It occurs in tears of variable size or in broken irregular pieces having a somewhat crystalline appearance. It is pale yellow to pinkish brown, translucent, and horny, and is sometimes admixed with a few darker fragments and occasional pieces of bark. The gum has a slightly acetous odor and a mucilaginous and slightly acetous taste. In the powdered form it is light gray to pinkish gray. Karaya gum is insoluble in alcohol, but it swells in water to form a gel.

REQUIREMENTS

Identification

A. Add 2 g to 50 ml of water. It swells to form a granular, stiff, slightly opalescent mucilage.
B. Add a few drops of Millon's Reagent to a 1 in 100 solution of the gum. A white curdy precipitate forms.

Arsenic (as As) Not more than 3 ppm.
Ash (Acid-Insoluble) Not more than 1.0%.
Foreign Gums Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Insoluble Matter Not more than 3%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 20%.
Starch Passes test.
Viscosity of a 1% Solution Not less than the minimum or within the range claimed by the vendor.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Ash (Acid-Insoluble) Determine as directed in the general method, page 466.
Foreign Gums The gum swells in 60% alcohol (*distinction from other gums*).
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Insoluble Matter Transfer about 5 g, accurately weighed, into a 250-ml Erlenmeyer flask, add a mixture of equal parts of diluted hydrochloric acid TS and water, cover the flask with a watch glass, and boil gently until the mixture loses its viscosity. Filter the solution through a tared filtering crucible, wash the residue with water until the washings are free from acid, dry at 105° for 1 h, and weigh.
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Powder an unground sample until it passes through a No. 40 sieve, mix well before weighing, and dry at 105° for 5 h.
Starch To a 1 in 10 solution of the gum add a few drops of iodine TS. No blue color is produced.
Viscosity Transfer a 4-g sample, finely powdered, into the container of a stirring apparatus equipped with blades capable of being adjusted to about 1000 rpm. Add 10 ml of alcohol to the sample, swirl to wet the gum uniformly, and then add 390 ml of water, avoiding the formation of lumps. Stir the mixture for 7 min, pour the resulting dispersion into a 500-ml bottle, insert a stopper, and allow to stand for about 12 h in a water bath at 25°. Determine the apparent viscosity at this temperature with a model LVF Brookfield or equivalent-type viscometer (see *Viscosity of Sodium Carboxymethylcellulose*, page 550) using a suitable spindle, speed, and factor.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Kelp

DESCRIPTION

The dehydrated seaweed obtained from the class *Phaeophyceae* (brown algae) of the genera *Macrocystis* (including *M. pyrifera* and related species) and *Laminaria* (including *L. digitata, L. cloustoni*, and *L. saccharina*). The seaweed may be chopped to provide coarse particles and/or it may be ground to provide a fine powder. It is dark green to olive brown in color, and has a salty, characteristic taste.

REQUIREMENTS

Arsenic (as As, inorganic) Not more than 3 ppm.
Ash (Total) Not more than 45%.
Heavy Metals (as Pb) Not more than 0.004%.
Iodine Content Between 0.1% and 0.5%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 13%.

TESTS

Arsenic Assemble the special distillation apparatus for determination of inorganic arsenic as shown in Fig. 5 on page 465 of the general method. Weigh accurately 2.00 g of kelp, which has been previously ground to pass a 60-mesh screen, and transfer to the distillation flask (*A*) of the apparatus. To the flask add 50 ml of distillation-reducing solution (prepared on the day of use by dissolving 36 g of ACS low-arsenic ferrous chloride, $FeCl_2 4H_2O$, in 500 ml of 6.6 *N* hydrochlo-

ric acid), connect the flask to the receiver chamber (*B*), then complete the assembly of the apparatus, and begin circulating tap water through the condenser (*C*). Half-fill the lower two bulbs of the splash head (*D*) with distilled water.

With the stopcock in position such that the receiver chamber drains into the distillation flask, heat the flask until the temperature above the solution reaches 106° to 108°, and continue refluxing at this temperature for 45 min. Close the stopcock, continue heating at 108° to 110°, and collect 30 to 33 ml of distillate in the receiver chamber. Remove the heating source and allow the temperature to drop to about 80°.

Drain the distillate from the receiver chamber into a 250-ml beaker that is contained in an ice-water bath. Close the stopcock, and add a second 50-ml portion of the distillation-reducing solution through the thermometer opening to the distillation flask. Replace the thermometer, increase the temperature to 108° to 110°, and collect a second 30- to 33-ml portion of distillate in the receiver chamber.

Drain the second distillate into the beaker containing the first portion, and continue cooling in the ice-water bath until the combined distillate cools to room temperature. Remove the splash head, and wash its contents into the beaker. Also, wash down the insides of the condenser and receiver chamber with water, collecting the washings in the beaker. Filter the distillate plus washings through a Whatman No. 40 or equivalent filter paper, collecting the filtrate in a 300-ml Erlenmeyer flask having a 24/40 standard-taper joint, to be used later as the arsine generator flask. Wash the filter three times with water so that the final volume of filtrate measures 200 ml.

Add 2 ml of potassium iodide TS and 0.5 ml of *Stannous Chloride Solution*, and continue as directed in the *Procedure*, page 465, under the *Arsenic Test*, beginning with "Allow the mixture to stand for 30 min at room temperature . . . " but using 6.0 ml, rather than 3.0 ml, of *Standard Arsenic Solution* in the preparation of the standard.

Ash (Total) Determine as directed in the general method, page 466.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodine Content Transfer about 2 g of the sample, accurately weighed, into a large porcelain crucible, and mix thoroughly with 10 g of potassium carbonate. Ignite the sample in a muffle furnace, starting with low heat, and then ignite at 500° to 600° for 20 min or until combustion is complete. Dissolve the ash in about 200 ml of boiling water, filter, and wash the filter paper with two 15-ml portions of boiling water, adding the washings to the filtrate. Cool to room temperature, neutralize to methyl red TS with approximately 20 ml of 85% phosphoric acid diluted with 20 ml of water, and then add 5 ml in excess. Cool the reaction mixture on an ice bath, and add bromine TS (about 5 ml) until a permanent yellow color is obtained. Gently boil the solution to remove all free bromine, adding water if necessary to maintain a volume of 200 ml or more. Boil for an additional 5 min after the bromine color has been completely dissipated. Add a few mg of salicylic acid, stir, and cool to about 20°. Add 1 ml of the diluted phosphoric acid solution and 5 ml of potassium iodide TS, and titrate immediately with 0.01 *N* sodium thiosulfate, using starch TS as the indicator. Each ml of 0.01 *N* sodium thiosulfate is equivalent to 211.5 µg of I.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Dietary supplement (source of iodine).

Labdanum Oil

DESCRIPTION

The volatile oil obtained by steam distillation from crude labdanum gum extracted from the perennial shrub *Cistus ladaniferus* L. (Fam. *Cistaceae*). It is a golden yellow, viscous liquid having a powerful balsamic odor, which on dilution is reminiscent of ambergris. It turns dark brown on standing. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 596, using the same test conditions as specified therein.

Acid Value Between 18 and 86.

Angular Rotation Between +0°15′ and +7°.

Ester Value Between 31 and 86.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.492 and 1.507 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.905 and 0.993.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Determine as directed in the general method, page 501, using about 1 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is

saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 0.5 ml of 90% alcohol, but the solution usually becomes opalescent or turbid upon further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Lactated Mono-Diglycerides

DESCRIPTION

A mixture of partial lactic and fatty acid esters of glycerin. It varies in consistency from a soft to a hard, waxy solid. It is dispersible in hot water, and is moderately soluble in hot isopropanol, in xylene, and in cottonseed oil.

REQUIREMENTS

Identification

Transfer into a 25-ml glass-stoppered test tube 1 ml of the solution of the sample remaining after titrating with 0.1 *N* potassium hydroxide in the determination of *Total Lactic Acid*, add 0.1 ml of cupric sulfate solution (1 g of $CuSO_4.5H_2O$ in 25 ml of water) and 6 ml of sulfuric acid, and mix. Stopper loosely, heat in a boiling water bath for 5 min, and then cool in an ice bath for 5 min. Remove from the ice bath, add 0.1 ml of *p*-phenylphenol solution (75 mg dissolved in 5 ml of sodium hydroxide TS), and mix. Allow to stand at room temperature for 1 min, then heat in a boiling water bath for 1 min. A deep, blue violet color indicates the presence of lactic acid.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

The following specifications should conform to the representations of the vendor: **1-Monoglyceride Content, Total Lactic Acid, Acid Value, Free Glycerin,** and **Water.**

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Free Glycerin Determine as directed in the general method, page 504.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

1-Monoglyceride Content Determine as directed in the general method, page 506.

Total Lactic Acid Transfer an accurately weighed portion of the melted sample, equivalent to between 140 and 170 mg of lactic acid, into a 250-ml Erlenmeyer flask. Pipet 20 ml of 0.5 *N* alcoholic potassium hydroxide into the flask, connect an air condenser at least 65 cm in length, and reflux for 30 min. Run a blank determination using the same volume of alkali. Add 20 ml of water to each flask, then disconnect the condensers, evaporate to a volume of 20 ml, and cool to about 40°. Add methyl red TS to each flask, and titrate the blank with 0.5 *N* hydrochloric acid. Add exactly the same volume of 0.5 *N* hydrochloric acid to the sample flask, with swirling. To each flask add 50 ml of hexane, swirl vigorously to dissolve the fatty acids in the sample flask, then transfer quantitatively the contents of each flask into separate 250-ml separators and shake for 30 s. Collect the aqueous phases in 300-ml Erlenmeyer flasks, wash the hexane solutions with 50 ml of water, and combine the wash solutions with the original aqueous phases in the Erlenmeyer flasks, discarding the hexane solutions. Add phenolphthalein TS and titrate with 0.1 *N* potassium hydroxide to a pink color that persists for at least 30 s. Each ml of 0.1 *N* potassium hydroxide is equivalent to 9.008 mg of lactic acid ($C_3H_6O_3$).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; stabilizer.

Lactic Acid

2-Hydroxypropionic Acid

DESCRIPTION

A colorless or yellowish, nearly odorless, syrupy liquid consisting of a mixture of lactic acid ($C_3H_6O_3$) and lactic acid lactate ($C_6H_{10}O_5$). It is obtained by the lactic fermentation of sugars or is prepared synthetically. It is usually available in solutions containing the equivalent of from 50% to 90% of lactic acid. It is hygroscopic, and when concentrated by boiling, the acid condenses to form lactic acid lactate, 2-(lactoyloxy)propanoic acid, which on dilution and heating hydrolyzes to lactic acid. It is miscible with water and with alcohol.

REQUIREMENTS

Identification

It gives positive tests for *Lactate*, page 517.

Assay Not less than 95.0% and not more than 105.0% of the labeled concentration of $C_3H_6O_3$.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.2%.
Citric, Oxalic, Phosphoric, or Tartaric Acid Passes test.
Cyanide Passes test (approximately 5 ppm).
Heavy Metals (as Pb) Not more than 10 ppm.
Iron Not more than 10 ppm.
Residue on Ignition Not more than 0.1%.
Sugars Passes test.
Sulfate Not more than 0.25%.

TESTS

Assay Weigh accurately a portion of the sample equivalent to about 3 g of lactic acid, transfer to a 250-ml flask, add 50.0 ml of 1 *N* sodium hydroxide, mix, and boil for 20 min. Add phenolphthalein TS, titrate the excess alkali in the hot solution with 1 *N* sulfuric acid, and perform a blank determination (see page 2). Each ml of 1 *N* sodium hydroxide is equivalent to 90.08 mg of $C_3H_6O_3$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride Dissolve about 5 g, accurately weighed, in 50 ml of water, and neutralize to litmus paper with sodium hydroxide solution (1 in 4). Add 2 ml of potassium chromate TS, and titrate with 0.1 *N* silver nitrate to the first appearance of a red tinge. Each ml of 0.1 *N* silver nitrate is equivalent to 3.545 mg of Cl.

Citric, Oxalic, Phosphoric, or Tartaric Acid Dilute 1 g to 10 ml with water, add 40 ml of calcium hydroxide TS, and boil for 2 min. No turbidity is produced.

Cyanide

p-Phenylenediamine-Pyridine Mixed Reagent Dissolve 200 mg of *p*-phenylenediamine hydrochloride in 100 ml of water, warming to effect solution. Cool, allow the solids to settle, and use the supernatant liquid to make the mixed reagent. Dissolve 128 ml of pyridine in 365 ml of water, add 10 ml of hydrochloric acid, and mix. To prepare the mixed reagent, mix 30 ml of the *p*-phenylenediamine solution with all of the pyridine solution, and allow to stand for 24 h before using. The mixed reagent is stable for about three weeks when stored in an amber bottle.

Sample Solution Transfer an accurately weighed quantity of the sample, equivalent to 20.0 g of 100% lactic acid, into a 100-ml volumetric flask, dilute to volume with water, and mix.

Cyanide Standard Solution Dissolve 2.5 g of potassium cyanide in 1000 ml of 0.1 *N* sodium hydroxide. Transfer a 10-ml aliquot into a 100-ml volumetric flask, dilute to volume with 0.1 *N* sodium hydroxide, and mix. Each ml of this solution contains 10 µg of CN.

Procedure Pipet a 10-ml aliquot of the *Sample Solution* into a 50-ml beaker. Into a second 50-ml beaker pipet 1.0 ml of the *Cyanide Standard Solution*, and add 10 ml of water. Place the beakers in an ice bath, and adjust the pH to between 9 and 10 with 20% sodium hydroxide, stirring slowly and adding the reagent slowly to avoid overheating. Allow the solutions to stand for 3 min, and then slowly add 10% phosphoric acid to a pH between 5 and 6. Transfer the solutions into 100-ml separators containing 25 ml of cold water, and rinse the beakers and pH meter electrodes with a few ml of cold water, collecting the washings in the respective separator. Add 2 ml of bromine TS, stopper, and mix. Add 2 ml of 2% sodium arsenite solution, stopper, and mix. To the clear solutions add 10 ml of *n*-butanol, stopper, and mix. Finally, add 5 ml of *p-Phenylenediamine-Pyridine Mixed Reagent*, mix, and allow to stand for 15 min. Remove and discard the aqueous phases, and filter the alcohol phases into 10-mm cells. The absorbance of the sample, determined at 480 nm with a suitable spectrophotometer, is no greater than that of the standard.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron To the ash obtained in the test for *Residue on Ignition* add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, dilute to 40 ml with water, and add about 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 20 ml of *Iron Standard Solution* (20 µg Fe) in an equal volume of solution containing the quantities of reagents used in the test.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Sugars Add 5 drops of the sample to 10 ml of hot alkaline cupric tartrate TS. No red precipitate is formed.

Sulfate Transfer about 50 g, accurately weighed, into a 600-ml beaker, dissolve in 200 ml of water, and neutralize to between pH 4.5 and 6.5 with sodium hydroxide solution (1 in 2), making the final adjustment with a more dilute alkali solution. Filter, if necessary, and heat the filtrate or clear solution to just below the boiling point. Add 10 ml of barium chloride TS, stirring vigorously, boil the mixture gently for 5 min, and allow to stand for at least 2 h, or preferably overnight. Collect the precipitate of barium sulfate on a tared Gooch crucible, wash until free from chloride, dry, and ignite at 600° to constant weight. The weight of barium sulfate so obtained, multiplied by 0.412, represents the weight of SO_4 in the sample taken.

Packaging and Storage Store in tight containers.
Functional Use in Foods Acid.

Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol

Propylene Glycol Lactostearate

DESCRIPTION

A mixture of partial lactic and fatty acid esters of propylene glycol and glycerin produced by the lactylation of a product

obtained by reacting edible fats or oils with propylene glycol. It varies in consistency from a soft solid to a hard, waxy solid. It is dispersible in hot water, and is moderately soluble in hot isopropanol, in benzene, in chloroform, and in soybean oil.

REQUIREMENTS

Identification

Place about 150 mg of melted sample in a 16- × 125-mm tube equipped with a screw cap having a Teflon liner, and add 4 ml of absolute methanol, 4 drops of a 25% sodium methoxide solution in absolute methanol, and a boiling chip. Cap the tube, reflux for 15 min, and cool to room temperature. Add 8 drops of a 15% potassium acid sulfate solution, 4 ml of water, and 4 ml of *n*-hexane, cap the tube, shake for 1 min, and centrifuge for 30 to 60 s. Decant and discard the *n*-hexane layer, and repeat the extraction with three additional 4-ml portions of *n*-hexane, discarding each extract. Transfer the aqueous alcoholic phase from the tube to a 50-ml round bottom glass-stoppered flask, place the flask in a water bath at 50° to 55°, and evaporate to near dryness (about 0.5 ml) with a rotary film evaporator under full water aspirator vacuum. (*Caution:* Do not heat above 55°.) Remove the flask from the evaporator, add 1 ml of a 1:1 methanol–0.5 *N* hydrochloric acid solution, swirl for several min, and decant the clear solution into a small flask. Inject a portion of this solution into a suitable gas chromatograph (see page 471), and obtain the chromatogram, observing the following operating conditions or equivalent conditions: *detector*, flame ionization; *carrier gas*, helium, flowing at a rate of 50 ml per min; *column*, 1.8-m × 3-mm (id) packed with 80/100-mesh Porapak Q (ethylvinylbenzene-divinylbenzene polymer porous beads); *column temperature*, 175° to 210°, heated at a rate of 4° per min and holding at 210° until the glycerin is eluted; *inlet port temperature*, 310°; *detector temperature*, 385°; *recorder*, 0–1 mV range, with 1-s full-scale deflection at a chart speed of 6.5 mm per min; *sample size*, 2 to 3 µl. From the chromatogram so obtained, identify the peaks by their relative positions on the chart. The major peaks, representing propylene glycol, methyl lactate, lactic acid, and glycerin in the order listed, may be identified with suitable reference substances. Major peaks may also be identified by their relative retention times using a suitable internal standard.

Acid Value Not more than 12.0.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Water-Insoluble Combined Lactic Acid Between 14.0% and 18.0%.

The following specifications should conform to the representations of the vendor: **1-Monoglyceride Content, Total Lactic Acid, Free Lactic Acid, Free Glycerin,** and **Water.**

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Free Glycerin Determine as directed in the general method, page 504.

Free Lactic Acid Transfer about 15 g of the sample, accurately weighed, into a beaker, dissolve in about 75 ml of benzene, and transfer the solution into a 500-ml glass-stoppered graduate. Wash the beaker with about 125 ml of benzene in divided portions, adding the washings to the graduate. Add 200 ml of water to the graduate, and shake vigorously for 1 min. After 125 ml or more of the aqueous phase has separated, pipet 100.0 ml of the aqueous phase into an Erlenmeyer flask, add 1 ml of phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a slight pink color. Calculate the percentage of free lactic acid in the sample by the formula

$$9.008 \times V \times N/(0.5 \times W),$$

in which V is the volume, in ml, of 0.5 *N* sodium hydroxide required, N is the exact normality of the sodium hydroxide solution, and W is the weight of the sample, in g.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

1-Monoglyceride Content Determine as directed in the general method, page 506.

Total Lactic Acid Transfer about 3 g of the sample, accurately weighed, into a 250-ml glass-stoppered flask, pipet 50.0 ml of 0.7 *N* alcoholic potassium hydroxide into the flask, attach an air condenser, and boil gently on a steam bath for 30 min or until the sample is completely saponified. Remove the flask from the steam bath, immediately remove the air condenser, and allow the solution to cool until it begins to jell. Add 75.0 ml of 0.5 *N* hydrochloric acid, mix, and transfer the solution to a 500-ml separator, washing the flask with two 15-ml portions of water. Cool to 35° or lower, and extract with 100 ml of diethyl ether. Transfer the aqueous layer to a second 500-ml separator, and wash the ether layer with two 20-ml portions of water, adding the wash water to the original aqueous phase in the second separator. Retain the ether solution. Extract the aqueous phase with a second 100-ml portion of diethyl ether, and transfer the aqueous phase to a 500-ml Erlenmeyer flask. Combine and wash the ether extracts with five 20-ml portions of water, and add the wash water to the flask. To the combined aqueous phases in the Erlenmeyer flask, add 1 ml of phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a slight pink color. Perform a blank determination (see page 2), and calculate the percentage of total lactic acid by the formula

$$9.008(S - B)(N)/W,$$

in which $(S - B)$ represents the difference, in ml, between the volumes of 0.5 *N* sodium hydroxide required for the sample and blank, respectively, N is the exact normality of the sodium hydroxide solution, and W is the weight of the sample, in g.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Water-Insoluble Combined Lactic Acid Transfer about 3 g of the sample, accurately weighed, into a 250-ml separator with the aid of 100 ml of benzene, and wash with three 30-ml portions of water, discarding the washings. Transfer the benzene layer to a 250-ml glass-stoppered Erlenmeyer flask, wash the separator with a few ml of benzene, and completely evaporate the combined benzene solution to dryness. Pipet 50.0 ml of 0.7 *N* alcoholic potassium hydroxide into the flask, attach an air condenser, boil gently on a steam bath for 30 min or until the sample is completely saponified, and remove the flask from the steam bath. Immediately remove the air condenser, and allow the solution to cool until it begins to jell. Add 75.0 ml of 0.5 *N* hydrochloric acid, mix, and transfer the solution into a 500-ml separator, washing the flask with two 15-ml portions of water. Cool to 35° or lower, and extract with 100 ml of diethyl ether. Transfer the water layer to a second 500-ml separator, and wash the diethyl ether with two 20-ml portions of water, adding the wash water to the original aqueous phase in the second separator. Retain the ether solution. Extract the aqueous phase with a second 100-ml portion of diethyl ether, and transfer the aqueous phase to a 500-ml Erlenmeyer flask. Combine and wash the ether extracts with five 20-ml portions of water, and add the wash water to the flask. To the combined aqueous phases in the flask add 1 ml of phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a slight pink color. Perform a blank determination (see page 2), and calculate the percentage of water-insoluble combined lactic acid in the sample by the formula

$$9.008(S - B)(N)/W,$$

in which $(S - B)$ represents the difference, in ml, between the volumes of 0.5 *N* sodium hydroxide required for the sample and blank, respectively, N is the exact normality of the sodium hydroxide solution, and W is the weight of the sample, in g.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; stabilizer; whipping agent; plasticizer; surface-active agent.

Lactylic Esters of Fatty Acids

DESCRIPTION

Lactylic esters of fatty acids are mixed fatty acid esters of lactic acid and its polymers, with minor quantities of free lactic acid, polylactic acid, and fatty acids. They vary in consistency from liquids to hard, waxy solids. They are dispersible in hot water and are soluble in organic solvents and in vegetable oils. They conform to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

A. Transfer into a 25-ml glass-stoppered test tube 1 ml of the solution obtained in the test for *Total Lactic Acid* after titrating with 0.1 *N* potassium hydroxide. Add 0.1 ml of cupric sulfate solution (1 g of $CuSO_4.5H_2O$ in 25 ml of water) and 6 ml of sulfuric acid, and mix. Stopper loosely, heat in a boiling water bath for 5 min, then cool in an ice bath for 5 min, and remove from the bath. Add 0.1 ml of *p*-phenylphenol TS, mix, allow to stand at room temperature for 1 min, and then heat in a boiling water bath for 1 min. A deep, blue violet color indicates the presence of lactic acid.

B. Assemble a suitable apparatus for ascending thin-layer chromatography. Prepare a slurry of chromatographic silica gel containing about 13% of calcium sulfate as the binder (use 1 g of $CaSO_4$ to each 2 ml of water), apply a uniformly thin layer to glass plates of convenient size, dry in the air for 10 min, and activate by drying at 100° for 1 h. Store the cool plates in a clean, dry place until ready for use.

Transfer 1 g of the sample into a 10-ml volumetric flask, dissolve, and dilute to volume with chloroform. Transfer 250 mg of stearic acid into another 10-ml volumetric flask, dissolve, and dilute to volume with chloroform.

Spot 2 µl of the sample solution and 1 µl of the stearic acid solution approximately 1.5 cm from the bottom of the plate, allow the spots to dry, and then place the plate in a suitable chromatographic chamber containing a mixture of 4 volumes of acetone, 4 volumes of acetic acid, and 92 volumes of hexane. Develop by ascending chromatography until the solvent front travels 15 cm beyond the sample spot. Remove the plate from the chamber, dry thoroughly in air, and spray evenly with a saturated solution of chromium trioxide in sulfuric acid. Immediately place the sprayed plate on a hot plate maintained at about 200° in a hood, char until white fumes of sulfur trioxide cease, and cool on an asbestos mat at room temperature. The spots from the sample are located according to the following R_f values: stearic acid, 1.00; fatty acid, 1.00; acylated monolactic acid, 0.84; acylated dilactic acid, 0.76; acylated trilactic acid, 0.68; and tetralactic acid, 0.62.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

The following specifications should conform to the representations of the vendor: **Acylated Monolactic Acid, Acylated Polylactic Acid, Free Fatty Acid, Total Lactic Acid, Acid Value, Saponification Value,** and **Water.**

TESTS

Assay for Acylated Monolactic Acid, Acylated Polylactic Acid, and Free Fatty Acid This assay is performed by gas-liquid chromatography (see page 475) using an instrument containing a thermal conductivity or flame ionization detector and

helium as the carrier gas. The operating conditions of the apparatus may vary, depending upon the particular instrument used, but a suitable chromatogram is obtained with a 1.2-m × 6.3-mm column packed with 20% SE-30 or SE-52, or other comparable grades of silicone rubber gums, on Chromosorb P or W or Diatoport S, or other comparable grades of diatomaceous material. The column should be programmed between 150° and 310°, using a heating rate of 4° per min, the inlet port temperature should be 335°, and the detector temperature should be 315°. The recorder should be equipped with an attenuator switch and should be operated in the 0- to 1-mV range, with 1-s full-scale deflection at a chart speed of 12.7 mm per min. A constant gas flow rate of about 54 ml per min should be established and maintained throughout the determination.

Diazomethane Reagent [*Caution:* Diazomethane is both toxic and potentially explosive. Its preparation and use should be carried out in a hood.] Place 1 ml of potassium hydroxide solution (4 in 10), followed by 2.5 ml of methanol, in a 25-ml distilling flask fitted with a dropping funnel and an efficient spiral water-cooled condenser set downward for distillation. Connect the condenser to a 50-ml receiving flask that is cooled in ice and vented to the hood. Heat the distilling flask in a water bath to 65°, add 2 ml of ether through the dropping funnel, saturating the distillation apparatus with ether vapor, and close the stopcock. Place in the dropping funnel a solution containing 2.15 g of *N*-methyl-*N*-nitroso-*p*-toluene sulfonamide in 13 ml of ether, and adjust the stopcock so that the rate of distillation is about equal to the rate of addition from the funnel. When the funnel is empty, add another 2 ml of ether, and continue the distillation until the distillate is colorless. The ether-alcohol solution of diazomethane so obtained should be used immediately or stored at −10° until used.

Procedure To approximately 50 mg of the sample add the *Diazomethane Reagent* until a yellow color persists. Carefully evaporate the ether at 50° under a stream of clean, dry nitrogen. Inject 0.5 to 2.0 µl of the melted methyl esters so obtained into the gas chromatographic apparatus, using a 10-µl capacity Hamilton fixed needle or equivalent. The sample size should be adjusted so that the major peak is not attenuated more than ×8. From the chromatogram so obtained, identify the peaks by their relative position on the chart. The esters, appearing in the order of increasing number of carbon atoms in the fatty acid and in order of increasing length of the polymer, are eluted as follows: myristate, palmitate, stearate, palmitoyl lactylate (2-palmitoyloxypropionate), stearoyl lactylate (2-stearoyloxypropionate), palmitoyl lactoyl lactylate, stearoyl lactoyl lactylate, palmitoyl dilactoyl lactylate, stearoyl dilactoyl lactylate, palmitoyl trilactoyl lactylate, stearoyl trilactoyl lactylate, and palmitoyl tetralactoyl lactylate. Other esters may be determined by interpolation of a conventional carbon number–retention plot.

Determine the composition of the sample, using the area normalization method, by the formula

$$\%_i = 100A_i/\Sigma(A_1 + \cdot\cdot\cdot + A_n),$$

in which i represents the component of interest, A_i is the equalized area for the component of interest, and $\Sigma(A_i + \cdot\cdot\cdot + A_n)$ is the sum of the equalized areas.

If free and polylactic acids are present, as determined below, the results should be corrected by multiplying $\%_i$ by [(100 − % free and polylactic acid)/100].

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Saponification Value Determine as directed in the general procedure, page 509.

Total Free and Polylactic Acids Weigh accurately about 500 mg of the sample, previously melted, transfer into a 50-ml glass-stoppered separator with the aid of 15 ml of benzene, and add 10 ml of water. Invert the funnel 10 times, and allow to stand until the layers have separated. Filter the aqueous layer through a plug of glass wool into a 125-ml flask, wash the benzene with two 10-ml portions of water, and combine the aqueous layers. To the flask add 5.0 ml of 0.1 *N* sodium hydroxide, and then heat on a steam bath for 15 min under a nitrogen atmosphere. Add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid to the disappearance of the pink color. Conduct a blank determination, using 30 ml of water and 5.0 ml of 0.1 *N* sodium hydroxide, and calculate the percentage of free and polylactic acids in the sample by the formula

$$(B - S) \times 9.0/W,$$

in which $B - S$ represents the difference, in ml, between the volumes of 0.1 *N* hydrochloric acid required for the blank and the sample, respectively, 9.0 is an equivalence factor for the lactic acid, and W is the weight, in g, of the sample.

Total Lactic Acid Transfer an accurately weighed portion of the melted sample, equivalent to between 140 mg and 170 mg of lactic acid, into a 250-ml Erlenmeyer flask, and add to the flask 20.0 ml of 0.5 *N* alcoholic potassium hydroxide. Connect an air condenser, at least 65 cm in length, to the flask, and reflux for 30 min. Add 20 ml of water through the condenser, disconnect the condenser, and prepare a blank containing 20.0 ml of the alkali and 20 ml of water. Evaporate the contents of each flask to a volume of about 20 ml, cool to about 40°, add methyl red TS to the flask containing the blank, and titrate the blank with 0.5 *N* hydrochloric acid. Add exactly the same volume of the acid to the sample flask, with swirling, and then add to the sample flask 50 ml of hexane, swirling vigorously to dissolve the fatty acids. Transfer quantitatively the contents of the sample flask into a 250-ml separator, and shake for 30 s. Collect the aqueous phase in an Erlenmeyer flask, wash the hexane solution with 50 ml of water, and combine the wash solution with the original aqueous phase in the flask, discarding the hexane solution. Add phenolphthalein TS, and titrate with 0.1 *N* potassium hydroxide to a pink color that persists for at least 30 s. Each ml of 0.1 *N* potassium hydroxide is equivalent to 9.008 mg of lactic acid ($C_3H_6O_3$).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight, plastic-lined containers in a cool, dry place.

Functional Use in Foods Emulsifier; surface-active agent.

Lanolin, Anhydrous

Wool Fat

DESCRIPTION

A purified, yellowish white, semisolid, fatlike substance extracted from the wool of sheep. It is insoluble in water, but mixes with about twice its weight of water without separation. It is soluble in chloroform and in ether.

REQUIREMENTS

Acid Value Not more than 1.12.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Iodine Value Between 18 and 36.
Lead Not more than 3 ppm.
Loss on Heating Not more than 0.5%.
Melting Range Between 36° and 42°.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Iodine Value Determine by the *Wijs Method*, page 505.

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Heating Heat a 5-g sample on a steam bath, with frequent stirring, to constant weight.

Melting Range Determine as directed in the general procedure, page 519.

Packaging and Storage Store in well-closed containers, preferably at a temperature not exceeding 30°.

Functional Use in Foods Masticatory substance in chewing gum base.

Laurel Leaf Oil

Bay Leaf Oil

DESCRIPTION

The oil obtained by steam distillation from the leaves of *Laurus nobilis* L. (Fam. *Lauraceae*). It is a light yellow to yellow liquid having an aromatic and spicy odor. It is soluble in most fixed oils, and is soluble with cloudiness in mineral oil and in propylene glycol. It is insoluble in glycerin.

NOTE: The oil from *Laurus nobilis* L. should not be confused with that of the West Indian bay tree, or California bay laurel.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 597, using the same test conditions as specified therein.

Acid Value Not more than 3.0.
Angular Rotation Between −10° and −19°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.465 and 1.470 at 20°.
Saponification Value Between 15 and 45.
Saponification Value after Acetylation Between 36 and 85.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.905 and 0.929.

TESTS

Acid Value Determine as directed in the general procedure, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Saponification Value after Acetylation Proceed as directed under *Total Alcohols*, page 499, using about 2.5 g of acetylated oil, accurately weighed. Calculate the saponification value by the formula $28.05 \times A/B$, in which A is the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the titration, and B is the weight of the acetylated oil, in g.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 80% alcohol and remains in solution upon dilution to 10 ml.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or aluminum containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Lauric Acid

Dodecanoic Acid

$$CH_3(CH_2)_{10}COOH$$

$C_{12}H_{24}O_2$ Mol wt 200.32

DESCRIPTION

A solid organic acid obtained from coconut oil and other vegetable fats. It occurs as a white or faintly yellowish, somewhat glossy, crystalline solid or powder. It is practically insoluble in water, but is soluble in alcohol, in chloroform, and in ether.

REQUIREMENTS

Acid Value Between 252 and 287.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Not more than 3.0.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 253 and 287.
Titer (Solidification Point) Between 26° and 44°.
Unsaponifiable Matter Not more than 0.3%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Residue on Ignition, page 533 Ignite 10 g as directed in the general method.
Saponification Value Determine as directed in the general method, page 509, using about 3 g, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.
Unsaponifiable Matter Determine as directed in the general method, page 509.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Component in the manufacture of other food-grade additives; defoaming agent.

Lavandin Oil, Abrial Type

DESCRIPTION

An oil obtained by steam distillation of the fresh flowering tops of a hybrid, *Lavandula abrialis* unofficial (Fam. *Labiatae*), of true lavender, *Lavandula officinalis*, or of spike lavender, *Lavandula latifolia*. It is a pale yellow to yellow liquid having a slight camphoraceous odor that is strongly suggestive of lavender. It is soluble in most fixed oils and in propylene glycol. It is soluble with opalescence in mineral oil, but it is relatively insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 597, using the same test conditions as specified therein.

Assay Not less than 28.0% and not more than 35.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −2° and −5°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.460 and 1.464 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.885 and 0.893.

TESTS

Assay Weigh accurately about 3 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is

saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol. A slight opalescence sometimes develops on further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or good-quality galvanized containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Lavender Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh flowering tops of *Lavandula officinalis* Chaix ex Villars (*Lavandula vera* De Candolle) (Fam. *Labiatae*). It is a colorless or yellow liquid having the characteristic odor and taste of lavender flowers.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 597, using the same test conditions as specified therein.

Assay Not less than 35.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).

Alcohol Passes test.

Angular Rotation Between $-3°$ and $-10°$.

Foreign Water-Soluble Esters Passes test.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.459 and 1.470 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.875 and 0.888.

TESTS

Assay Weigh accurately about 5 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.

Alcohol Transfer 5 ml to a narrow, graduated, glass-stoppered, 10-ml cylinder, add 5 ml of water, and shake. The volume of the oil does not diminish.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Foreign Water-Soluble Esters Mix 20 ml of the sample with 40 ml of 5% alcohol in a glass-stoppered, 100-ml cylinder. When the mixture has cleared, pipet 30 ml of the alcohol layer into a 125-ml Erlenmeyer flask. Add phenolphthalein TS, and neutralize the solution with 0.5 *N* sodium hydroxide. Add 5.0 ml of 0.5 *N* sodium hydroxide and heat the mixture on a boiling water bath under a reflux condenser for 1 h. Allow the mixture to cool, and titrate the excess alkali with 0.5 *N* hydrochloric acid. Not less than 4.7 ml of the acid is required to neutralize the mixture.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Lecithin

DESCRIPTION

Food-grade lecithin is obtained from soybeans and other plant sources. It is a complex mixture of acetone-insoluble phosphatides that consists chiefly of phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl inositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates. Refined grades of lecithin may contain any of these components in varying proportions and combinations depending on the type of fractionation used. In its oil-free form, the preponderance of triglycerides and fatty acids is removed and the product contains 90% or more of phosphatides representing all or certain fractions of the total phosphatide complex. The consistency of both natural grades and refined grades of lecithin may vary from plastic to fluid, depending upon free fatty acid and oil content, and upon the presence or absence of other diluents. Its color varies from light yellow to brown, depending on the source, on crop variations, and on whether it is bleached or unbleached. It is odorless or has a characteristic, slight nutlike odor and a bland taste. Edible diluents, such as cocoa butter and vegetable oils, often replace soybean oil to improve functional and flavor characteristics. Lecithin is only partially soluble in water, but it readily hydrates to form emulsions. The oil-free phosphatides are soluble in fatty acids, but are practically insoluble in fixed oils. When all phosphatide fractions are present, lecithin is partially soluble in alcohol and practically insoluble in acetone.

REQUIREMENTS

Acetone-Insoluble Matter (phosphatides) Not less than 50.0%.
Acid Value Not more than 36.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Hexane-Insoluble Matter Not more than 0.3%.
Lead Not more than 10 ppm.
Peroxide Value Not more than 100.
Water Not more than 1.5%.

TESTS

Acetone-Insoluble Matter (phosphatides)

Purification of Phosphatides Dissolve 5 g of phosphatides from previous *Acetone-Insoluble Matter* determinations in 10 ml of petroleum ether, and add 25 ml of acetone to the solution. Transfer approximately equal portions of the precipitate to each of two 40-ml centrifuge tubes using additional portions of acetone to facilitate the transfer. Stir thoroughly, dilute to 40 ml with acetone, stir again, chill for 15 min in an ice bath, stir again, and then centrifuge for 5 min. Decant the acetone, crush the solids with a stirring rod, refill the tube with acetone, stir, chill, centrifuge, and decant as before. The solids after the second centrifugation require no further purification and may be used for preparing the *Phosphatide-Acetone Solution*. Five g of the purified phosphatides are required to saturate about 16 L of acetone.

Phosphatide-Acetone Solution Add a quantity of purified phosphatides to sufficient acetone, previously cooled to a temperature of about 5°, to form a saturated solution, and maintain the mixture at this temperature for 2 h, shaking it vigorously at 15-min intervals. Decant the solution through a rapid filter paper, avoiding the transfer of any undissolved solids to the paper and conducting the filtration under refrigerated conditions (not above 5°).

Procedure If it is plastic or semisolid, soften a portion of the lecithin by warming it in a water bath at a temperature not exceeding 60° and then mixing it thoroughly. Transfer 2 g of a well-mixed sample, accurately weighed, into a 40-ml centrifuge tube, previously tared with a glass stirring rod, and add 15 ml of *Phosphatide-Acetone Solution* from a buret. Warm the mixture in a water bath until the lecithin melts, but avoid evaporation of the acetone. Stir until the sample is completely disintegrated and dispersed, and then transfer the tube into an ice bath, chill for 5 min, remove from the ice bath, and add about one half of the required volume of *Phosphatide-Acetone Solution*, previously chilled for 5 min in an ice bath. Stir the mixture to complete dispersion of the sample, dilute to 40 ml with chilled *Phosphatide-Acetone Solution* (5°), again stir, and return the tube and contents to the ice bath for 15 min. At the end of the 15-min chilling period, stir again while still in the ice bath, remove the stirring rod, temporarily supporting it in a vertical upside-down position, and centrifuge the mixture immediately at about 2000 rpm for 5 min. Decant the supernatant liquid from the centrifuge tube, crush the centrifuged solids with the same stirring rod previously used, and refill the tube to the 40-ml mark with chilled (5°) *Phosphatide-Acetone Solution*, and repeat the chilling, stirring, centrifugation, and decantation procedure previously followed. After the second centrifugation and decantation of the supernatant acetone, again crush the solids with the assigned stirring rod, and place the tube and its contents in a horizontal position at room temperature until the excess acetone has evaporated. Mix the residue again, dry the centrifuge tube and its contents at 105° for 45 min in a forced-draft oven, cool, and weigh. Calculate the percentage of acetone-insoluble substances by the formula $(100R/S) - B$, in which R is the weight of residue, S is the weight of the sample, and B is the percentage of *Hexane-Insoluble Matter* determined as directed in this monograph.

Acid Value If it is plastic or semisolid, soften a portion of the lecithin by warming it in a water bath at a temperature not exceeding 60°, and then mix it thoroughly. Transfer about 2 g of a well-mixed sample into a 250-ml Erlenmeyer flask, and dissolve it in 50 ml of petroleum ether. To this solution add 50 ml of alcohol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide, and mix well. Add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for 5 s. Calculate the number of mg of potassium hydroxide required to neutralize the acids in 1 g of the sample by multiplying the number of ml of 0.1 *N* sodium hydroxide consumed in the titration by 5.6 and dividing the result by the weight of the sample.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hexane-Insoluble Matter If plastic or semisolid, soften a portion of the lecithin by warming it at a temperature not exceeding 60°, and then mix it thoroughly. Weigh 10 g of a previously well-mixed sample into a 250-ml wide-mouth Erlenmeyer flask, add 100 ml of solvent hexane, and shake until the lecithin is dissolved. Filter the solution through a 30-ml Corning "C" porosity or equivalent filtering funnel that previously has been dried at 105° for 1 h, cooled in a desiccator, and weighed. Wash the flask with two successive 25-ml portions of solvent hexane, and pass the washings through the filter. Dry the funnel at 105° for 1 h, cool to room temperature in a desiccator, and weigh. From the gain in weight of the funnel, calculate the percentage of the hexane-insoluble matter in the sample.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Peroxide Value Determine as directed under *Hydroxylated Lecithin*, page 148.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Antioxidant; emulsifier.

Lemongrass Oil

DESCRIPTION

A volatile oil prepared by steam distillation of freshly cut and partially dried cymbopogon grasses indigenous to tropical and subtropical areas. Two types of lemongrass oil are commercially available. The *East Indian* type, also known as Cochin, Native, and British Indian lemongrass oil, is usually a dark yellow to light brownish red liquid having a pronounced heavy lemonlike odor. The *West Indian* type, also known as Madagascar, Guatemala, or other country of origin lemongrass oil, is light yellow to light brown in color and has a lemonlike odor of a lighter character than the East Indian type oil. Lemongrass oils are soluble in mineral oil, freely soluble in propylene glycol, but practically insoluble in water and in glycerin. The East Indian variety dissolves readily in alcohol, but the West Indian oil yields cloudy solutions.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 597, using the same test conditions as specified therein.

Assay Not less than 75%, by volume, of aldehydes as citral.
Angular Rotation Between $-3°$ and $+1°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.483 and 1.489.
Solubility in Alcohol Passes test.
Specific Gravity *East Indian type:* between 0.894 and 0.904; *West Indian type:* between 0.869 and 0.894.
Steam-Volatile Oil Not less than 93%, by volume.

TESTS

Assay Mix 50.0 ml of the sample with 500 mg of tartaric acid, shake for 5 min, and filter. Dry the filtered oil over anhydrous sodium sulfate, and then pipet 10.0 ml of the clear, treated oil into a 150-ml cassia flask. (NOTE: Retain the remaining oil for the *Steam-Volatile Oil* test.) Add 75 ml of a 30% solution of sodium bisulfite, stopper the flask, and shake until a semisolid to solid sodium bisulfite addition product has formed. Allow the mixture to stand at room temperature for 5 min, then loosen the stopper, and immerse the flask in a water bath heated to between 85° and 90°. Maintain the water bath at this temperature, shaking the flask occasionally, until the addition product dissolves, and then continue the heating and intermittent shaking for another 30 min. When the liquids have separated completely, add enough of the sodium bisulfite solution to raise the lower level of the oily layer within the graduated portion of the neck of the flask. Calculate the percentage, by volume, of the citral by the formula

$$100 - (V \times 10),$$

in which V is the number of ml of separated oil in the graduated neck of the cassia flask.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. *East Indian type:* one ml dissolves in 3 ml of 70% alcohol, usually with slight turbidity; *West Indian type:* yields a cloudy solution with 70%, 80%, 90%, and 95% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Steam-Volatile Oil Proceed as directed under *Volatile Oil Content*, page 503, using 25.0 ml of the oil prepared as directed in the *Assay*.

Packaging and Storage Store in a cool place in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers, or in black iron unlined drums. If stored in glass containers, avoid exposure to light.

Labeling Label lemongrass oil to indicate whether it is the East Indian or West Indian type.

Functional Use in Foods Flavoring agent.

Lemon Oil, Coldpressed

Lemon Oil, Expressed

DESCRIPTION

A volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of *Citrus limon* L. Burmann filius (Fam. *Rutaceae*), with or without the previous separation of the pulp and the peel. It is a pale to deep yellow or greenish yellow liquid having the characteristic odor and taste of the outer part of fresh lemon peel. It is miscible with dehydrated alcohol and with glacial acetic acid. It may contain a suitable antioxidant.

NOTE: Do not use lemon oil that has a terebinthine odor.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths

(or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 598, using the same test conditions as specified therein.

Assay *California type:* not less than 2.2% and not more than 3.8% of aldehydes, calculated as citral ($C_{10}H_{16}O$); *Italian type:* not less than 3.0% and not more than 5.5% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +57° and +65.6°.
Arsenic (as As) Not more than 3 ppm.
Foreign Oils Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.473 and 1.476 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.849 and 0.855.
Ultraviolet Absorbance *California type:* not less than 0.2; *Italian type:* not less than 0.49.

TESTS

Assay Weigh accurately a 5-ml sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500. Allow the mixture to stand for 15 min, with occasional shaking, before titrating, and use 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Foreign Oils Transfer 50 ml to a Ladenburg flask having four bulbs of the following approximate diameters: 6 cm, 3.5 cm, 3.0 cm, and 2.5 cm, respectively, in ascending order. The distance from the bottom of the flask to the side arm is 20 cm. Distil the oil at the rate of 1 drop per second until the distillate measures 5 ml. The angular rotation of the distillate is not more than 6° less than that of the original oil, and the refractive index is not less than 0.001 and not more than 0.003 lower than that of the original oil at 20°.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 95% alcohol.
Specific Gravity Determine by any reliable method (see page 3).
Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.
Labeling Label lemon oil, coldpressed, to indicate whether it is the California type or the Italian type.
Functional Use in Foods Flavoring agent.

Lemon Oil, Distilled

DESCRIPTION

The volatile oil obtained by distillation from the fresh peel or juice of the fruit of *Citrus limon* L. Burmann filius (Fam. *Rutaceae*), with or without the previous separation of the juice, pulp, and peel. It is a colorless to pale yellow liquid having the characteristic odor of fresh lemon peel. It is soluble in most fixed oils, in mineral oil, and in alcohol (with haze). It is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 598, using the same test conditions as specified therein.

Aldehydes Between 1.0% and 3.5% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +55° and +75°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.470 and 1.475 at 20°.
Specific Gravity Between 0.842 and 0.856.
Ultraviolet Absorbance Not more than 0.01.

TESTS

Aldehydes Weigh accurately about 5 ml of the sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, using 76.12 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand at room temperature for 1 h before titrating.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see page 3).
Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 250 mg of

sample, accurately weighed. The maximum absorbance occurs at 315 ± 5 nm.

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Lemon Oil, Desert Type, Coldpressed

Lemon Oil Arizona

DESCRIPTION

The volatile oil obtained by expression, without the aid of heat, from the fresh peel of the fruit of *Citrus Limon* L. Burmann filius (Fam. *Rutaceae*), with or without the previous separation of the pulp and peel. It is a pale to deep yellow or greenish yellow liquid having the characteristic odor and taste of the outer part of fresh lemon peel. It is miscible with dehydrated alcohol and with glacial acetic acid. It may contain a suitable antioxidant.

NOTE: Do not use if it has a terebinthine odor.

REQUIREMENTS

Assay Not less than 1.7% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +67° and +78°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.473 and 1.476.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.846 and 0.851.
Ultraviolet Absorbance Not less than 0.20.

TESTS

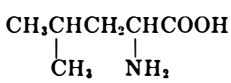
Assay Weigh accurately about 5 ml of the sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500. Allow the mixture to stand for 15 min, with occasional shaking, before titrating, and use 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Refractive Index, page 530 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of alcohol, sometimes with a slight haze.
Specific Gravity Determine by any reliable method (see page 3).
Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Functional Use in Foods Flavoring agent.

DL-Leucine

DL-2-Amino-4-methylvaleric Acid



$C_6H_{13}NO_2$ Mol wt 131.17

DESCRIPTION

Small white crystals or a crystalline powder. It is odorless and has a slightly bitter taste. It is freely soluble in water, slightly soluble in alcohol, and insoluble in ether. It melts with decomposition at about 290°. The pH of a 1 in 100 solution is between 5.5 and 7.0.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A reddish purple or bluish purple color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_6N_{13}NO_2$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 400 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid. Add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to the first appearance of a pure green color or until the blue color

disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

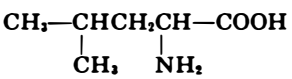
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Leucine

L-2-Amino-4-methylvaleric Acid



$C_6H_{13}NO_2$ Mol wt 131.18

DESCRIPTION

Small, white, lustrous plates, or a white crystalline powder. One g dissolves in about 40 ml of water and in about 100 ml of acetic acid. It is sparingly soluble in alcohol, but is soluble in dilute hydrochloric acid and in solutions of alkali hydroxides and carbonates.

REQUIREMENTS

Identification

It sublimes at about 150°.

Assay Not less than 98.5% of $C_6H_{13}NO_2$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.003%.

Iron Not more than 0.005%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.1%.

Methionine Passes test.

Nitrogen (Total) Not less than 10.6%.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]_D^{25°}$: Between +15.0° and +16.0°; $[\alpha]_D^{20°}$: between +14.9° and +16.5°.

Tyrosine Passes test.

TESTS

Assay Transfer about 400 mg, previously dried at 105° for two h and accurately weighed, into a 250-ml flask. Dissolve the sample in about 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 13.12 mg of $C_6H_{13}NO_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 670-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron To the ash obtained in the test for *Residue on Ignition* add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, and dilute with water to 50 ml. Dilute 10 ml of this solution to 40 ml with water, and add 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 µg Fe) in an equal volume of a solution containing the quantities of the reagents used in the test.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Methionine Dissolve 100 mg in 0.3 ml of sulfuric acid saturated with anhydrous cupric sulfate. No yellow color is produced within 2 min.

Nitrogen (Total) Proceed as directed under *Nitrogen Determination*, page 521, using about 300 mg of the sample previously dried and accurately weighed.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Specific Rotation, page 530 $[\alpha]_D^{25°}$: Determine in a solution containing 2 g in sufficient 6 *N* hydrochloric acid to make 100 ml; $[\alpha]_D^{20°}$: determine in a solution containing 4 g in sufficient 6 *N* hydrochloric acid to make 100 ml.

Tyrosine Dissolve 100 g in 3 ml of diluted sulfuric acid TS, and add to it 3 ml of a 1 in 10 solution of mercuric sulfate in diluted sulfuric acid TS and 0.5 ml of a 1 in 20 solution of sodium nitrite. No red or pink color appears within 15 min.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Lime Oil, Coldpressed

Lime Oil, Expressed

DESCRIPTION

The volatile oil obtained by expression from the fresh peel or crushed whole fruit of *Citrus aurantifolia* Swingle (Mexican type) or *Citrus latifolia* (Tahitian type). It is a yellow to brownish green to green liquid and often shows a waxy separation. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectra under *Infrared Spectra of Essential Oils*, pages 598 and 610, using the same test conditions as specified therein.

Assay *Mexican type:* not less than 4.5% and not more than 8.5% of aldehydes (as citral); *Tahitian type:* not less than 3.2% and not more than 7.5% of aldehydes (as citral).
Angular Rotation *Mexican type:* between +35° and +41°; *Tahitian type:* between +38° and +53°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index *Mexican type:* between 1.482 and 1.486; *Tahitian type:* between 1.476 and 1.486.
Residue on Evaporation *Mexican type:* between 10.0% and 14.5%; *Tahitian type:* between 5.0% and 12.0%.
Specific Gravity *Mexican type:* between 0.872 and 0.881; *Tahitian type:* between 0.858 and 0.876.
Ultraviolet Absorbance *Mexican type:* not less than 0.45; *Tahitian type:* not less than 0.24.

TESTS

Assay Weigh accurately about 5 ml of the sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500. Allow the mixture to stand for 1 h, with occasional shaking, before titrating, and use 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Residue on Ignition Proceed as directed in the general method, page 533, using a 3-g sample and heating for 6 h.
Specific Gravity Determine by any reliable method (see page 3).
Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 20 mg of sample, accurately weighed. The maximum absorbance occurs at 315 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.
Labeling Label lime oil, coldpressed, to indicate whether it is the Mexican or Tahitian type.
Functional Use in Foods Flavoring agent.

Lime Oil, Distilled

DESCRIPTION

The volatile oil obtained by distillation from the juice, or the whole crushed fruit, of *Citrus aurantifolia* Swingle. It is a colorless to greenish yellow liquid. It is soluble in most fixed oils and in mineral oil. It is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 598, using the same test conditions as specified therein.

Aldehydes Between 0.5% and 2.5% of aldehydes, calculated as citral ($C_{10}H_{16}O$).
Angular Rotation Between +34° and +47°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.474 and 1.477 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.855 and 0.863.

TESTS

Aldehydes Weigh accurately about 5 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, using 76.12 as the equivalence factor (*e*) in the calculation. Allow the

mixture to stand at room temperature for 15 min before titrating.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 5 ml of 90% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, galvanized, or black iron containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Limestone, Ground

DESCRIPTION

Ground limestone consists essentially of calcium carbonate. It is obtained by crushing, grinding, and classifying of naturally occurring limestone benefited by flotation and/or air classification. It is produced as a fine, white to off-white, microcrystalline powder. It is odorless and tasteless and is stable in air. It is practically insoluble in water and in alcohol. The presence of any ammonium salt or carbon dioxide increases its solubility in water, but the presence of any alkali hydroxide reduces the solubility.

REQUIREMENTS

Identification

It dissolves with effervescence in diluted acetic acid TS, in diluted hydrochloric acid TS, and in diluted nitric acid TS, and the resulting solutions, after boiling, give positive tests for *Calcium*, page 516.

Assay Not less than 94.0% of $CaCO_3$ after drying.
Acid-Insoluble Substances Not more than 2.5%.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Loss on Drying Not more than 2%.
Magnesium and Alkali Salts Not more than 3.5%.
Mercury Not more than 0.5 ppm.

TESTS

Assay Transfer about 200 mg, previously dried at 200° for 4 h and accurately weighed, into a 400-ml beaker, add 10 ml of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 ml of diluted hydrochloric acid TS from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the sample. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 ml with water. While stirring, preferably with a magnetic stirrer, add about 30 ml of 0.05 *M* disodium EDTA from a 50-ml buret, then add 15 ml of potassium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 5.004 mg of $CaCO_3$.

Acid-Insoluble Substances Suspend 5 g in 25 ml of water, cautiously add with agitation 25 ml of dilute hydrochloric acid (1 in 2), then add water to make a volume of about 200 ml. Heat the solution to boiling, cover, digest on a steam bath for 1 h, cool, and filter. Wash the precipitate with water until the last washing shows no chloride with silver nitrate TS, and then ignite it. The weight of the residue does not exceed 125 mg.

Arsenic A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed under *Method II* in the general procedure, page 511.

Sample Solution for the Determination of Heavy Metals and Lead Cautiously dissolve 5 g in 25 ml of dilute hydrochloric acid (1 in 2) and evaporate to dryness on a steam bath. Dissolve the residue in about 15 ml of water and dilute to 25 ml (1 ml = 200 mg).

Heavy Metals Neutralize 2.5 ml (500 mg) of the *Sample Solution* with sodium hydroxide TS, using phenolphthalein as the indicator, and dilute to 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 5-ml portion of the *Sample Solution* (1 g) meets the requirements of the *Lead Limit Test*, page 518, using 3 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 200° for 4 h.

Magnesium and Alkali Salts Mix 1 g with 40 ml of water, carefully add 5 ml of hydrochloric acid, mix, and boil for 1 min. Rapidly add 40 ml of oxalic acid TS, and stir vigorously until precipitation is well established. Immediately add 2 drops of methyl red TS, then add ammonia TS, dropwise, until the mixture is just alkaline, and cool. Transfer the mixture to a 100-ml cylinder, dilute with water to 100 ml, let it stand for 4 h or overnight, then decant the clear, supernatant liquid through a dry filter paper. To 50 ml of the clear filtrate in a platinum dish add 0.5 ml of sulfuric acid, and evaporate the mixture on a steam bath to a small volume. Carefully evaporate the remaining liquid to dryness over a free flame, and continue heating until the ammonium salts have been completely decomposed and volatilized. Finally,

ignite the residue to constant weight. The weight of the residue does not exceed 17.5 mg.

Mercury Determine as directed under *Mercury Limit Test*, page 520, using the following as the *Sample Preparation*: Transfer 4.0 g of the sample into a 50-ml beaker, cautiously dissolve in 10 ml of dilute hydrochloric acid solution (1 in 2), add 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with sodium hydroxide TS. Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Texturizing and release agent and modifier for chewing gum base and chewing gum.

Linaloe Wood Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the wood of *Bursera delpechiana* Poiss. (Fam. *Burseraceae*) and other *Bursera* species. It is a colorless to yellow liquid having a pleasant flowery odor. It is soluble in most fixed oils and in propylene glycol. It is soluble in mineral oil, but becomes opalescent or turbid on dilution. It is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 599, using the same test conditions as specified therein.

Assay Not less than 85.0% of alcohols, calculated as linalool ($C_{10}H_{18}O$).

Acid Value Not more than 3.0.

Angular Rotation Between $-5°$ and $-13°$.

Ester Value Between 40 and 75.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.459 and 1.463 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.876 and 0.883.

TESTS

Assay Proceed as directed under *Linalool Determination*, page 501, using about 1.5 g of acetylated oil, accurately weighed, for the saponification.

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Determine as directed in the general method, page 501, using about 2.5 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 5 ml of 60% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Locust (Carob) Bean Gum

Locust Bean Gum; Carob Bean Gum

DESCRIPTION

A gum obtained from the ground endosperms of *Ceratonia siliqua* (L.) Taub. (Fam. *Leguminosae*). It consists chiefly of a high-molecular-weight hydrocolloidal polysaccharide, composed of galactose and mannose units combined through glycosidic linkages, which may be described chemically as a galactomannan. It is a white to yellowish white, nearly odorless powder. It is dispersible in either hot or cold water, forming a sol having a pH between 5.4 and 7.0, which may be converted to a gel by the addition of small amounts of sodium borate.

REQUIREMENTS

Identification

A. Transfer a 2-g sample into a 400-ml beaker, moisten it with about 4 ml of isopropyl alcohol, add with vigorous stirring 200 ml of cold water, and continue the stirring until the gum is uniformly dispersed. An opalescent, slightly viscous solution is formed.

B. Transfer 100 ml of the solution prepared in *Identification Test A* into another 400-ml beaker, heat the mixture in a boiling water bath for about 10 min, and then cool to room temperature. An appreciable increase in viscosity is produced (*distinction from guar gum*), except for the flash-ground gum.

Acid-Insoluble Matter Not more than 5%.

Arsenic (as As) Not more than 3 ppm.

Ash (Total) Not more than 1.2%.

Galactomannans Not less than 73.0%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 15%.
Protein Not more than 8%.
Starch Passes test.

TESTS

Acid-Insoluble Matter Transfer 1.5 g of the sample, accurately weighed, into a 250-ml beaker containing 150 ml of water and 1.5 ml of concentrated sulfuric acid. Cover the beaker with a watch glass, and heat the mixture on a steam bath for 6 h, rubbing down the wall of the beaker frequently with a rubber-tipped stirring rod and replacing any water lost by evaporation. Then add about 500 mg of a suitable filter aid, accurately weighed, and filter through a tared Gooch crucible. Wash the residue several times with hot water, dry the crucible and its contents at 105° for 3 h, cool in a desiccator, and weigh. The difference between the weight of the filter aid and that of the residue is the weight of the *Acid-Insoluble Matter*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Total) Determine as directed in the general method, page 466.

Galactomannans The remainder, after subtracting from 100% the sum of the percentages of *Acid-Insoluble Matter*, *Total Ash*, *Loss on Drying*, and *Protein*, represents the percentage of galactomannans in the sample.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 5 h.

Protein Transfer about 3.5 g, accurately weighed, into a 500-ml Kjeldahl flask, and proceed as directed under *Nitrogen Determination*, page 521. The percentage of nitrogen determined, multiplied by 6.25, gives the percentage of protein in the sample.

Starch To a 1 in 10 solution of the gum add a few drops of iodine TS. No blue color is produced.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Lovage Oil

DESCRIPTION

The volatile oil obtained by steam distillation of the fresh root of the plant *Levisticum officinale* L. Koch syn. *Angelica levisticum*, Baillon (Fam. *Umbelliferae*). It is a yellow-greenish brown to deep brown liquid having a strong characteristic aromatic odor and taste. It is soluble in most fixed oils and slightly soluble, with opalescence, in mineral oil, but it is relatively insoluble in glycerin and in propylene glycol.

NOTE: This oil becomes darker and more viscous under the influence of air and light.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 599, using the same test conditions as specified therein.

Acid Value Between 2.0 and 16.0.
Angular Rotation Between −1° and +5°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.536 and 1.554 at 20°.
Saponification Value Between 238 and 258.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.034 and 1.057.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using 1.5 g accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 80% alcohol, sometimes with slight turbidity. The age of the oil has an adverse effect upon solubility.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

L-Lysine Monohydrochloride

2,6-Diaminohexanoic Acid Hydrochloride

$$NH_2(CH_2)_4CH(NH_2)COOH.HCl$$

$C_6H_{14}N_2O_2.HCl$ Mol wt 182.65

DESCRIPTION

A white or nearly white, practically odorless, free-flowing, crystalline powder. It is freely soluble in water, but is almost insoluble in alcohol and in ether. It melts at about 260° with decomposition.

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 100 solution with 1 ml of ninhydrin TS. A violet color is produced.
B. A 1 in 20 solution gives positive tests for *Chloride*, page 516.

Assay Not less than 98.0% of $C_6H_{14}N_2O_2.HCl$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 1%.
Residue on Ignition Not more than 0.2%.
Specific Rotation $[\alpha]_D^{25°}$: Between +19.6° and +21.6°.

TESTS

Assay Transfer about 150 mg, accurately weighed, into a 150-ml beaker, and dissolve in 8 ml of mercuric acetate TS, heating on a steam bath to effect solution. Cool, add 100 ml of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 9.133 mg of $C_6H_{14}N_2O_2.HCl$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition Ignite 2 g as directed in the general method, page 533.
Specific Rotation, page 530 Determine in a solution containing 2.5 g in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Mace Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the ground, dried arillode of the ripe seed of *Myristica fragrans* Houtt. (Fam. *Myristicaceae*). Two types of oil, the East Indian and the West Indian, are commercially available. It is a colorless to pale yellow liquid having the characteristic odor and taste of nutmeg. It is soluble in most fixed oils and in mineral oil, but it is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 599, using the same test conditions as specified therein.

Angular Rotation *East Indian type:* between +2° and +30°; *West Indian type:* between +20° and +45°.
Heavy Metals (as Pb) Passes test.
Refractive Index *East Indian type:* between 1.474 and 1.488; *West Indian type:* between 1.469 and 1.480 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity *East Indian type:* between 0.880 and 0.930; *West Indian type:* between 0.854 and 0.880.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 90% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, glass, tin-lined, or other suitably lined containers in a cool place protected from light.
Labeling Label mace oil to indicate whether it is the East Indian or West Indian type.
Functional Use in Foods Flavoring agent.

Magnesium Carbonate

DESCRIPTION

Magnesium carbonate is a basic hydrated magnesium carbonate or a normal hydrated magnesium carbonate. It occurs as light, white, friable masses, or as a bulky, white powder. It is odorless, and is stable in air. It is practically insoluble in water, to which, however, it imparts a slightly alkaline reaction. It is insoluble in alcohol, but is dissolved by dilute acids with effervescence.

REQUIREMENTS

Identification

When treated with diluted hydrochloric acid TS, it dissolves with effervescence, and the resulting solution gives positive tests for *Magnesium*, page 517.

Assay The equivalent of not less than 40.0% and not more than 43.5% of MgO.
Acid-Insoluble Substances Not more than 0.05%.
Arsenic (as As) Not more than 3 ppm.
Calcium Oxide Not more than 0.6%.
Heavy Metals (as Pb) Not more than 0.003%.
Lead Not more than 10 ppm.
Soluble Salts Not more than 1%.

TESTS

Assay Dissolve about 1 g, accurately weighed, in 30.0 ml of 1 *N* sulfuric acid, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed, deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the weight of the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the magnesium oxide present. Each ml of 1 *N* sulfuric acid is equivalent to 20.16 mg of MgO and to 28.04 mg of CaO.

Acid-Insoluble Substances Mix 5.0 g with 75 ml of water, add hydrochloric acid in small portions, with agitation, until no more of the sample dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool, and weigh.

Arsenic A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Calcium Oxide Dissolve about 1 g, accurately weighed, in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a Gooch crucible containing an asbestos mat that previously has been washed with diluted sulfuric acid TS, water, and alcohol, and ignited and weighed. Wash the crystals on the mat several times with a mixture of 2 volumes of alcohol and 1 volume of diluted sulfuric acid TS. Ignite the crucible and contents at a dull red heat, cool, and weigh. The weight of calcium sulfate so obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

Heavy Metals Dissolve 667 mg in 10 ml of diluted hydrochloric acid TS, and evaporate the solution to dryness on a steam bath. Toward the end of the evaporation, stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue in 20 ml of water, and evaporate to dryness in the same manner as before. Redissolve the residue in 25 ml of water, and filter if necessary. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Soluble Salts Mix 2.0 g with 100 ml of a mixture of equal volumes of *n*-propyl alcohol and water. Heat the mixture to the boiling point with constant stirring, cool to room temperature, add water to make 100 ml, and filter. Evaporate 50 ml of the filtrate on a steam bath to dryness, and dry at 105° for 1 h. The weight of the residue does not exceed 10 mg.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Alkali; drying agent; color-retention agent; anticaking agent; carrier.

Magnesium Chloride

$MgCl_2.6H_2O$ Mol wt 203.30

DESCRIPTION

Colorless, odorless flakes or crystals. It is very deliquescent. It is very soluble in water and freely soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Magnesium*, page 517, and for *Chloride*, page 516.

Assay Not less than 99.0% and not more than the equivalent of 105.0% of $MgCl_2.6H_2O$.
Ammonium Not more than 0.005%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Sulfate Not more than 0.02%.

TESTS

Assay Dissolve about 450 mg, accurately weighed, in 25 ml of water, add 5 ml of ammonia-ammonium chloride buffer TS and 0.1 ml of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA until the solution is blue in color. Each ml

of 0.05 *M* disodium EDTA is equivalent to 10.16 mg of $MgCl_2.6H_2O$.

Ammonium Dissolve 1 g in 90 ml of water, and slowly add 10 ml of a freshly boiled and cooled solution of sodium hydroxide (1 in 10). Allow to settle, then decant 20 ml of the supernatant liquid into a color comparison tube, dilute to 50 ml with water, and add 2 ml of Nessler's reagent. Any color does not exceed that produced by 10 µg of ammonium (NH_4) ion in 48 ml of water and 2 ml of the sodium hydroxide solution.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Sulfate, page 471 Any turbidity produced by a 1-g sample does not exceed that shown in a control containing 200 µg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Functional Use in Foods Color-retention agent; firming agent.

Magnesium Hydroxide

$Mg(OH)_2$ Mol wt 58.32

DESCRIPTION

A white, bulky powder. It dissolves in dilute acids, but is practically insoluble in water and in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution in diluted hydrochloric acid TS gives positive tests for *Magnesium*, page 517.

Assay Not less than 95.0% of $Mg(OH)_2$ after drying.

Alkalies (Free) and Soluble Salts Passes test.

Arsenic (as As) Not more than 3 ppm.

Calcium Oxide Not more than 1%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 2%.

Loss on Ignition Between 30.0% and 33.0%.

TESTS

Assay Transfer about 400 mg, previously dried at 105° for 2 h and accurately weighed, into an Erlenmeyer flask. Add 25.0 ml of 1 *N* sulfuric acid, and, after solution is complete, add methyl red TS and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed, deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the sample taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the $Mg(OH)_2$ in the sample of magnesium hydroxide taken. Each ml of 1 *N* sulfuric acid is equivalent to 29.16 mg of $Mg(OH)_2$ and to 28.04 mg of CaO.

Alkalies (Free) and Soluble Salts Boil 2 g with 100 ml of water for 5 min in a covered beaker, then filter while hot. Titrate 50 ml of the cooled filtrate with 0.1 *N* sulfuric acid, using methyl red TS as the indicator. Not more than 2 ml of the acid is consumed. Evaporate 25 ml of the filtrate to dryness, and dry at 105° for 3 h. Not more than 10 mg of residue remains.

Arsenic A solution of 1 g in 25 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Calcium Oxide Dissolve about 500 mg, accurately weighed, in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of alcohol, and allow the mixture to stand overnight. Warm the mixture to about 50°, if necessary, to dissolve any crystals of magnesium sulfate, and filter through a Gooch crucible containing an asbestos mat that has been previously washed with diluted sulfuric acid TS, water, and alcohol, and ignited. Wash the crystals on the mat several times with a mixture of 3 volumes of alcohol and 1 volume of water. Ignite the crucible and contents at a dull red heat, cool, and weigh. The weight of calcium sulfate thus obtained, multiplied by 0.4119, gives the equivalent of calcium oxide (CaO).

Heavy Metals Dissolve 1 g in 10 ml of diluted hydrochloric acid TS, and evaporate to dryness on a steam bath. Toward the end of the evaporation, stir the residue frequently, disintegrate it to obtain a dry powder, dissolve the powder in 20 ml of water, and filter. A 10-ml portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 20 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Loss on Ignition Transfer about 500 mg, accurately weighed, to a tared platinum crucible, and ignite, increasing the heat gradually, to constant weight.

Packaging and Storage Store in tight containers.

Functional Use in Foods Alkali; drying agent; color-retention agent.

Magnesium Oxide

MgO Mol wt 40.30

DESCRIPTION

A very bulky, white powder known as light magnesium oxide or a relatively dense, white powder known as heavy magnesium oxide. Five g of light magnesium oxide occupy a volume of approximately 40 to 50 ml, while 5 g of heavy magnesium oxide occupy a volume of approximately 10 to 20 ml. It is practically

insoluble in water and is insoluble in alcohol. It is soluble in dilute acids.

REQUIREMENTS

Identification

A solution of magnesium oxide in diluted hydrochloric acid TS gives positive tests for *Magnesium*, page 517.

Assay Not less than 96.0% of MgO after ignition.
Acid-Insoluble Substances Not more than 0.1%.
Alkalies (Free) and Soluble Salts Passes test.
Arsenic (as As) Not more than 3 ppm.
Calcium Oxide Not more than 1.5%.
Heavy Metals (as Pb) Not more than 0.04%.
Lead Not more than 10 ppm.
Loss on Ignition Not more than 10%.

TESTS

Assay Ignite about 500 mg to constant weight at 800° to 825° in a tared platinum crucible, weigh the residue accurately, dissolve it in 30.0 ml of 1 *N* sulfuric acid, boil gently to remove any carbon dioxide, cool, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. From the volume of 1 *N* sulfuric acid consumed deduct the volume of 1 *N* sulfuric acid corresponding to the content of calcium oxide in the magnesium oxide taken for the assay. The difference is the volume of 1 *N* sulfuric acid equivalent to the MgO in the portion of magnesium oxide taken. Each ml of 1 *N* sulfuric acid is equivalent to 20.15 mg of MgO and to 28.04 mg of CaO.

Acid-Insoluble Substances Mix 2 g with 75 ml of water, add hydrochloric acid in small portions, with agitation, until no more dissolves, and boil for 5 min. If an insoluble residue remains, filter, wash well with water until the last washing is free from chloride, ignite, cool, and weigh.

Alkalies (Free) and Soluble Salts Boil 2 g with 100 ml of water for 5 min in a covered beaker, and filter while hot. Add methyl red TS, and titrate 50 ml of the cooled filtrate with 0.1 *N* sulfuric acid. Not more than 2 ml of the acid is consumed. Evaporate 25 ml of the filtrate to dryness, and dry at 105° for 1 h. Not more than 10 mg of residue remains.

Arsenic A solution of 1 g in 20 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Calcium Oxide Dissolve about 400 mg, accurately weighed, in a mixture of 3 ml of sulfuric acid and 22 ml of water. Add 50 ml of alcohol, and allow the mixture to stand overnight. If crystals of magnesium sulfate separate, warm the mixture to about 50° to dissolve them. Filter through a Gooch crucible containing an asbestos mat that previously has been washed with diluted sulfuric acid TS, water, and alcohol, and ignited and weighed. Wash the crystals on the mat several times with a mixture of 2 volumes of alcohol and 1 volume of diluted sulfuric acid TS. Ignite the crucible and contents at a dull red heat, cool, and weigh. The weight of calcium sulfate obtained, multiplied by 0.4119, gives the equivalent of calcium oxide in the sample taken for the test.

Heavy Metals Dissolve 500 mg in 20 ml of diluted hydrochloric acid TS, and evaporate the solution to dryness on a steam bath. Toward the end of the evaporation, stir frequently to disintegrate the residue so that finally a dry powder is obtained. Dissolve the residue in 20 ml of water and evaporate to dryness in the same manner as before. Redissolve the residue in 20 ml of water and filter if necessary. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 20 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Ignition Weigh accurately about 500 mg in a tared covered platinum crucible. Ignite at between 800° and 825° for 15 min, cool, and weigh.

Packaging and Storage Store in tight containers.

Labeling Label magnesium oxide to indicate whether it is light magnesium oxide or heavy magnesium oxide.

Functional Use in Foods Alkali; neutralizer.

Magnesium Phosphate, Dibasic

Dimagnesium Phosphate

$MgHPO_4 \cdot 3H_2O$ Mol wt 174.33

DESCRIPTION

A white, odorless crystalline powder. It is slightly soluble in water and insoluble in alcohol, but is soluble in dilute acids.

REQUIREMENTS

Identification

A. Dissolve about 200 mg in 10 ml of diluted nitric acid TS, and add, dropwise, ammonium molybdate TS. A greenish yellow precipitate of ammonium phosphomolybdate forms that is soluble in ammonia TS.

B. Dissolve 100 mg in 0.5 ml of diluted acetic acid TS and 20 ml of water. Add 1 ml of ferric chloride TS, let stand for 5 min, and filter. The filtrate gives a positive test for *Magnesium*, page 517.

Assay Not less than 96.0% of $Mg_2P_2O_7$, calculated on the ignited basis.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 0.003%.
Lead Not more than 5 ppm.
Loss on Ignition Between 29% and 36%.

TESTS

Assay Weigh accurately about 500 mg of the residue obtained in the test for *Loss on Ignition*, and dissolve it by heating in a mixture of 50 ml of water and 2 ml of hydrochloric acid. Cool, dilute to 100.0 ml with water, and mix. Transfer 50.0 ml of this solution into a 400-ml beaker, add 100 ml of water, and heat to 55° to 60°. From a buret add 15 ml of 0.1 *M* disodium EDTA, add a magnetic stirring bar, and adjust with sodium hydroxide TS to pH 10 while stirring. Add 10 ml of ammonia-ammonium chloride buffer TS and 12 drops of eriochrome black TS, and continue the titration with 0.1 *M* disodium EDTA until the wine-red color changes to pure blue. Calculate the weight, in mg, of $Mg_2P_2O_7$ in the residue taken by the formula

$$2 \times 11.13 \times V,$$

in which V is the volume, in ml, of 0.1 *M* disodium EDTA required in the titration of the 50.0-ml aliquot.

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in *Method III* under *Fluoride Limit Test*, page 511 (except in the *Procedure* use 10 ml of 1 *N* hydrochloric acid to dissolve the sample), or use the following procedure: Transfer 5.0 g of the sample into a 200-ml distilling flask connected with a condenser and carrying a thermometer and a dropping funnel equipped with a stopcock. Dissolve the sample in 25 ml of dilute sulfuric acid (1 in 4), add 6 glass beads, and connect the apparatus for distillation, using a 600-ml beaker to collect the distillate. Add 40 ml of the dilute sulfuric acid to the flask through the dropping funnel, then fill the funnel with water, heat the solution to boiling, and continue heating until the temperature reaches 165°. Adjust the stopcock of the dropping funnel so that the temperature is maintained at 165° ± 5°, and continue the distillation until about 300 ml has been collected. Rinse the condenser and condenser arm with water, collecting the rinsings in the beaker. Add sodium hydroxide TS to the distillate to make it alkaline to litmus paper, and then add 5 ml in excess. Add 5 ml of 30% hydrogen peroxide and 6 glass beads to the beaker, boil until a volume of about 30 ml is reached, and cool. Transfer the condensed distillate, including the glass beads, into a 125-ml distilling flask connected with a condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Add 30 ml of perchloric acid, and continue as directed under the *Fluoride Limit Test, Method I*, page 510, beginning with "Connect a small dropping funnel or a steam generator to the capillary tube. . . . "

Heavy Metals, page 512 Suspend 1.33 g in 20 ml of water, and add hydrochloric acid, dropwise, until the sample just dissolves. Adjust the pH to between 3 and 4, filter, and dilute the filtrate to 40 ml with water. For the control (*Solution A*), add 20 µg of lead ion (Pb) to 10 ml of the filtrate, and dilute to 40 ml. For the sample (*Solution B*), dilute the remaining 30 ml of the filtrate to 40 ml. Add 10 ml of hydrogen sulfide TS to each solution, and allow to stand for 5 min. *Solution B* is no darker than *Solution A*.

Lead Dissolve 1 g in 20 ml of diluted hydrochloric acid TS, evaporate the solution to a volume of about 10 ml on a steam bath, dilute to about 20 ml with water, and cool. This solution meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Ignition Weigh accurately about 1 g, and ignite, preferably in a muffle furnace, at 800° ± 25° to constant weight.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Magnesium Phosphate, Tribasic

Trimagnesium Phosphate

$Mg_3(PO_4)_2 \cdot xH_2O$ Mol wt (anhydrous) 262.86

DESCRIPTION

Tribasic magnesium phosphate may contain four, five, or eight molecules of water of hydration. It occurs as a white, odorless, tasteless crystalline powder. It is readily soluble in dilute mineral acids, but is almost insoluble in water.

REQUIREMENTS

Identification

A. Dissolve about 200 mg in 10 ml of diluted nitric acid TS, and add, dropwise, ammonium molybdate TS. A greenish yellow precipitate of ammonium phosphomolybdate forms that is soluble in ammonia TS.

B. Dissolve 100 mg in 0.7 ml of diluted acetic acid TS and 20 ml of water. Add 1 ml of ferric chloride TS, let stand for 5 min, and filter. The filtrate gives a positive test for *Magnesium*, page 517.

Assay Not less than 98.0% and not more than the equivalent of 101.5% of $Mg_3(PO_4)_2$, calculated on the ignited basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 5 ppm.

Loss on Heating $Mg_3(PO_4)_2 \cdot 4H_2O$: between 15% and 23%; $Mg_3(PO_4)_2 \cdot 5H_2O$: between 20% and 27%; $Mg_3(PO_4)_2 \cdot 8H_2O$: between 30% and 37%.

TESTS

Assay Weigh accurately about 200 mg of the sample, and dissolve it in a mixture of 25 ml of water and 10 ml of diluted nitric acid TS. Filter, if necessary, wash any precipitate, then dissolve the precipitate by the addition of 1 ml of diluted nitric acid TS. Adjust the temperature to about 50°, add 75 ml of ammonium molybdate TS, and maintain the temperature at about 50° for 30 min, stirring occasionally. Allow to stand for 16 h or overnight at room temperature. Wash the

precipitate once or twice with water by decantation, using from 30 to 40 ml each time, and pour these two washings through a filter. Transfer the precipitate to the same filter, and wash with potassium nitrate solution (1 in 100) until the last washing is not acid to litmus paper. Transfer the precipitate and filter to the precipitation vessel, add 50.0 ml of 1 *N* sodium hydroxide, agitate until the precipitate is dissolved, add 3 drops of phenolphthalein TS, and then titrate the excess alkali with 1 *N* sulfuric acid. Each ml of 1 *N* sodium hydroxide is equivalent to 5.714 mg of $Mg_3(PO_4)_2$.

Arsenic A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in the *Fluoride Limit Test* under *Magnesium Phosphate, Dibasic*, page 180.

Heavy Metals, page 512 Suspend 1.33 g in 20 ml of water, and add hydrochloric acid, dropwise, until the sample just dissolves. Adjust the pH to between 3 and 4, filter, and dilute the filtrate to 40 ml with water. For the control (*Solution A*), add 20 µg of lead ion (Pb) to 10 ml of the filtrate, and dilute to 40 ml. For the sample (*Solution B*), dilute the remaining 30 ml of the filtrate to 40 ml. Add 10 ml of hydrogen sulfide TS to each solution, and allow to stand for 5 min. *Solution B* is no darker than *Solution A*.

Lead Dissolve 1 g in 20 ml of diluted hydrochloric acid TS, evaporate the solution to a volume of about 10 ml on a steam bath, dilute to about 20 ml with water, and cool. This solution meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Heating Weigh accurately about 1 g and heat at about 425° to constant weight.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Magnesium Silicate

Synthetic Magnesium Silicate

DESCRIPTION

A synthetic, usually amorphous form of magnesium silicate in which the molar ratio of magnesium oxide to silicon dioxide is approximately 2:5. It occurs as a very fine, white, odorless, tasteless powder, free from grittiness. It is insoluble in water and in alcohol, but is readily decomposed by mineral acids. The pH of a 1 in 10 slurry is between 7.0 and 10.8.

REQUIREMENTS

Identification

A. Mix about 500 mg with 10 ml of diluted hydrochloric acid TS, filter, and neutralize the filtrate to litmus paper with ammonia TS. The neutralized filtrate responds to the tests for *Magnesium*, page 517.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a Bunsen burner. Place the hot, transparent bead in contact with a sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.

Assay Not less than 15.0% of MgO and not less than 67.0% of SiO_2, calculated on the ignited basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Free Alkali (as NaOH) Not more than 1%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 15%.

Loss on Ignition Not more than 15%, determined on a dried sample.

Soluble Salts Not more than 3%.

TESTS

Assay for Magnesium Oxide Weigh accurately about 1.5 g, and transfer it into a 250-ml conical flask. Add 50.0 ml of 1 *N* sulfuric acid, and digest on a steam bath for 1 h. Cool to room temperature, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each ml of 1 *N* sulfuric acid is equivalent to 20.15 mg of MgO.

Assay for Silicon Dioxide Transfer about 700 mg, accurately weighed, into a 150-ml beaker. Add 20 ml of 1 *N* sulfuric acid, and heat on a steam bath for 1 h and 30 min. Decant the supernatant liquid through an ashless filter paper, and wash the residue, by decantation, three times with hot water. Treat the residue with 25 ml of water and digest on a steam bath for 15 min. Finally, transfer the residue to the filter and wash thoroughly with hot water. Transfer the filter paper and its contents to a platinum crucible. Heat to dryness, incinerate, then ignite strongly for 30 min, cool, and weigh. Moisten the residue with water, and add 6 ml of hydrofluoric acid and 3 drops of sulfuric acid. Evaporate to dryness, ignite for 5 min, cool, and weigh. The loss in weight represents the weight of SiO_2.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead Transfer 10.0 g of the sample into a 250-ml flask, and add 50 ml of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3, or equivalent, filter paper, into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine as directed in the test for *Fluoride* under *Calcium Silicate*, page 63.

Free Alkali Add 2 drops of phenolphthalein TS to 20 ml of diluted filtrate prepared in the test for *Soluble Salts*, representing 1 g of magnesium silicate. If a pink color is produced, not more than 2.5 ml of 0.1 *N* hydrochloric acid is required to discharge it.

Heavy Metals A 5-ml portion of the *Sample Solution* diluted to 25 ml with water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control.

Lead A 10-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h. Retain the sample for determination of *Loss on Ignition*.

Loss on Ignition Ignite the sample, retained from the test for *Loss on Drying*, at 900° to 1000° for 20 min.

Soluble Salts Boil 10 g with 150 ml of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. To 75 ml of the clear filtrate add 25 ml of water. Evaporate 50 ml of this solution, representing 2.5 g of magnesium silicate, in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. The weight of the residue does not exceed 50 mg.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Anticaking agent; filter aid.

Magnesium Stearate

DESCRIPTION

Magnesium stearate is a compound of magnesium with a mixture of solid organic acids obtained from edible sources, and consists chiefly of variable proportions of magnesium stearate and magnesium palmitate. It occurs as a fine, white, bulky powder having a faint, characteristic odor. It is unctuous, and is free from grittiness. It is insoluble in water, in alcohol, and in ether. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for salts of fatty acids and fatty acids derived from edible fats sources.

REQUIREMENTS

Identification

A. Heat 1 g with a mixture of 25 ml of water and 5 ml of hydrochloric acid. Fatty acids are liberated, floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Magnesium*, page 517.

B. Mix 25 g of the sample with 200 ml of hot water, then add 60 ml of diluted sulfuric acid TS, and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see page 538).

Assay Not less than the equivalent of 6.8% and not more than the equivalent of 8.3% of MgO.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 4%.

TESTS

Assay Boil about 1 g of the sample, accurately weighed, with 50 ml of 0.1 *N* hydrochloric acid for about 30 min, or until the separated fatty acid layer is clear, adding water if necessary to maintain the original volume. Cool, filter, and wash the filter and the container thoroughly with water until the last washing is not acid to litmus. Neutralize the filtrate with 1 *N* sodium hydroxide to litmus. While stirring, preferably with a magnetic stirrer, titrate with 0.05 *M* disodium EDTA as follows: Add about 30 ml of the titrant from a 50-ml buret, then add 5 ml of ammonia-ammonium chloride buffer TS and 0.15 ml of eriochrome black TS, and continue the titration to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 2.015 mg of MgO.

Arsenic Mix 1 g of the sample with 10 ml of hydrochloric acid and 8 drops of bromine TS, and heat on a steam bath until a transparent layer of melted fatty acid forms. Add 50 ml of water, boil down to about 25 ml, and filter while hot. Cool, neutralize with a 1 in 2 solution of sodium hydroxide, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals, page 512 Place 750 mg of the sample in a porcelain dish, place 250 mg of the sample in a second dish for the control, and to each add 5 ml of a 1 in 4 solution of magnesium nitrate in alcohol. Cover the dishes with 7.6-cm short-stem funnels so that the stems are straight up. Heat for 30 min on a hot plate at the low setting, then heat for 30 min at the medium setting, and cool. Remove the funnels, add 20 µg of lead ion (Pb) to the control, and heat each dish over an Argand burner until most of the carbon is burned off. Cool, add 10 ml of nitric acid, and transfer the solutions into 250-ml beakers. Add 5 ml of 70% perchloric acid, evaporate to dryness, then add 2 ml of hydrochloric acid to the residues, and wash down the inside of the beakers with water. Evaporate carefully to dryness again, swirling near the dry point to avoid spattering. Repeat the hydrochloric acid treatment, then cool, and dissolve the residues in about 10 ml of water. To each solution add 1 drop of phenolphthalein TS and sufficient sodium hydroxide TS until the solutions just turn pink, and then add diluted hydrochloric acid TS until the solutions become colorless. Add 1 ml of diluted acetic acid TS and a small amount of charcoal to each solution, and filter through Whatman No. 2, or equivalent, filter paper into 50-ml Nessler tubes. Wash with water, dilute to 40 ml, and add 10 ml of hydrogen sulfide TS to each tube. The color in

the solution of the sample does not exceed that produced in the control.

Lead, page 518 Ignite 500 mg in a silica crucible in a muffle furnace at 475° to 500° for 15 to 20 min. Cool, add 3 drops of nitric acid, evaporate over a low flame to dryness, and re-ignite at 475° to 500° for 30 min. Dissolve the residue in 1 ml of a mixture of equal parts by volume of nitric acid and water, and wash into a separator with several successive portions of water. Add 3 ml of *Ammonium Citrate Solution* and 0.5 ml of *Hydroxylamine Hydrochloride Solution*, and make alkaline to phenol red TS with stronger ammonia TS. Add 10 ml of *Potassium Cyanide Solution*. Immediately extract the solution with successive 5-ml portions of *Dithizone Extraction Solution*, draining off each extract into another separator, until the last portion of dithizone solution retains its green color. Shake the combined extracts for 30 s with 20 ml of dilute nitric acid (1 in 100), and discard the chloroform layer. Add to the acid solution exactly 4 ml of *Ammonia-Cyanide Solution* and 2 drops of *Hydroxylamine Hydrochloride Solution*. Add 10 ml of *Standard Dithizone Solution*, and shake the mixture for 30 s. Filter the chloroform layer through an acid-washed filter paper into a Nessler tube, and compare the color with that of a standard prepared as follows: To 20 ml of dilute nitric acid (1 in 100), add 5 µg of lead ion (Pb), 4 ml of *Ammonia-Cyanide Solution*, and 2 drops of *Hydroxylamine Hydrochloride Solution*, and shake for 30 s with 10 ml of *Standard Dithizone Solution*. Filter through an acid-washed filter paper into a Nessler tube. The color of the sample solution does not exceed that in the control.

Loss on Drying, page 518 Dry at 105° to constant weight, using 2-h increments of heating.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Anticaking agent; binder; emulsifier.

Magnesium Sulfate

Epsom Salt

$MgSO_4 \cdot 7H_2O$ Mol wt 246.47

DESCRIPTION

Small, colorless crystals, usually needlelike, with a cooling, saline, bitter taste. It is freely soluble in water, slowly soluble in glycerin, and sparingly soluble in alcohol. It effloresces in warm, dry air. Its solutions are neutral.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Magnesium*, page 517, and for *Sulfate*, page 517.

Assay Not less than 99.5% of $MgSO_4$ after ignition.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Ignition Between 40% and 52%.
Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 500 mg of the residue obtained in the test for *Loss on Ignition*, dissolve it in a mixture of 50 ml of water and 1 ml of hydrochloric acid, dilute to 100.0 ml with water, and mix. Transfer 50.0 ml of this solution into a 250-ml Erlenmeyer flask, add 10 ml of ammonia–ammonium chloride buffer TS and 12 drops of eriochrome black TS, and titrate with 0.1 *M* disodium EDTA until the wine-red color changes to pure blue. Each ml of 0.1 *M* disodium EDTA is equivalent to 12.04 mg of $MgSO_4$.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

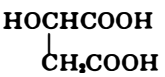
Loss on Ignition Weigh accurately about 1 g in a crucible, heat at 105° for 2 h, then ignite in a muffle furnace at 450° ± 25° to constant weight.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Malic Acid

DL-Malic Acid; Hydroxysuccinic Acid



$C_4H_6O_5$ Mol wt 134.09

DESCRIPTION

White or nearly white, crystalline powder or granules having a strongly acid taste. One g dissolves in 0.8 ml of water and in 1.4 ml of alcohol. Its solutions are optically inactive. It melts at about 130°.

REQUIREMENTS

Identification

Dissolve a few mg of the sample in 1 ml of diluted sulfuric acid TS, add 1 ml of a 0.003% solution of 2-naphthol in concentrated sulfuric acid, and mix. The solution shows a yellowish color by transmitted light, and it has a blue fluorescence.

Assay Not less than 99.0% of $C_4H_6O_5$.
Arsenic (as As) Not more than 3 ppm.
Fumaric Acid Not more than 1.0%.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Maleic Acid Not more than 0.05%.
Residue on Ignition Not more than 0.1%.
Water-Insoluble Matter Not more than 0.1%.

TESTS

Assay Dissolve about 2 g, accurately weighed, in 40 ml of recently boiled and cooled water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide to the first appearance of a faint pink color that persists for at least 30 s. Each ml of 1 *N* sodium hydroxide is equivalent to 67.04 mg of $C_4H_6O_5$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Fumaric and Maleic Acids

Buffer Solution A Dissolve 74.5 g of potassium chloride in 500 ml of water in a 1000-ml volumetric flask, add 100 ml of hydrochloric acid, and dilute to volume with water.

Buffer Solution B Dissolve 171.0 g of dibasic potassium phosphate, $K_2HPO_4.3H_2O$, in 1000 ml of water, and add monobasic potassium phosphate, KH_2PO_4, until the pH is exactly 7.0.

Maxima Suppressor Dissolve, with the aid of a magnetic stirrer, 1 g of gelatin in 65 ml of hot, boiled water, and after cooling add 35 ml of anhydrous ethanol as a preservative.

Standard Solution Weigh accurately about 20 g of the sample, 100 mg of fumaric acid of the highest purity available, and 10 mg of maleic acid of the highest purity available, and transfer into a 500-ml volumetric flask. Add 300 ml of sodium hydroxide TS and a few drops of phenolphthalein TS, and then continue the neutralization with sodium hydroxide TS to a faint pink color that persists for at least 30 s. Dilute to volume with water, and mix.

Sample Solution Transfer about 4 g of the sample, accurately weighed, into a 100-ml volumetric flask, and dissolve in 25 ml of water. Add phenolphthalein TS, and neutralize with sodium hydroxide TS as directed for the *Standard Solution*. Dilute to volume with water, and mix.

Procedure Transfer 25.0-ml portions of the *Sample Solution* into separate 100-ml volumetric flasks. Dilute one flask (*Sample A*) to volume with *Buffer Solution A*. To the other flask (*Sample B*) add 50 ml of *Buffer Solution B*, and dilute to volume with water.

Rinse a polarograph cell with a portion of *Sample A*, add a suitable volume of the solution to the cell, immerse it in a water bath regulated at 24.5° to 25.5°, add 2 drops of the *Maxima Suppressor*, and then deaerate by bubbling nitrogen through the solution for at least 5 min. Insert the dropping mercury electrode (negative polarity) of a suitable polarograph, adjust the current sensitivity as necessary, and record the polarogram from −0.1 to −0.8 V at the rate of 0.2 V per min, using a saturated calomel electrode as the reference electrode. Transfer 25.0 ml of the *Standard Solution* into a 100-ml volumetric flask, and dilute to volume with *Buffer Solution A*. Obtain the polarogram of this standard (*Standard A*) in the same manner as directed for *Sample A*. In each polarogram, determine the height of the maleic acid plus fumaric acid wave occurring at the half-wave potential near −0.56 V, recording that for the sample as i_u and that for the standard as i_s.

In the same manner, obtain polarograms from *Sample B* and *Standard B*, except record the polarogram from −1.05 to −1.7 V at the rate of 0.1 V per min. In each polarogram, determine the height of the maleic acid wave occurring at the half-wave potential near −1.33 V, recording that for the sample as i_u' and that for the standard as i_s'.

Calculation Calculate the weight, in mg, of combined maleic acid and fumaric acid in the sample by the formula

$$500C \times i_u/(i_s - i_u),$$

in which C is the concentration, in mg per ml, of combined maleic acid and fumaric acid in the *Standard Solution*. Similarly, calculate the weight, in mg, of maleic acid in the sample by the formula

$$500C' \times i_u'/(i_s' - i_u'),$$

in which C' is the concentration, in mg per ml, of maleic acid in the *Standard Solution*. Finally, calculate the weight of fumaric acid in the sample from the difference in these values.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

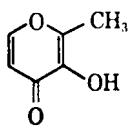
Residue on Iguition Ignite 2 g as directed in the general method, page 533.

Water-Insoluble Matter Dissolve 25 g in 100 ml of water, and filter through a tared Gooch crucible. Wash the filter with hot water, dry at 100° to constant weight, cool, and weigh.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Acidifier; flavoring agent.

Maltol

3-Hydroxy-2-methyl-4-pyrone



$C_6H_6O_3$ Mol wt 126.11

DESCRIPTION

A white, crystalline powder having a characteristic caramel-butterscotch odor, and suggestive of a fruity-strawberry aroma

in dilute solution. One g dissolves in about 82 ml of water, 21 ml of alcohol, 80 ml of glycerin, and 28 ml of propylene glycol.

REQUIREMENTS

Identification

A 1 in 100,000 solution in 0.1 *N* hydrochloric acid exhibits an absorbance maximum at 274 ± 2 nm.

Assay Not less than 99.0% of $C_6H_6O_3$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Melting Range Between 160° and 164°.
Residue on Ignition Not more than 0.2%.
Water Not more than 0.5%.

TESTS

Assay

Standard Solution Weigh accurately about 50 mg of FCC Maltol Reference Standard, dissolve it in sufficient 0.1 *N* hydrochloric acid to make 250.0 ml, and mix. Transfer 5.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Assay Solution Weigh accurately about 50 mg of the sample, dissolve it in sufficient 0.1 *N* hydrochloric acid to make 250.0 ml, and mix. Transfer 5.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with 0.1 *N* hydrochloric acid, and mix.

Procedure Determine the absorbance of each solution in a 1-cm quartz cell at the wavelength of maximum absorption at about 274 nm, with a suitable spectrophotometer, using 0.1 *N* hydrochloric acid as the blank. Calculate the quantity, in mg, of $C_6H_6O_3$ in the sample taken by the formula $5C(A_U/A_S)$, in which C is the concentration, in µg per ml, of FCC Maltol Reference Standard in the *Standard Solution*, A_U is the absorbance of the *Assay Solution*, and A_S is the absorbance of the *Standard Solution*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Melting Range Determine as directed in *Procedure for Class Ia*, page 519.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Flavoring agent.

Mandarin Oil, Coldpressed

Mandarin Oil, Expressed

DESCRIPTION

The oil obtained by expression of the peels of the ripe fruit of the mandarin orange, *Citrus reticulata* Blanco var. *Mandarin*. It is a clear, dark orange to reddish yellow, or brownish orange liquid with a pleasant orangelike odor. It often shows a bluish fluorescence in diffused light. Oils produced from unripe fruit often show a green color. It is soluble in most fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is insoluble in glycerin. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 599, using the same test conditions as specified therein.

Assay Not less than 0.4% and not more than 1.8% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).
Angular Rotation Between +63° and +78°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.473 and 1.477 at 20°.
Residue on Evaporation Between 2% and 5%.
Specific Gravity Between 0.847 and 0.853.

TESTS

Assay Weigh accurately about 10 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, using 156.26 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 30 min at room temperature before titrating.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Residue on Evaporation Proceed as directed in the general method, page 533, using about 5 g, accurately weighed, and heating for 5 h.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, galvanized, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Manganese Chloride

$MnCl_2.4H_2O$ Mol wt 197.90

DESCRIPTION

Large, irregular, pink, translucent crystals. It is freely soluble in water at room temperature and very soluble in hot water.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Manganese*, page 517, and for *Chloride*, page 516.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $MnCl_2.4H_2O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Matter Not more than 0.005%.

Iron Not more than 5 ppm.

pH of a 5% Solution Between 4.0 and 6.0.

Substances Not Precipitated by Sulfide Not more than 0.2%, after ignition.

Sulfate Not more than 0.005%.

TESTS

Assay Transfer about 4 g, accurately weighed, into a 250-ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Transfer 25.0 ml of this solution into a 400-ml beaker, and add 10 ml of a 1 in 10 solution of hydroxylamine hydrochloride, 25 ml of 0.05 *M* disodium EDTA measured from a buret, 25 ml of ammonia-ammonium chloride buffer TS, and 5 drops of eriochrome black TS. Heat the solution to between 55° and 65°, and titrate from the buret to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 9.896 mg of $MnCl_2.4H_2O$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Matter Dissolve about 20 g, accurately weighed, in 200 ml of water, and allow to stand on a steam bath for 1 h. Filter through a tared sintered-glass crucible, wash thoroughly with hot water, dry at 105° for 1 h, cool, and weigh.

Iron Dissolve 2.0 g in 20 ml of water, add 1 ml of hydrochloric acid, and dilute to 50 ml with water. Add about 40 mg of ammonium persulfate crystals and 3 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 µg Fe) in an equal volume of a solution containing the quantities of the reagents used in the test.

pH of a 5% Solution Determine by the *Potentiometric Method*, page 531.

Substances Not Precipitated by Sulfide Dissolve 2.0 g in about 90 ml of water, add 4 ml of ammonium hydroxide, heat to 80°, and pass hydrogen sulfide through the solution to completely precipitate the manganese. Dilute to 100 ml, mix, and allow the precipitate to settle. Decant the supernatant liquid through a filter, and evaporate 50 ml of the filtrate to dryness in a tared dish. Add 0.5 ml of sulfuric acid, ignite to constant weight, cool, and weigh.

Sulfate Dissolve 10.0 g in 100 ml of water, add 1 ml of diluted hydrochloric acid TS, mix, and filter. Heat to boiling, then add 10 ml of barium chloride TS, and allow to stand overnight. Filter out any precipitate in a tared crucible, wash, ignite gently, cool, and weigh. The weight of the ignited precipitate should not be more than 1.2 mg greater than the weight obtained in a complete blank test.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Manganese Gluconate

$[CH_2OH(CHOH)_4COO]_2Mn.2H_2O$

$C_{12}H_{22}MnO_{14}.2H_2O$ Mol wt 481.27

DESCRIPTION

A slightly pink-colored powder. It is very soluble in hot water and is very slightly soluble in alcohol.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Manganese*, page 517.

B. It meets the requirements of *Identification Test B* under *Copper Gluconate*, page 90.

Assay Not less than 98.0% of $C_{12}H_{22}MnO_{14}.2H_2O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Reducing Substances Not more than 0.5%.

TESTS

Assay Dissolve about 600 mg, accurately weighed, in 50 ml of water in a 250-ml porcelain casserole, add 1 g of hydroxyl-

amine hydrochloride, 10 ml of ammonia-ammonium chloride buffer TS, and 5 drops of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA to a deep blue color. Each ml of 0.05 *M* disodium EDTA is equivalent to 24.06 mg of $C_{12}H_{22}MnO_{14}.2H_2O$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 500 mg in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 25 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Reducing Substances Determine as directed in the test for *Reducing Substances* under *Copper Gluconate*, page 90.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Manganese Glycerophosphate

$C_3H_7MnO_6P.xH_2O$ Mol wt 225.00

DESCRIPTION

A white or pinkish white powder. It is odorless and nearly tasteless. One g dissolves in about 5 ml of citric acid solution (1 in 4). It is slightly soluble in water, and is insoluble in alcohol.

REQUIREMENTS

Identification

A. A 1 in 20 solution in diluted hydrochloric acid TS gives positive tests for *Manganese*, page 517.

B. Heat a mixture of 100 mg of the sample with 500 mg of potassium bisulfate. Pungent vapors of acrolein are evolved.

Assay Not less than 98.0% of $C_3H_7MnO_6P$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 12%.

TESTS

Assay Dissolve about 1 g, previously dried at 110° to constant weight and accurately weighed, in 1.5 ml of nitric acid and 5 ml of warm water. Dilute to 125 ml, add 2.0 g of dibasic ammonium phosphate and a few drops of methyl red TS, and heat to boiling. While the solution is boiling, slowly add stronger ammonia TS, dropwise and with constant stirring, until alkaline, and then add 2.0 ml in excess. Let stand 2 h at room temperature. Filter through a tared Gooch crucible, and wash the precipitate with dilute ammonia TS (1 in 100). Dry at 105°, ignite at a bright red heat, cool in a desiccator, and weigh. Each g of manganese pyrophosphate so obtained is equivalent to 1.585 g of $C_3H_7MnO_6P$.

Arsenic A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 500 mg in 5 ml of diluted hydrochloric acid TS, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Mix 1 g with 3 ml of dilute nitric acid (1 in 2) and 10 ml of water, and boil until brown fumes appear. Add 10 ml of water, boil for 2 min, cool, and dilute to 100 ml with water. A 25-ml portion of this solution meets the requirements of the *Lead Limit Test*, page 518, using 25 ml of *Ammonium Citrate Solution*, 1 ml of *Potassium Cyanide Solution*, 0.5 ml of *Hydroxylamine Hydrochloride Solution*, and 2.5 µg of lead ion (Pb).

Loss on Drying, page 518 Dry at 110° to constant weight.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Manganese Hypophosphite

$Mn(PH_2O_2)_2.xH_2O$ Mol wt 184.91

DESCRIPTION

A pink, granular or crystalline powder that is stable in the air. It is odorless and nearly tasteless. One g dissolves in about 6.5 ml of water at 25° or in about 6 ml of boiling water. It is soluble in alcohol.

Caution: Mix manganese hypophosphite with nitrates, chlorates, or other oxidizing agents very carefully, as an explosion may occur if it is triturated or heated.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Manganese*, page 517, and for *Hypophosphite*, page 517.

Assay Not less than 97.0% of $Mn(PH_2O_2)_2$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 9%.

TESTS

Assay Transfer about 120 mg, previously dried at 105° for 1 h and accurately weighed, into a 100-ml volumetric flask, dissolve in water, and dilute with water to volume. Transfer 50.0 ml of this solution into a 250-ml glass-stoppered iodine flask, add 50.0 ml of 0.1 *N* bromine and 20 ml of diluted sulfuric acid TS, and stopper the flask. Place a few ml of a

saturated solution of potassium iodide in the lip around the stopper, shake the flask well, and allow to stand for 3 h. Place the flask in an ice bath for 5 min, then carefully remove the stopper and allow the potassium iodide solution to be drawn into the flask. Add 2 g of potassium iodide dissolved in 10 ml of recently boiled water, shake the flask, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 *N* bromine is equivalent to 2.311 mg of $Mn(PH_2O_2)_2$.

Arsenic Dissolve 1 g in 15 ml of water, add 3 ml of nitric acid, evaporate to dryness on a steam bath, and dissolve the residue in 35 ml of water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 500 mg in 15 ml of water, add 3 ml of nitric acid, evaporate to dryness on a steam bath, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dissolve 250 mg in 10 ml of water, add 2 ml of dilute nitric acid (1 in 2), and boil until brown fumes appear. Add 10 ml of water, boil for 2 min, then cool and dilute with water to about 25 ml. This solution meets the requirements of the *Lead Limit Test*, page 518, using 25 ml of *Ammonium Citrate Solution*, 1 ml of *Potassium Cyanide Solution*, 0.5 ml of *Hydroxylamine Hydrochloride Solution*, and 2.5 µg of lead ion (Pb).

Loss on Drying, page 518 Dry at 105° for 1 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Manganese Sulfate

$MnSO_4.H_2O$ Mol wt 169.01

DESCRIPTION

A pale pink, granular, odorless powder. It is freely soluble in water and is insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Manganese*, page 517, and for *Sulfate*, page 517.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $MnSO_4.H_2O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Heating Between 10% and 13%.

Selenium Not more than 0.003%.

TESTS

Assay Transfer about 4 g, accurately weighed, into a 250-ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Transfer a 25.0-ml portion of this solution into a 400-ml beaker, and add 10 ml of a 1 in 10 solution of hydroxylamine hydrochloride, 25 ml of 0.05 *M* disodium EDTA measured from a buret, 25 ml of ammonia-ammonium chloride buffer TS, and 5 drops of eriochrome black TS. Heat the solution to between 55° and 65°, and titrate from the buret to a blue endpoint. Each ml of 0.05 *M* disodium EDTA is equivalent to 8.450 mg of $MnSO_4.H_2O$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 500 mg in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dissolve 1 g in 3 ml of dilute nitric acid (1 in 2) and 10 ml of water, and boil for 2 min. Cool, and dilute to 100 ml with water. A 25-ml portion of this solution meets the requirements of the *Lead Limit Test*, page 518, using 25 ml of *Ammonium Citrate Solution*, 1 ml of *Potassium Cyanide Solution*, 0.5 ml of *Hydroxylamine Hydrochloride Solution*, and 2.5 µg of lead ion (Pb).

Loss on Heating Heat about 1 g, accurately weighed, in a crucible tared in a stoppered weighing bottle, to constant weight at 400° to 500°. Cool in a desiccator, transfer to the stoppered weighing bottle, and weigh.

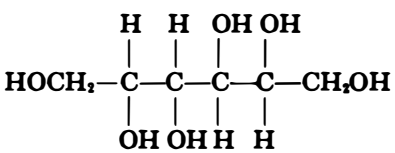
Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200-mg of sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Mannitol

D-Mannitol; Mannite; 1,2,3,4,5,6-Hexanehexol



$C_6H_{14}O_6$ Mol wt 182.17

DESCRIPTION

A white, crystalline solid consisting of D-mannitol and a small quantity of sorbitol. It is odorless and has a sweet taste. It is soluble in water, very slightly soluble in alcohol, and practically insoluble in most other common organic solvents.

REQUIREMENTS

Identification

A. Add 5 drops of a saturated solution of the sample to 1 ml of ferric chloride TS. Add 5 drops of water to a second tube containing 1 ml of ferric chloride TS. Add 5 drops of sodium hydroxide solution (1 in 5) to each tube. A brown precipitate of ferric hydroxide forms in the tube containing no mannitol, and a yellow precipitate forms in the tube containing mannitol. Shake the tubes vigorously. A clear solution results in the tube containing mannitol, but the precipitate persists in the other tube. Additional sodium hydroxide solution does not cause precipitation in the tube containing mannitol, but further precipitation occurs in the other.

B. Place a 500-mg sample in a test tube, and add 3 ml of acetic anhydride and 1 ml of pyridine. Heat the mixture in a boiling water bath for 15 min, with frequent shaking, or until solution is complete, continue heating for 5 min, and cool. Add 20 ml of water, mix well, allow to stand for 5 min, and collect the precipitate on a sintered-glass filter. The mannitol hexaacetate so obtained, after drying at 60° under vacuum for 1 h or after recrystallization from ether, melts between 119° and 124° (see page 519).

Assay Not less than 96.0% and not more than the equivalent of 101.0% of $C_6H_{14}O_6$ (mannitol), calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.007%.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Melting Range Between 165° and 168°.

Reducing Sugars Passes test.

Specific Rotation $[\alpha]_D^{25°}$: Between +23.3° and +24.3°.

Sulfate Not more than 0.01%.

TESTS

Assay

Adsorbent Mix intimately, as by mechanical rolling or tumbling for 12 h, 9 parts, by weight, of very fine chromatographic fuller's earth and 2 parts, by weight, of chromatographic siliceous earth. Use only that portion that passes a 100-mesh sieve.

Chromatographic Column Insert a pledget of cotton on the removable porous plate of a slightly tapered, 38- × 230-mm chromatographic tube, the constricted end of which is fitted to a 500-ml filtering flask. Apply a vacuum of between 20 and 30 mm of Hg, and maintain the vacuum within this range until the chromatogram is developed. Add the *Adsorbent* through a powder funnel in a steady stream until the tube is filled. Tap the tube from bottom to top, around its circumference, with a wooden rod to ensure uniform packing. Adjust the column height to within about 20 mm of the top of the tube, and level the top of the column with a rubber stopper.

Developing Solvent Mix 15 parts, by volume, of water with 85 parts, by volume, of isopropyl alcohol.

Standard Sorbitol Solution Weigh accurately 450.0 mg of sorbitol of greater than 99.0% purity, and transfer into a 100-ml volumetric flask. Dissolve in about 60 ml of the *Developing Solvent*, dilute to volume with the *Developing Solvent*, and mix.

Assay Preparation Weigh accurately 450.0 mg of the sample, and transfer into a 100-ml volumetric flask. Dissolve in about 60 ml of the *Developing Solvent*, heating gently to effect solution. Add 5.0 ml of the *Standard Sorbitol Solution*, cool to room temperature, dilute to volume with *Developing Solvent*, and mix.

Procedure Pour 20 ml of the *Developing Solvent* onto the prepared chromatographic column, leaving a 5-mm layer of the solvent above the top of the column. Pipet 10.0 ml of the *Assay Preparation* into the tube, and, after it has practically disappeared into the column, add two 10-ml portions of the *Developing Solvent*, allowing each portion to just enter the column. (*Caution:* Do not allow the column to go dry.) Attach a separator containing 305 ml of the *Developing Solvent* to the tube by means of a rubber stopper, and adjust the flow of eluate to maintain a solvent layer about 5 to 10 mm above the top of the column.

After all of the *Developing Solvent* has passed through the column, maintain the vacuum until the column shrinks from the walls of the tube but does not become dry, and then disconnect the source of vacuum rapidly so as to cause a flow of air between the column and the walls of the tube. Place a pledget of cotton on the surface of the column, invert the tube, and tap the tube on a solid surface to loosen the column. Extrude the column onto a strip of aluminum foil, using a cork stopper attached to a wooden rod inserted through the constricted end of the tube. Streak the entire length of the column, from top to bottom, at three places equidistant around its circumference, using a solution consisting of 1 g of potassium permanganate and 10 g of sodium hydroxide dissolved in 100 ml of water.

The position of the polyol zones on the chromatogram is indicated by a color change of the streaks from green to brown, with the leading edge of the mannitol zone located about 15 cm from the top of the column. The leading edge of the sorbitol zone appears about 6 cm from the top of the column. (Sorbitol, which is present in FCC mannitol at too low a concentration to be detectable on the potassium permanganate streaks, is added in order to yield a readily detectable polyol zone.) The trailing end of the mannitol zone will discolor the potassium permanganate streaks very slowly and indistinctly; therefore, the distinct leading edge of the sorbitol zone must be used as a guide in cutting the zones from the column. Mark the leading edges of the sorbitol and mannitol zones, and, using a sharp blade, cut the column at the leading edge of the sorbitol zone and about 20 mm below the leading edge of the mannitol zone. Discard the top and bottom portions of the column. Cut the mannitol zone into small pieces, and allow it to dry overnight, protected from drafts.

Powder the dried mannitol zone with a spatula, pack it into a clean, dry chromatographic tube as directed under *Chroma-*

tographic Column, and elute the mannitol by passing 150 ml of warm water through the column. Transfer the eluate into a 200-ml volumetric flask, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a 250-ml iodine flask, add 50.0 ml of sodium periodate solution (prepared by dissolving 4.5 g of sodium periodate in 500 ml of water, adding 2 ml of sulfuric acid, and diluting to 1000 ml with water), and heat for 20 min in a water bath maintained at 80° to 85°. Cool to room temperature in an ice bath, add 3 g of sodium bicarbonate and 10 ml of potassium iodide solution (1 in 5), and titrate immediately with 0.05 *N* sodium arsenite, adding starch TS as the endpoint is approached. Perform a blank determination, using water in place of the mannitol eluate, and make any necessary corrections (see page 2). Each ml of 0.05 *N* sodium arsenite is equivalent to 910.9 μg of mannitol ($C_5H_{14}O_6$).

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 200-mg sample does not exceed that shown in a control containing 14 μg of chloride ion (Cl).

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 4 h.

Melting Range Determine as directed in the general procedure, page 519.

Reducing Sugars Add 1 ml of a saturated solution to 5 ml of alkaline cupric citrate TS, and heat for 5 min in a boiling water bath. No more than a very slight precipitation occurs.

Specific Rotation Transfer 10.0 g of the sample, accurately weighed, into a 100-ml volumetric flask, and add sodium borate equivalent to 12.8 g of $Na_2B_4O_7.10H_2O$, the purity of which is determined by assay as follows: Dissolve about 3 g of sodium borate decahydrate, accurately weighed, in 50 ml of water, add methyl red TS, and titrate with 0.5 *N* hydrochloric acid, each ml of which is equivalent to 95.34 mg of $Na_2B_4O_7.10H_2O$. To the flask add sufficient water to make about 90 ml, allow to stand for 1 h with occasional shaking, then dilute to volume with water, and mix. Determine the specific rotation of this solution as directed in the general procedure, page 530.

Sulfate, page 471 Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 200 μg of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement; texturizing agent.

Marjoram Oil, Spanish Type

DESCRIPTION

A volatile oil obtained by steam distillation from the flowering plant *Thymus mastichina* L. (Fam. *Labiatae*). It is a slightly yellow liquid having a camphoraceous note. It is soluble in most fixed oils, but it is insoluble in glycerin, in propylene glycol, and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 600, using the same test conditions as specified therein.

Assay Not less than 49% and not more than 65% of cineole.
Acid Value Not more than 2.0.
Angular Rotation Between −5° and +10°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.463 and 1.468 at 20°.
Saponification Value Between 5 and 20.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.904 and 0.920.

TESTS

Assay Dry a sample over anhydrous sodium sulfate, and transfer 3 g of the dried oil, accurately weighed, into a test tube as directed for *Solidification Point*, page 538. Add 2.1 g of melted *o*-cresol. The *o*-cresol must be pure and dry and have a solidification point not below 30°. Insert the thermometer, stir, and warm the tube gently until the mixture is completely melted. Proceed as directed under *Solidification Point*, page 539. Repeat the procedure until two successive readings agree within 0.1°. Compute the percentage of cineole from the *Percentage of Cineole* table, page 501.

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 10 g, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502, One ml dissolves in 1 ml of 80% alcohol

and remains in solution on further addition of the alcohol to a total volume of 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Marjoram Oil, Sweet

DESCRIPTION

The volatile oil obtained by steam distillation of the dried herb of the marjoram shrub, *Marjoram hortensis* L. (Fam. *Labiatae*). It is a yellow or greenish yellow oil having a spicy or cardamom note. It is soluble in most fixed oils and in mineral oil (with turbidity). It is only partly soluble in propylene glycol, and is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 600, using the same test conditions as specified therein.

Acid Value Not more than 2.5.
Angular Rotation Between +14° and +24°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.470 and 1.475 at 20°.
Saponification Value Between 23 and 40.
Saponification Value after Acetylation Between 68 and 86.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.890 and 0.906.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Saponification Value after Acetylation Proceed as directed under *Total Alcohols*, page 499, using about 2.5 g of acetylated oil, accurately weighed. Calculate the saponification value by the formula $28.05 \times A/B$, in which A is the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the titration, and B is the weight of the acetylated oil, in g.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 80% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Masticatory Substances, Natural

Coagulated or Concentrated Latices of Vegetable Origin

DESCRIPTION

The masticatory substances of vegetable origin are comprised of the gums from the trees of *Sapotaceae*, *Apocynaceae*, *Moraceae*, and *Euphorbiaceae* as listed below. The coagulated material varies in color from white to brown, depending on its moisture content and heat treatment during purification. The gums are purified by extensive treatment either alone or in combination with other gums or food-grade materials. They are heat treated and then clarified by centrifuging or by any other appropriate means of filtration.

Family	*Genus and Species*
Sapotaceae	
Chicle	*Manilkara zapotilla* Gilly and *Manilkara chicle* Gilly
Chiquibul	*Manilkara zapotilla* Gilly
Crown gum	*Manilkara zapotilla* Gilly and *Manilkara chicle* Gilly
Gutta hang kang	*Palaquium leiocarpum* Boerl. and *Palaquium oblongifolium* Burck
Gutta Katiau	*Palaquium ganua moteleyana* Clarke (also known as *Sideroxylon glabrescens*)
Massaranduba balata (and the solvent-free resin extract of Massaranduba balata)	*Manilkara huberi* (Ducke) Chevalier
Massaranduba chocolate	*Manilkara solimoesensis* Gilly
Nispero	*Manilkara zapotilla* Gilly and *Manilkara chicle* Gilly
Rosidinha (rosadinha)	*Micropholis* (also known as *Sideroxylon*) spp.
Venezuelan chicle	*Manilkara williamsii* Standley and related spp.

Apocynaceae	
Jelutong	*Dyera costulata* Hook. F. and *Dyera lowii* Hook. F.
Leche caspi (sorva)	*Couma macrocarpa* Barb. Rodr.
Pendare	*Couma macrocarpa* Barb. Rodr. and *Couma utilis* (Mart.) Muell. Arg.
Perillo	*Couma macrocarpa* Barb. Rodr. and *Couma utilis* (Mart.) Muell. Arg.
Moraceae	
Leche de vaca	*Brosimum utile* (H.B.K.) Pittier and *Poulsenia* ssp.; also *Lacmellea standleyi* (Woodson), *Monachino* (Apocynaceae)
Niger Gutta	*Ficus platyphylla* Del.
Tunu (tuno)	*Castilla fallax* Cook
Euphorbiaceae	
Chilte	*Cnidoscolus* (also known as *Jatropha*) *elasticus* Lundell and *Cnidoscolus tepiquensis* (Cost. and Gall.) McVaugh
Natural rubber (latex solids)	*Hevea brasiliensis*

REQUIREMENTS

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.

The following specifications, where applicable, should conform to the representations of the vendor: cleanliness, color, texture, odor, and loss on drying.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Mentha Arvensis Oil, Partially Dementholized

Cornmint Oil, Partially Dementholized

DESCRIPTION

The portion of oil remaining after the partial removal of menthol, by freezing operations only, from the oil of *Mentha arvensis* var. *piperascens* Holmes (forma piperascens Malinvaud). It is a colorless to yellow liquid having a characteristic minty odor. It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 600, using the same test conditions as specified therein.

Assay Not less than 40.0% and not more than 60.0% of total alcohols, calculated as menthol ($C_{10}H_{20}O$).
Angular Rotation Between −20° and −35°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.458 and 1.465 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.888 and 0.908.
Total Esters Between 5.0% and 20.0%, calculated as menthyl acetate ($C_{12}H_{22}O_2$).
Total Ketones Between 30.0% and 50.0%, calculated as menthone ($C_{10}H_{18}O$).

TESTS

Assay Proceed as directed under *Total Alcohols*, page 499, using about 1.5 g of the acetylated oil, accurately weighed, for the saponification. Calculate the percentage of alcohol, as menthol, in the sample by the formula

$$A \times 7.813(1 - 0.0021E)/(B - 0.021A),$$

in which A is the number of ml of 0.5 N alcoholic potassium hydroxide consumed in the saponification; B is the weight of the acetylated oil taken, in g; and E is the percentage of esters, as menthyl acetate, determined as directed under *Total Esters* below.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method. One ml dissolves in 2.5 to 4 ml of 80% alcohol and may become hazy upon further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Total Esters Weigh accurately about 10 g, and proceed as directed under *Ester Determination*, page 500, using 99.15 as the equivalence factor (*e*) in the calculation.

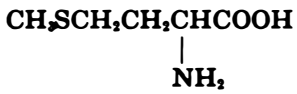
Total Ketones Weigh accurately about 1 g, and proceed as directed for ketones under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, using 77.12 as the equivalence factor (*e*) in the calculation.

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

DL-Methionine

DL-2-Amino-4-(methylthio)butyric Acid



$C_5H_{11}NO_2S$ Mol wt 149.21

DESCRIPTION

White, crystalline platelets or powder having a characteristic odor. One g dissolves in about 30 ml of water. It is soluble in dilute acids and in solutions of alkali hydroxides. It is very slightly soluble in alcohol, and practically insoluble in ether. It is optically inactive. The pH of a 1 in 100 solution is between 5.6 and 6.1.

REQUIREMENTS

Identification

A. Add 25 mg of a previously dried sample to 1 ml of a saturated solution of anhydrous cupric sulfate in sulfuric acid. A yellow color appears.

B. To 10 ml of a 1 in 1000 solution add successively, shaking after each addition, 1 ml of a 1 in 5 solution of sodium hydroxide, 1 ml of a 1 in 100 glycine solution, and 0.3 ml of a freshly prepared 1 in 10 solution of sodium nitroferricyanide. Keep the mixture at about 40° for 10 min, cool in an ice bath for 2 min, then add 2 ml of 20% hydrochloric acid and shake the mixture. A red or orange red color appears.

C. To 1 ml of a 1 in 30 solution add 1 ml of ninhydrin TS and 100 mg of sodium acetate, and heat to boiling. An intense violet blue color is formed (distinction from hydroxy analog).

Assay Not less than 99.0% of $C_5H_{11}NO_2S$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 300 mg, accurately weighed, into a glass-stoppered flask. Add 100 ml of water, 5 g of dibasic potassium phosphate, 2 g of monobasic potassium phosphate, and 2 g of potassium iodide, and mix well to dissolve. Add exactly 50 ml of 0.1 *N* iodine, stopper the flask, mix well, allow to stand for 30 min, and then titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* iodine is equivalent to 7.461 mg of $C_5H_{11}NO_2S$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

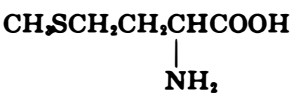
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Methionine

L-2-Amino-4-(methylthio)butyric Acid



$C_5H_{11}NO_2S$ Mol wt 149.21

DESCRIPTION

Colorless or white lustrous plates, or a white crystalline powder. It has a slight, characteristic odor. It is soluble in water, in alkali solutions, and in dilute mineral acids. It is slightly soluble in alcohol and practically insoluble in ether.

REQUIREMENTS

Identification

L-Methionine responds to *Identification Tests A*, *B*, and *C* under DL-*Methionine*, page 193.

Assay Not less than 99.0% of $C_5H_{11}NO_2S$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{25°}$: Between $-6.8°$ and $-8.2°$; $[\alpha]_D^{20°}$: between $+21.0°$ and $+25.0°$.

TESTS

Assay Transfer about 300 mg, accurately weighed, into a glass-stoppered flask. Add 100 ml of water, 5 g of dibasic potassium phosphate, 2 g of monobasic potassium phosphate, and 2 g of potassium iodide, and mix well to dissolve. Add exactly 50 ml of 0.1 *N* iodine, stopper the flask, mix well, allow to stand for 30 min, and then titrate the excess iodine with 0.1 *N* sodium thiosulfate. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* iodine is equivalent to 7.461 mg of $C_5H_{11}NO_2S$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 2 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 $[\alpha]_D^{25°}$: Determine in a solution containing 4 g in sufficient water to make 100 ml; $[\alpha]_D^{20°}$: determine in a solution containing 2 g in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Methyl Alcohol

Methanol

CH_3OH Mol wt 32.04

DESCRIPTION

A clear, colorless, flammable liquid having a characteristic odor. It is miscible with water, with ethyl alcohol, and with ether. Its refractive index at 20° is about 1.329.

REQUIREMENTS

Assay Not less than 99.85% of CH_3OH, by weight.
Acetone and Aldehydes Not more than 0.003%.
Acidity (as formic acid) Not more than 0.0015%.
Alkalinity (as NH_3) Not more than 3 ppm.
Distillation Range Within a range of 1°, including 64.6° ± 0.1°.
Heavy Metals (as Pb) Not more than 1 ppm.
Nonvolatile Residue Not more than 10 ppm.
Readily Carbonizable Substances Passes test.
Solubility in Water Passes test.
Substances Reducing Permanganate Passes test.
Water Not more than 0.1%.

TESTS

Assay Its specific gravity, determined by any reliable method (see page 3), is not greater than 0.7893 at 25°/25° (equivalent to 0.7928 at 20°/20°).
Acetone and Aldehydes To 1.25 ml (about 1 g) of the sample add 3.75 ml of water and 5.0 ml of alkaline mercuric-potassium iodide TS. Any turbidity does not exceed that produced in a standard containing 30 µg of acetone.
Acidity To a mixture of 10 ml of alcohol and 25 ml of water add 0.5 ml of phenolphthalein TS, and titrate with 0.02 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Add 19 ml (about 15 g) of the sample, mix, and titrate with 0.02 *N* sodium hydroxide until the pink color is restored. Not more than 0.25 ml is required.
Alkalinity Add 1 drop of methyl red TS to 25 ml of water, add 0.02 *N* sulfuric acid until a red color just appears, then add 29 ml (about 22.5 g) of the sample, and mix. Not more than 0.2 ml of 0.02 *N* sulfuric acid is required to restore the red color.
Distillation Range Proceed as directed in the general method, page 478.
Heavy Metals Evaporate 25 ml (about 20 g) of the sample to dryness on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Nonvolatile Residue Evaporate 125 ml (about 100 g) of the

sample to dryness in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Readily Carbonizable Substances, page 532 A mixture of 25 ml of sulfuric acid TS (cooled to 10°) and 25 ml of the sample has no more color than 3.5 ml of platinum-cobalt CS, diluted to 50 ml with water (equivalent to not more than 35 APHA color units).

Solubility in Water Mix 15 ml of the sample with 45 ml of water. After 1 h, the solution is as clear as an equal volume of water.

Substances Reducing Permanganate Transfer 20 ml of the sample, previously cooled to 15°, to a glass-stoppered cylinder, add 0.1 ml of 0.1 *N* potassium permanganate, mix, and allow to stand for 5 min. The pink color is not entirely discharged.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers remote from heat, sparks, and open flames.

Functional Use in Foods Extraction solvent.

Methylcellulose

DESCRIPTION

Methylcellulose is the methyl ether of cellulose in the form of a white, fibrous powder or granules. It is soluble in water and in a limited number of organic solvent systems. Aqueous solutions of methylcellulose are surface active, form films upon drying, and undergo a reversible transformation from sol to gel upon heating and cooling, respectively.

REQUIREMENTS

Identification

A. Add 1 g to 100 ml of water. It swells and disperses to form a clear to opalescent mucilaginous solution, depending upon the intrinsic viscosity, which is stable in the presence of most electrolytes and alcohol in concentrations up to 40%.

B. Heat a few ml of the solution prepared for *Identification Test A*. The solution becomes cloudy, and a flaky precipitate appears that redissolves as the solution cools.

C. Pour a few ml of the solution prepared for *Identification Test A* onto a glass plate, and allow the water to evaporate. A thin, self-sustaining film results.

Assay Not less than 27.5% or more than 31.5% of methoxyl groups (—OCH_3), calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 5%.

Residue on Ignition Not more than 1.5%.

Viscosity The apparent viscosity of a solution containing 2 g in each 100 ml is not less than 80% and not more than 120% of that stated on the label for viscosity types of 100 centipoises or less, and not less than 75% and not more than 140% of that stated on the label for viscosity types higher than 100 centipoises.

TESTS

Assay

Caution: Perform all steps involving hydriodic acid carefully, in a well-ventilated hood. Use goggles, acid-resistant gloves, and other appropriate safety equipment. Be extremely careful when handling the hot vials, since they are under pressure. In the event of hydriodic acid exposure, wash with copious amounts of water, and seek medical attention at once.

Internal Standard Solution Transfer about 2.5 g of toluene, accurately weighed, into a 100-ml volumetric flask containing 10 ml of *o*-xylene, dilute with *o*-xylene to volume, and mix.

Standard Preparation Transfer about 135 mg of adipic acid into a suitable serum vial, add 4.0 ml of hydriodic acid followed by 4.0 ml of the *Internal Standard Solution*, and close the vial securely with a septum stopper. Weigh the vial and its contents accurately, add 90 µl of methyl iodide with a syringe through the septum, again weigh, and calculate the weight of methyl iodide added. Shake well, and allow the layers to separate.

Assay Preparation Transfer about 0.065 g of the sample, accurately weighed, into a 5-ml vial equipped with a pressure-tight septum closure, add an amount of adipic acid equal to the weight of the sample, and pipet 2 ml of the *Internal Standard Solution* into the vial. Cautiously pipet 2 ml of hydriodic acid into the mixture, immediately secure the closure, and weigh accurately. Shake the vial for 30 s, heat at 150° for 20 min, remove from the heat, shake again, using extreme caution, and heat at 150° for 40 min. Allow the vial to cool for about 45 min, and then weigh. If the weight loss is greater than 10 mg, discard the mixture and prepare another *Assay Preparation*.

Chromatographic System Use a suitable gas chromatograph equipped with a thermal conductivity detector. Under typical conditions, the instrument contains a 1.8-m × 4-mm glass column packed with 10% methylsilicone oil (UCW 982 or equivalent) on 100/120-mesh flux-calcined chromatographic siliceous earth (Chromosorb WHP or equivalent). The column is maintained at 100°, and the injection port and detector at 200°, and helium is used as the carrier gas flowing at the rate of 20 ml per min.

Calibration Inject about 2 µl of the upper layer of the *Standard Preparation* into the chromatograph, and record the chromatogram. The retention times for methyl iodide, toluene, and *o*-xylene are approximately 3, 7, and 13 min, respectively. Calculate the relative response factor, *F*, of equal weights of toluene and methyl iodide by the formula *Q/A*, in which *Q* is the quantity ratio of methyl iodide to toluene in the *Standard Preparation*, and *A* is the peak area

ratio of the methyl iodide to toluene obtained from the *Standard Preparation.*

Procedure Inject about 2 µl of the upper layer of the *Assay Preparation* into the chromatograph, and record the chromatogram. Calculate the percentage of methoxyl groups (—OCH_3) in the sample by the formula

$$2 \times (31/142) \times F \times a \times (W/w),$$

in which 31/142 is the ratio of the formula weights of methoxyl to methyl iodide; a is the ratio of the area of the methyl iodide peak to that of the toluene peak obtained from the *Assay Preparation*; W is the weight of the toluene in the *Internal Standard Solution*, in g; and w is the weight of sample taken, in g.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, adding 1 ml of hydroxylamine hydrochloride solution (1 in 5) to the solution of the residue. Any color does not exceed that produced in a control (*Solution A*) containing 20 µg of lead ion (Pb) and 1 ml of the hydroxylamine hydrochloride solution.

Loss on Drying, page 518 Dry a 3-g sample at 105° for 2 h.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Viscosity Weigh accurately a sample, equivalent to 2 g of solids on the dried basis, transfer to a wide-mouth 250-ml centrifuge bottle, and add 98 g of water previously heated to between 80° and 90°. Stir with a mechanical stirrer for 10 min, then place the bottle in an ice bath until solution is complete, adjust the weight of the solution to 100 g, if necessary, and centrifuge it to expel any entrapped air. Adjust the temperature of the solution to 20° ± 0.1°, and determine the viscosity as directed under *Viscosity of Methylcellulose*, page 549.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Thickener; stabilizer; emulsifier; bodying agent; bulking agent; binder; film former.

Methylene Chloride

Dichloromethane

CH_2Cl_2 Mol wt 84.93

DESCRIPTION

A clear, colorless, nonflammable liquid having an odor resembling that of chloroform. It is soluble in about 50 parts of water, and is miscible with alcohol, with acetone, with chloroform, with ether, and with carbon tetrachloride. Its refractive index at 20° is about 1.424.

REQUIREMENTS

Acidity (as HCl) Not more than 10 ppm.
Distillation Range Between 39.5° and 40.5°.
Free Halogens Passes test.
Foreign Odor Passes test.
Heavy Metals (as Pb) Not more than 1 ppm.
Nonvolatile Residue Not more than 0.002%.
Specific Gravity Between 1.318 and 1.323.
Water Not more than 0.02%.

TESTS

Acidity Transfer 100 ml (about 132 g) of the sample into a separator, add 100 ml of neutralized water, and shake vigorously for 2 min. Allow the layers to separate, transfer the aqueous phase into an Erlenmeyer flask, add 4 drops of bromothymol blue TS, and titrate with 0.01 N sodium hydroxide. Not more than 3.6 ml is required.

Distillation Range Proceed as directed in the general method, page 478.

Free Halogens Transfer 10 ml of the sample to a separator, add 25 ml of water, and shake vigorously for 1 min. Allow the layers to separate completely, and then remove and discard the lower sample layer. To the aqueous phase add 1 ml of potassium iodide TS and a few drops of starch TS, and allow to stand for 5 min. A blue color does not develop.

Foreign Odor Pour a few ml of the sample onto a clean filter paper, observe the odor at once, and then allow to evaporate in air at room temperature. No foreign odor is perceptible after the last traces of the sample have evaporated from the paper.

Heavy Metals Evaporate 15 ml (about 20 g) of the sample to dryness in a glass evaporating dish (*Caution:* use hood) on a steam bath. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Filter if necessary through a small filter, wash the evaporating dish and the filter with about 10 ml of water, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Evaporate 38 ml (about 50 g) of the sample to dryness (*Caution:* use hood) in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Specific Gravity Determine by any reliable method (see page 3).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Extraction solvent.

Methyl Ester of Rosin, Partially Hydrogenated

DESCRIPTION

A light amber–colored liquid resin produced by the esterification of rosin with methanol, followed by partial hydrogenation and purification by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify partially hydrogenated methyl ester of rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 717. The sample is prepared for analysis neat (as is) on a cesium bromide plate.

Acid Value Between 4 and 8.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Refractive Index Between 1.517 and 1.520 at 20°.
Viscosity Between 23 and 76 poises.

TESTS

Acid Value Determine as directed in the general procedure, page 533.
Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Viscosity Determine as directed in the general procedure, page 537.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Masticatory substance in chewing gum base.

Methyl Ethyl Cellulose

DESCRIPTION

Methyl ethyl cellulose is the methyl ether of ethyl cellulose in which both the methyl and ethyl groups are attached to the anhydroglucose units by ether linkages. It occurs as a white or pale cream–colored, fibrous solid or powder. It is practically odorless and disperses in cold water to form aqueous solutions that undergo a reversible transformation from sol to gel upon heating and cooling, respectively.

REQUIREMENTS

Identification

A. Add 1 g to 100 ml of water. It disperses to form an opalescent, fibrous sol.
B. Heat a few ml of the sol prepared for *Identification Test A* to about 60°. The sol becomes cloudy and a gelatinous precipitate forms that redissolves upon cooling.
C. The remaining sol from *Identification Test A*, when whipped as for egg white in a kitchen-type mixer, produces a stable air/liquid foam.

Assay Not less than 14.5% and not more than 19.0% of ethoxyl groups ($—OC_2H_5$) and not less than 3.5% and not more than 6.5% of methoxyl groups ($—OCH_3$).
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 5 ppm.
Loss on Drying *Fibrous form*: not more than 15%; *powdered form*: not more than 10%.
Residue on Ignition Not more than 0.6%.
Viscosity The apparent viscosity of a solution containing the equivalent of 2.5 g of dry sample in each 100 g of solution is not less than 80% and not more than 120% of that stated on the label or otherwise represented by the vendor. The usual range of viscosity types is between 20 and 60 centipoises.

TESTS

Assay for Ethoxyl Groups Proceed as directed for *Hydroxypropoxyl Determination*, page 514. Each ml of 0.02 *N* sodium hydroxide is equivalent to 0.9 mg of ethoxyl groups ($—OC_2H_5$).
Assay for Methoxyl Groups Place about 50 mg, previously dried at 105°, in a tared gelatin capsule, and weigh accurately. Proceed as directed for *Methoxyl Determination*, page 521, but calculate the total alkoxyl content as ethoxyl groups ($—OC_2H_5$). Each ml of 0.1 *N* sodium thiosulfate is equivalent to 0.7510 mg of ethoxyl groups ($—OC_2H_5$). Now calculate the methoxyl groups ($—OCH_3$) by the formula

$$(A - B) \times 31/45,$$

in which A is the total alkoxyl content calculated as $—OC_2H_5$, B is the $—OC_2H_5$ determined in the *Assay for*

Ethoxyl Groups, 31 is the molecular weight of —OCH_3, and 45 is the molecular weight of —OC_2H_5.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, adding 1 ml of a 1 in 5 hydroxylamine hydrochloride solution to the solution of the residue. Any color does not exceed that produced in a control (*Solution A*) containing 20 µg of lead ion (Pb) and 1 ml of the hydroxylamine hydrochloride solution.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry a 3-g sample at 105° for 4 h.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Viscosity Transfer an accurately weighed sample, equivalent to 5.0 g on the dried basis, into a 250-ml beaker. Adjust the rotor of a variable-speed stirrer about an inch above the sample, add 195 ml of recently boiled and cooled water, and stir at a speed that will avoid undue aeration. Continue the stirring for about 1.5 h, then either set aside for 3 h or overnight, or centrifuge to expel any entrapped air. Adjust the temperature to 20° ± 0.1°, and determine as directed under *Viscosity of Methylcellulose*, page 549, using a *Viscometer for High Viscosity*.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; stabilizer; foaming agent.

Methyl Formate

$HCOOCH_3$

$C_2H_4O_2$ Mol wt 60.05

DESCRIPTION

A colorless, flammable liquid having a pleasant odor. It is miscible with water and with practically all organic solvents. It boils at about 32°, and its specific gravity is about 0.96.

Caution: Methyl formate is very flammable. Do not use where it may be ignited.

REQUIREMENTS

Identification

Place about 25 mg of the sample in a small test tube, and add 0.1 *N* sodium hydroxide to make the mixture just alkaline to litmus paper. Add 1 drop of mercuric chloride TS and 1 drop of buffer solution (0.1 ml of glacial acetic acid and 100 mg of sodium acetate in 10 ml of water), and mix. Place the tube in a steam bath, evaporate to dryness, and cool. Add 1 drop each of 0.1 *N* ammonium hydroxide and water, and mix. An intense black to gray black color forms.

Assay Not less than 97.0% of $C_2H_4O_2$.

Acidity (as formic acid) Not more than 0.04%.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Nonvolatile Residue Not more than 0.002%.

Water Not more than 0.05%.

TESTS

Assay Transfer 100.0 ml of 0.5 *N* sodium hydroxide from a buret into a 100-ml volumetric flask. For the blank, similarly transfer 100.0 ml into a 500-ml Erlenmeyer flask. Weigh the volumetric flask and its contents, pipet 2 ml of the sample into the flask, mix, and reweigh to obtain the weight of the sample added. Quantitatively transfer the contents of the volumetric flask into a second 500-ml Erlenmeyer flask with the aid of several small portions of water. Add sufficient water to the blank flask to match the volume of the sample flask, then add phenolphthalein TS to each flask, and titrate the excess alkali with 0.5 *N* hydrochloric acid (see page 2). Each ml of 0.5 *N* hydrochloric acid is equivalent to 30.03 mg of $C_2H_4O_2$.

Acidity Transfer 50 ml of the sample into a 300-ml Erlenmeyer flask containing 1 ml of bromocresol purple TS and 50 ml of methanol that has been previously titrated with 0.1 *N* alcoholic potassium hydroxide to the first appearance of a bluish purple color, and titrate the sample with the alkali to the same color. Not more than 4.2 ml is required.

Sample Solution for the Determination of Arsenic and Heavy Metals Evaporate a 4.2-ml (4-g) sample to dryness with 20 mg of sodium carbonate, heat gently to volatilize any organic matter, and dissolve the residue in 40 ml of water.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A 20-ml portion of the *Sample Solution*, diluted to 25 ml with water, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Dry a 125-ml platinum dish at 105° for at least 15 min, cool in a desiccator, and weigh. Evaporate four 50-ml portions of the sample in the dish on a steam bath, but do not allow the dish to go dry between additions of the sample. After all of the sample has evaporated, heat in an oven at 105° for 30 min, cool in a desiccator, and weigh. Not more than 3.9 mg of residue remains.

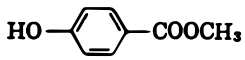
Water Determine by the *Karl Fischer Titrimetric Method*, page 552, using 50 ml of methanol and 50 ml of the sample. Not more than 24 mg of water is found.

Packaging and Storage Store in tight containers, and observe the safety precautions stated on the label.

Functional Use in Foods Fumigant.

Methylparaben

Methyl *p*-Hydroxybenzoate



$C_8H_8O_3$ Mol wt 152.15

DESCRIPTION

Small, colorless crystals or a white, crystalline powder. It is odorless or has a faint, characteristic odor and a slight, burning taste. One g dissolves in about 400 ml of water at 25°, in about 50 ml of water at 80°, in 2.5 ml of alcohol, in about 7 ml of ether, and in about 4 ml of propylene glycol. It is slightly soluble in glycerin, fixed oils, benzene, and carbon tetrachloride.

REQUIREMENTS

Identification

Dissolve 500 mg in 10 ml of sodium hydroxide TS, boil for 30 min, allow the solution to evaporate to a volume of about 5 ml, and cool. Acidify the solution with diluted sulfuric acid TS, collect the crystals on a filter, wash several times with small portions of water, and dry in a desiccator over silica gel. The *p*-hydroxybenzoic acid so obtained melts between 212° and 217° (see page 519).

Assay Not less than 99.0% of $C_8H_8O_3$, calculated on the dried basis.
Acidity Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Melting Range Between 125° and 128°.
Residue on Ignition Not more than 0.05%.

TESTS

Assay Transfer about 2 g, accurately weighed, into a flask, add 40.0 ml of 1 *N* sodium hydroxide, and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, cool, and titrate the excess sodium hydroxide with 1 *N* sulfuric acid to pH 6.5. Perform a blank determination with the same quantities of the same reagents in the same manner, and make any necessary correction (see page 2). Each ml of 1 *N* sodium hydroxide is equivalent to 152.2 mg of $C_8H_8O_3$, calculated on the dried basis.

Acidity Heat 750 mg with 15 ml of water at 80° for 1 min, cool, and filter. The filtrate is acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 *N* sodium hydroxide and 2 drops of methyl red TS. The solution is yellow, without even a light cast of pink.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals, page 512 Dissolve 2 g in 23 ml of acetone, and add 2 ml of diluted acetic acid TS, 2 ml of water, and 10 ml of hydrogen sulfide TS. Any color does not exceed that produced in a control (*Solution A*) made with 23 ml of acetone, 2 ml of diluted acetic acid TS, 2 ml of *Standard Lead Solution* (20 µg Pb ion), and 10 ml of hydrogen sulfide TS.

Loss on Drying, page 518 Dry over silica gel for 5 h.

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition Ignite 4 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Preservative; antimicrobial agent.

Mineral Oil, White

Liquid Petrolatum

DESCRIPTION

A mixture of refined liquid hydrocarbons, essentially paraffinic and naphthenic in nature, obtained from petroleum. It occurs as a colorless, transparent, oily liquid, free or nearly free from fluorescence. It is odorless and tasteless when cold, and develops not more than a faint odor of petroleum when heated. It is insoluble in water and in alcohol, is soluble in volatile oils, and is miscible with most fixed oils, but not with castor oil. It may contain any antioxidant permitted in food by the federal Food and Drug Administration, in an amount not greater than that required to produce its intended effect.

REQUIREMENTS

Readily Carbonizable Substances Passes test.
Specific Gravity Not less than that stated, or within the range claimed by the vendor.
Sulfur Compounds Passes test.
Ultraviolet Absorbance (polynuclear hydrocarbons) Passes test.
Viscosity Not less than that stated, or within the range claimed by the vendor.

TESTS

Readily Carbonizable Substances Place 5 ml of the sample in a glass-stoppered test tube that previously has been rinsed with chromic acid cleaning mixture (200 g of sodium dichromate dissolved in about 100 ml of water to which 1500 ml of sulfuric acid has been added slowly with stirring), then rinsed with water, and dried. Add 5 ml of sulfuric acid containing between 94.5% and 94.9% of H_2SO_4, and heat in a boiling water bath for 10 min. After the test tube has been in the bath for 30 s, remove it quickly, and, while holding the stopper in place, give three vigorous vertical shakes over an

amplitude of about 5 in. Repeat every 30 s. Do not keep the test tube out of the bath longer than 3 s for each shaking period. At the end of 10 min from the time when first placed in the water bath, remove the test tube. The sample remains unchanged in color, and the acid does not become darker than standard color produced by mixing in a similar test tube 3 ml of ferric chloride CS, 1.5 ml of cobaltous chloride CS, and 0.5 ml of cupric sulfate CS, this mixture being overlaid with 5 ml of mineral oil.

Specific Gravity Determine by any reliable method (see page 3).

Sulfur Compounds Prepare a saturated solution of lead monoxide in a 1 in 5 solution of sodium hydroxide, and mix 2 drops of the clear solution with 4 ml of the sample and 2 ml of absolute alcohol. The mixture, after being heated at 70° for 10 min and then cooled, is no darker than a blank consisting of 4 ml of mineral oil and 2 ml of absolute alcohol.

Ultraviolet Absorbance (polynuclear hydrocarbons) All measurements are made with an ultraviolet spectrophotometer in 1-cm cells and in the wavelength range of 260 to 350 nm, under the same instrumental conditions. The standard reference absorbance is the absorbance at 275 nm of a standard reference solution of naphthalene (National Bureau of Standards Standard Material No. 577, or equivalent purity) containing a concentration of 7.0 mg/1000 ml, in purified isooctane, measured against isooctane of the same spectral purity in 1-cm cells. (The absorbance will be approximately 0.30.)

Hexane Use a pure grade of hexanes (predominantly *n*-hexane and methylcyclopentane) having an ultraviolet absorbance not exceeding 0.10 down to 220 nm and not exceeding 0.02 down to 260 nm. The purity should be such that the "solvent control," as defined under *Procedure*, has an absorbance curve compared to water showing no extraneous impurity peaks and no absorbance exceeding that of dimethyl sulfoxide compared to water at any wavelength in the range 260 to 350 nm, inclusive. If necessary to obtain the prescribed purities, the hexane may be passed through activated silica gel.

Dimethyl Sulfoxide Use a pure grade of dimethyl sulfoxide (99.9%, m.p. 18°) that is clear, water-white in appearance, having an absorbance curve compared to water not exceeding 1.0 at 264 nm and showing no extraneous impurity peaks in the wavelength range up to 350 nm. It should be stored in glass-stoppered bottles.

Apparatus Use 125-ml glass-stoppered separators equipped with tetrafluoroethylene polymer stopcocks or other suitable stopcocks that will not contaminate the solvents.

Procedure Transfer 25 ml of the sample and 25 ml of hexane to a separator, and mix. Add 5.0 ml of dimethyl sulfoxide, shake the mixture vigorously for at least 1 min, and allow to stand until the lower layer is clear. Completely transfer the lower layer to a second separator, add 2 ml of hexane, and shake the mixture vigorously. Allow to stand until the lower layer is clear, and then draw off the lower layer, designated as "mineral oil extract." Shake 5.0 ml of dimethyl sulfoxide with 25 ml of hexane vigorously for at least 1 min in a third separator, allow to stand until the lower layer is clear, and draw off this layer, designated as "solvent control." Determine the absorbance of the mineral oil extract in a 1-cm cell in the range 260 to 350 nm, inclusive, compared to the solvent control. The absorbance of the mineral oil extract does not exceed that of the solvent control at any wavelength in the specified range by more than one third of the standard reference absorbance. (NOTE: Suitable corrections of the absorbance should be made when testing samples containing added antioxidants.)

Viscosity Determine by any reliable method.

Packaging and Storage Store in tight containers.

Functional Use in Foods Binder; defoaming agent; lubricant; release agent; fermentation aid; protective coating.

Monoammonium L-Glutamate

Monoammonium Glutamate; Ammonium Glutamate

$$HOOCCH_2CH_2CH(NH_2)COONH_4H_2O$$

$C_5H_{12}N_2O_4.H_2O$ Mol wt 182.18

DESCRIPTION

A white, practically odorless, free-flowing, crystalline powder. It is freely soluble in water, but is practically insoluble in common organic solvents.

REQUIREMENTS

Identification

A. To 1 ml of a 1 in 30 solution add 1 ml of triketohydrindene hydrate TS and 100 mg of sodium acetate, and heat in a boiling water bath for 10 min. An intense, violet blue color is formed.

B. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 *N* hydrochloric acid. A white crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 *N* hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.

C. A 1 in 10 solution gives positive tests for *Ammonium*, page 515.

Assay Not less than 99.0% of $C_5H_{12}N_2O_4.H_2O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

pH of a 1 in 20 Solution Between 6.0 and 7.0.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]^{25°}_{546.1\ nm}$: Between +30.1° and +31.6°.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 9.109 mg of $C_5H_{12}N_2O_4.H_2O$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 50° for 4 h.

pH of a 1 in 20 Solution Determine by the *Potentiometric Method*, page 531.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation Determine in a solution containing 14.6 g in sufficient 2.3 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in tight containers.
Functional Use in Foods Flavor enhancer; salt substitute.

Mono- and Diglycerides

DESCRIPTION

Mono- and diglycerides consist of mixtures of glycerol mono- and diesters, with minor amounts of triesters, of edible fats or oils or edible fat-forming fatty acids. Those commercially available vary in consistency from yellow liquids through ivory-colored plastics to hard, ivory-colored solids having a bland odor and taste. They are insoluble in water, but are soluble in alcohol, in ethyl acetate, and in chloroform and other chlorinated hydrocarbons.

REQUIREMENTS

Acid Value Not more than 6.
Arsenic (as As) Not more than 3 ppm.
Free Glycerin Not more than 7%.
Heavy Metals (as Pb) Not more than 10 ppm.
Residue on Ignition Not more than 0.5%.

The following specifications should conform to the representations of the vendor: **1-Monoglyceride Content, Total Monoglycerides, Hydroxyl Value, Iodine Value,** and **Saponification Value.**

TESTS

1-Monoglyceride Content Determine as directed in the general method, page 506.

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Free Glycerin Determine as directed in the general method, page 504.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Iodine Value Determine by the *Wijs Method*, page 505.

Residue on Ignition Ignite 5 g as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509, using about 4 g, accurately weighed.

Total Monoglycerides Determine as directed in the general method, page 506.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Emulsifier; stabilizer.

Monoglyceride Citrate

DESCRIPTION

A mixture of glyceryl monooleate and its citric acid monoester, manufactured by the reaction of glyceryl monooleate with citric acid under controlled conditions. It occurs as a soft, white to ivory–colored, waxy solid having a lardlike consistency and a bland odor and taste. It is soluble in most common fat solvents and in alcohol, but is insoluble in water.

REQUIREMENTS

Acid Value Between 70 and 100.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Residue on Ignition Not more than 0.3%.
Saponification Value Between 260 and 265.
Total Citric Acid Between 14.0% and 17.0%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509.

Total Citric Acid

Standard Solution Transfer about 35 mg of sodium citrate dihydrate, accurately weighed, into a 100-ml volumetric flask, dissolve and dilute to volume with water, and mix. Calculate the concentration (C), in µg per ml, of citric acid in the final solution by the formula $1000 \times 0.6533W/100$, in which W is the weight, in mg, of the sodium citrate dihydrate taken, and 0.6533 is a factor converting sodium citrate dihydrate to citric acid.

Sample Solution Transfer about 150 mg of the sample, accurately weighed, into a saponification flask, add 50 ml of 4% alcoholic potassium hydroxide solution, and reflux for 1 h. Acidify the reaction mixture with hydrochloric acid to a pH of 2.8 to 3.2, transfer to a 400-ml beaker, and evaporate to dryness on a steam bath. Quantitatively transfer the contents of the beaker into a separator, using no more than 50 ml of water, and then extract with three 50-ml portions of petroleum ether (b.p. 30° to 60°), discarding the extracts. Transfer the water layer to a 100-ml volumetric flask, dilute to volume with water, and mix.

Procedure Pipet 2.0 ml each of the *Standard Solution* and of the *Sample Solution* into separate 40-ml graduated centrifuge tubes, and to each tube add 2 ml of dilute sulfuric acid (1 in 2) and 11 ml of water. Boil for 3 min, cool, and add 5 ml of bromine TS to each tube. Dilute to the 20-ml mark, allow to stand for 10 min, and centrifuge. Transfer 4.0 ml of each solution into separate 19- × 110-mm test tubes, add 1 ml of water, 0.5 ml of dilute sulfuric acid (1 in 2), and 0.3 ml of 1 *M* potassium bromide, and shake. Add 0.3 ml of 1.5 *N* potassium permanganate, shake, and allow to stand for 2 min. Add 1 ml of a saturated solution of ferrous sulfate, shake, allow to stand for 2 min, and then dilute to 10 ml with water. Add 10.0 ml of *n*-hexane (previously washed with sulfuric acid, followed by a water wash, and then dried over anhydrous sodium sulfate), shake vigorously for 2 min, and then centrifuge at a low speed for 1 min. Transfer 5.0 ml of the hexane extract into a 20- × 145-mm tube containing 10.0 ml of sodium sulfide solution (4 g of $Na_2S.9H_2O$ in each 100 ml of water), and shake vigorously briefly (3 oscillations only). Centrifuge the mixture at low speed for 1 min. Immediately determine the absorbance of each aqueous layer in a 1-cm cell at 450 nm with a suitable spectrophotometer, using a reagent blank in the reference cell. Calculate the quantity, in mg, of citric acid in the sample taken by the formula $0.1C \times A_U/A_S$, in which C is as defined under *Standard Solution*, A_U is the absorbance of the final solution from the *Sample Solution*, and A_S is the absorbance of the final solution from the *Standard Solution*.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Synergist and solubilizer for antioxidants.

Monopotassium L-Glutamate

Monopotassium Glutamate; Potassium Glutamate; MPG

$$HOOCCH_2CH_2CH(NH_2)COOK.H_2O$$

$C_5H_8KNO_4.H_2O$ Mol wt 203.24

DESCRIPTION

A white, practically odorless, free-flowing, crystalline powder. It is hygroscopic, is freely soluble in water, and is slightly soluble in alcohol.

REQUIREMENTS

Identification

A. To 1 ml of a 1 in 30 solution add 1 ml of triketohydrindene hydrate TS and 100 mg of sodium acetate, and heat in a boiling water bath for 10 min. An intense, violet blue color is formed.

B. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 *N* hydrochloric acid. A white, crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 *N* hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.

C. A 1 in 10 solution gives positive tests for *Potassium*, page 517.

Assay Not less than 99.0% of $C_5H_8KNO_4.H_2O$.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.1%.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.1%.

pH of a 1 in 50 Solution Between 6.7 and 7.3.

Specific Rotation $[\alpha]^{25°}_{546.1\ nm}$: Between +27.7° and +28.3°; $[\alpha]^{20°}_{D}$: between +22.5° and +24.0°.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 10.16 mg of $C_5H_8KNO_4.H_2O$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 20-mg

sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 60° in a vacuum for 2 h.

pH of a 1 in 50 Solution Determine by the *Potentiometric Method*, page 531.

Specific Rotation, page 530 $[\alpha]^{25°}_{546.1\ nm}$: Determine in a solution containing 16.3 g in sufficient 2.3 *N* hydrochloric acid to make 100 ml; $[\alpha]^{20°}_{D}$: determine in a solution containing 10 g in sufficient 2 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in tight containers.

Functional Use in Foods Flavor enhancer; salt substitute.

Monosodium L-Glutamate

Monosodium Glutamate; Sodium Glutamate; MSG

$HOOCCH_2CH_2CH(NH_2)COONa.H_2O$

$C_5H_8NNaO_4.H_2O$ Mol wt 187.13

DESCRIPTION

White, practically odorless, free-flowing crystals or crystalline powder. It is freely soluble in water, and is sparingly soluble in alcohol. It may have either a slightly sweet or a slightly salty taste.

REQUIREMENTS

Identification

A. To 1 ml of a 1 in 30 solution add 1 ml of triketohydrindene hydrate TS and 100 mg of sodium acetate and heat in a boiling water bath for 10 min. An intense, violet blue color is formed.

B. To 10 ml of a 1 in 10 solution add 5.6 ml of 1 *N* hydrochloric acid. A white crystalline precipitate of glutamic acid forms on standing. When 6 ml of 1 *N* hydrochloric acid is added to the turbid solution, the glutamic acid dissolves on stirring.

C. Prepare a 10% solution of the sample in 1 *N* hydrochloric acid. To 1 ml of this solution add 5 ml of cobalt-uranyl acetate TS, and agitate on a vortex mixer for 3 min. A golden yellow precipitate forms, indicating the presence of sodium.

Assay Not less than 99.0% of $C_5H_8NNaO_4.H_2O$.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.2%.

Clarity and Color of Solution Passes test.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

pH of a 1 in 20 Solution Between 6.7 and 7.2.

Specific Rotation $[\alpha]^{25°}_{546.1\ nm}$: Between +29.7° and +30.2°; $[\alpha]^{20°}_{D}$: between +24.8° and +25.3°.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 100 ml of glacial acetic acid. A few drops of water may be added prior to the addition of the acetic acid to effect faster dissolution of the sample. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 9.356 mg of $C_5H_8NNaO_4.H_2O$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 10-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Clarity and Color of Solution A solution of 1 g of the sample in 10 ml of water is colorless and has no more turbidity (see page 542) than a standard mixture prepared as follows: Dilute 0.2 ml of *Standard Chloride Solution* (see page 471) to 20 ml with water, add 1 ml of dilute nitric acid (1 in 3), 0.2 ml of a 1 in 50 solution of dextrin, and 1 ml of a 1 in 50 solution of silver nitrate, mix, and allow to stand for 15 min.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 100° for 5 h.

pH of a 1 in 20 Solution Determine by the *Potentiometric Method*, page 531.

Specific Rotation, page 530 $[\alpha]^{25°}_{546.1\ nm}$: Determine in a solution containing 15 g in sufficient 2.3 *N* hydrochloric acid to make 100 ml; $[\alpha]^{20°}_{D}$: determine in a solution containing 10 g in sufficient 2 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in tight containers.

Functional Use in Foods Flavor enhancer.

Myristic Acid

Tetradecanoic Acid

$$CH_3(CH_2)_{12}COOH$$

$C_{14}H_{28}O_2$ Mol wt 228.37

DESCRIPTION

A solid organic acid obtained from coconut oil and other fats. It occurs as a hard, white or faintly yellowish, somewhat glossy crystalline solid, or as a white or yellowish white powder. Myristic acid is practically insoluble in water, but is soluble in alcohol, in chloroform, and in ether.

REQUIREMENTS

Acid Value Between 242 and 249.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Not more than 1.0.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 242 and 251.
Titer (Solidification Point) Between 48° and 55.5°.
Unsaponifiable Matter Not more than 1%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.
Saponification Value Determine as directed in the general method, page 509, using about 3 g, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.
Unsaponifiable Matter, page 509 Determine as directed in the general method.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Component in the manufacture of other food-grade additives; defoaming agent.

Myrrh Oil

DESCRIPTION

The volatile oil obtained by steam distillation from myrrh gum obtained from several species of *Commiphora* (Fam. *Burseraceae*). It is a light brown or green liquid having the characteristic odor of the gum. It is soluble in most fixed oils, but is only slightly soluble in mineral oil. It is insoluble in glycerin and in propylene glycol. It becomes darker in color and more viscous under the influence of air and light.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 600, using the same test conditions as specified therein.

Acid Value Between 2 and 13.
Angniar Rotation Between −60° and −83°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.519 and 1.528 at 20°.
Saponification Value Between 9 and 35.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.985 and 1.014.

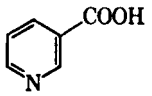
TESTS

Acid Value Determine as directed in the general method, page 499.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 509, using about 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 10 ml of 90% alcohol, occasionally with opalescence or turbidity.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Niacin

Nicotinic Acid; 3-Pyridinecarboxylic Acid



$C_6H_5NO_2$ Mol wt 123.11

DESCRIPTION

White or light yellow crystals or a crystalline powder. It is odorless or has a slight odor. One g dissolves in 60 ml of water. It is freely soluble in boiling water and in boiling alcohol, and also in solutions of alkali hydroxides and carbonates. It is almost insoluble in ether.

REQUIREMENTS

Identification

A. Triturate a sample with twice its weight of 2,4-dinitrochlorobenzene. Gently heat about 10 mg of the mixture in a test tube until melted, and continue the heating for a few seconds longer. Cool, and add 3 ml of alcoholic potassium hydroxide TS. A deep red to deep wine-red color is produced.

B. Dissolve about 50 mg in 20 ml of water, neutralize to litmus paper with 0.1 *N* sodium hydroxide, and add 3 ml of cupric sulfate TS. A blue precipitate gradually forms.

C. The infrared absorption spectrum of a mineral oil dispersion of the sample, previously dried at 105° for 1 h, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Niacin Reference Standard.

D. Determine the absorbance of a solution of the sample containing 20 µg in each ml of water in a 1-cm cell at 237 nm and 262 nm, using water as the blank. The ratio A_{237}/A_{262} is between 0.35 and 0.39.

Assay Not less than 99.5% and not more than 101.0% of $C_6H_5NO_2$, calculated on the dried basis.
Heavy Metals (as Pb) Not more than 0.002%.
Loss on Drying Not more than 1%.
Melting Range Between 234° and 238°.
Residue on Igultion Not more than 0.1%.

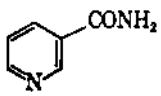
TESTS

Assay Dissolve about 300 mg, accurately weighed, in about 50 ml of water, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination (see page 2). Each ml of 0.1 *N* sodium hydroxide is equivalent to 12.31 mg of $C_6H_5NO_2$.
Heavy Metals Mix 1 g with 2 ml of diluted acetic acid TS, add water to make 25 ml, heat gently until solution is complete, and cool. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 105° for 1 h.
Melting Range Determine as directed in the general procedure, page 519.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Niacinamide

Nicotinamide



$C_6H_6N_2O$ Mol wt 122.13

DESCRIPTION

A white, crystalline powder. It is odorless or nearly so, and has a bitter taste. Its solutions are neutral to litmus. One g dissolves in about 1 ml of water, in about 1.5 ml of alcohol, and in about 10 ml of glycerin.

REQUIREMENTS

Identification

A. Transfer about 20 mg to a 1000-ml volumetric flask, dissolve and dilute with water to volume, mix, and determine the absorbance of the solution in a 1-cm cell at 245 nm and at 262 nm with a suitable spectrophotometer, using water as the blank. The ratio A_{245}/A_{262} is 0.65 ± 0.02.

B. To 20 mg in a test tube add 1 pellet of sodium hydroxide TS, and heat gently over an open flame. The odor of ammonia is perceptible. Upon heating more vigorously, the odor of pyridine is perceptible.

Assay Not less than 98.5% and not more than the equivalent of 101.0% of $C_6H_6N_2O$ after drying.
Heavy Metals (as Pb) Not more than 0.003%.
Loss on Drying Not more than 0.5%.
Melting Range Between 128° and 131°.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 300 mg, previously dried over silica gel for 4 h and accurately weighed, in 20 ml of glacial acetic acid, warming slightly if necessary to effect solution. Add 100 ml

of benzene and 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 12.21 mg of $C_6H_6N_2O$.

Heavy Metals Prepare and test a 670-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry over silica gel for 4 h.

Melting Range Determine as directed in the general procedure, page 519.

Readily Carbonizable Substances, page 532 Dissolve 200 mg in 5 ml of sulfuric acid TS. The solution has no more color than *Matching Fluid A*.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement.

Niacinamide Ascorbate

DESCRIPTION

A complex of ascorbic acid ($C_6H_8O_6$) and niacinamide ($C_6H_6N_2O$). It occurs as a lemon yellow–colored powder that is odorless or has a very slight odor. It may gradually darken upon exposure to air. One g is soluble in 3.5 ml of water and in about 20 ml of alcohol. It is very slightly soluble in chloroform and in ether, sparingly soluble in glycerin, and practically insoluble in benzene.

REQUIREMENTS

Identification

A. A 1 in 50 solution responds to the *Identification Tests* under *Ascorbic Acid*, page 27.

B. An 80-mg sample responds to *Identification Test B* under *Niacinamide*, page 205.

Assay Not less than 73.5% of ascorbic acid ($C_6H_8O_6$) and not less than 24.5% of niacinamide ($C_6H_6N_2O$), calculated on the anhydrous basis. The total of ascorbic acid and niacinamide is not less than 99.0%.

Heavy Metals (as Pb) Not more than 0.0025%.

Loss on Drying Not more than 0.5%.

Melting Range Between 141° and 145°.

Residue on Ignition Not more than 0.1%.

TESTS

Assay for Ascorbic Acid Proceed as directed in the *Assay* under *Ascorbic Acid*, page 27.

Assay for Niacinamide Proceed as directed in the *Assay* under *Niacinamide*, page 205, but use an undried sample and make the calculation on the anhydrous basis.

Heavy Metals Prepare and test an 800-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry to constant weight at 75°.

Melting Range, page 519 Determine as directed in *Procedure for Class Ia*.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Nutmeg Oil

Myristica Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the dried kernels of the ripe seed of *Myristica fragrans* Houttuyn (Fam. *Myristicaceae*). Two types of oil, the East Indian and the West Indian, are commercially available. It is a colorless or pale yellow liquid having the characteristic odor and taste of nutmeg. It is soluble in alcohol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 601, using the same test conditions as specified therein.

Angular Rotation *East Indian type:* between +8° and +30°; *West Indian type:* between +25° and +45°.

Heavy Metals (as Pb) Passes test.

Refractive Index *East Indian type:* between 1.474 and 1.488; *West Indian type:* between 1.469 and 1.476 at 20°.

Residue on Evaporation *East Indian type:* not more than 60 mg per 3 ml; *West Indian type:* not more than 50 mg per 3 ml.

Solubility in Alcohol Passes test.

Specific Gravity *East Indian type:* between 0.880 and 0.910; *West Indian type:* between 0.854 and 0.880.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is

saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Residue on Evaporation Proceed as directed in the general method, page 533, using 3 ml of sample. Heat on a steam bath for 5 h, and then heat at 105° for 1 h.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml of East Indian oil dissolves in 3 ml of 90% alcohol. One ml of West Indian oil dissolves in 4 ml of 90% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Labeling Label myristica oil to indicate whether it is the East Indian or West Indian type.

Functional Use in Foods Flavoring agent.

Octanoic Acid

Caprylic Acid

$$CH_3(CH_2)_6COOH$$

$C_8H_{16}O_2$ Mol wt 144.21

DESCRIPTION

A colorless oily liquid having a slight, unpleasant odor and a burning rancid taste. It is slightly soluble in water and soluble in most organic solvents. Its specific gravity is about 0.910.

REQUIREMENTS

Acid Value Between 366 and 396.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Not more than 2.0.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 366 and 398.
Titer (Solidification Point) Between 8° and 15°.
Unsaponifiable Matter Not more than 0.2%.
Water Not more than 0.4%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodine Value Determine by the *Wijs Method*, page 505.

Residue on Ignition, page 533 Ignite 10 g as directed in the general method.

Saponification Value Determine as directed in the general method, page 509, using about 2 g, accurately weighed.

Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.

Unsaponifiable Matter Determine as directed in the general method, page 509.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Component in the manufacture of other food-grade additives; defoaming agent.

Oleic Acid

cis-9-Octadecenoic Acid

$$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$$

$C_{18}H_{34}O_2$ Mol wt 282.46

DESCRIPTION

An unsaturated acid obtained from fats. Oleic acid is a colorless to pale yellow, oily liquid when freshly prepared, but upon exposure to air it gradually absorbs oxygen and darkens. It has a characteristic lardlike odor and taste. When strongly heated in air, it is decomposed with the production of acrid vapors. Its specific gravity is about 0.895. It is practically insoluble in water, but is miscible with alcohol, with ether, with benzene, and with fixed and volatile oils. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Acid Value Between 196 and 204.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Between 83 and 103.
Residue on Ignition Not more than 0.01%.
Saponification Value Between 196 and 206.
Titer (Solidification Point) Not above 10°.
Unsaponifiable Matter Not more than 2%.
Water Not more than 0.4%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodine Value Determine by the *Wijs Method*, page 505.

Residue on Ignition, page 533 Ignite 10 g as directed in the general method.

Saponification Value Determine as directed in the general method, page 509, using about 3 g, accurately weighed.

Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.

Unsaponifiable Matter, page 509 Determine as directed in the general method.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Component in the manufacture of other food-grade additives; defoaming agent; lubricant; binder.

Olibanum Oil

Oil of Frankincense

DESCRIPTION

The volatile oil distilled from a gum obtained from the trees *Boswellia carterii* Birdw. and other *Boswellia* species (Fam. *Burseraceae*). It is a pale yellow liquid having a balsamic odor with a faint lemon note. It is soluble in most fixed oils and, with a slight haze, in mineral oil. It is insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 601, using the same test conditions as specified therein.

Acid Value Not more than 4.0.

Angular Rotation Between −15° and +35°.

Ester Value Between 4 and 40.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.465 and 1.482 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.862 and 0.889.

TESTS

Acid Value Determine as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Ester Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 6 ml of 90% alcohol, occasionally with opalescence.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Onion Oil

DESCRIPTION

A volatile oil obtained by steam distillation of the bulbs of *Allium cepa* L. (Fam. *Liliaceae*). It is a clear, amber yellow to amber orange liquid having a strong pungent odor and taste characteristic of onion. It is soluble in most fixed oils, in mineral oil, and in alcohol. It is insoluble in glycerin and in propylene glycol.

NOTE: Onion oil is purchased mainly on the basis of its odor and flavor, which render definitive specifications of little value.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 601, using the same test conditions as specified therein.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.549 and 1.570 at 20°.

Specific Gravity Between 1.050 and 1.135.

TESTS

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added,

and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or aluminum containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Orange Oil, Coldpressed

Sweet Orange Oil

DESCRIPTION

The volatile oil obtained by expression, without the use of heat, from the fresh peel of the ripe fruit of *Citrus sinensis* L. Osbeck (Fam. *Rutaceae*). It is an intensely yellow, orange, or deep orange liquid having the characteristic odor and taste of the outer part of fresh, sweet orange peel. It is miscible with dehydrated alcohol and with carbon disulfide. It is soluble in glacial acetic acid. It may contain a suitable antioxidant.

NOTE: Do not use sweet orange oil that has a terebinthine odor.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 601, using the same test conditions as specified therein.

Assay Not less than 1.2% and not more than 2.5% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).

Angular Rotation Between +94° and +99°.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Refractive Index Between 1.472 and 1.474 at 20°.

Specific Gravity Between 0.842 and 0.846.

Ultraviolet Absorbance *California type:* not less than 0.130; *Florida type:* not less than 0.240.

TESTS

Assay Weigh accurately a 10-ml sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500. Allow the mixture to stand for 15 min, with occasional shaking, before titrating, and use 78.14 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 330 ± 3 nm.

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.

Labeling Label orange oil to indicate whether it is the California or Florida type.

Functional Use in Foods Flavoring agent.

Orange Oil, Bitter, Coldpressed

DESCRIPTION

The volatile oil obtained by expression, without the use of heat, from the fresh peel of the fruit of *Citrus aurantium* L. (Fam. *Rutaceae*). It is a pale yellow or yellowish brown liquid with the characteristic aromatic odor of the Seville orange and an aromatic somewhat bitter taste. It is miscible with absolute alcohol and with an equal volume of glacial acetic acid. It is soluble in fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is relatively insoluble in glycerin. It is affected by light, and its alcohol solutions are neutral to litmus. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 602, using the same test conditions as specified therein.

Aldehydes Not less than 0.5% and not more than 1.0% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).

Angular Rotation Between +88° and +98°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.472 and 1.476 at 20°.
Residue on Evaporation Between 2% and 5%.
Specific Gravity Between 0.845 and 0.851.

TESTS

Aldehydes Weigh accurately about 10 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, using 78.14 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand for 30 min at room temperature before titrating.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Proceed as directed in the general method, page 533, using 5 g of sample, and heat for 4.5 h.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or tin-lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Orange Oil, Distilled

DESCRIPTION

The volatile oil obtained by distillation from the fresh peel or juice of the fruit of *Citrus sinesis* L. Osbeck (Fam. *Rutaceae*), with or without the previous separation of the juice, pulp, or peel. It is a colorless to pale yellow liquid having the characteristic odor of fresh orange peel. It is soluble in most fixed oils, in mineral oil, and in alcohol (with haze). It is insoluble in glycerin and in propylene glycol. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 602, using the same test conditions as specified therein.

Aldehydes Between 1.0% and 2.5% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{20}O$).
Angular Rotation Between +94° and +99°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.471 and 1.474 at 20°.
Specific Gravity Between 0.840 and 0.844.
Ultraviolet Absorbance Not more than 0.01.

TESTS

Aldehydes Weigh accurately about 5 ml of the sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, using 78.14 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand at room temperature for 1 h before titrating.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see page 3).
Ultraviolet Absorbance Proceed as directed under *Ultraviolet Absorbance of Citrus Oils*, page 502, using about 250 mg of sample, accurately weighed. The maximum absorbance occurs at 330 ± 3 nm.

Packaging and Storage Store in full, tight containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Origanum Oil, Spanish Type

DESCRIPTION

The volatile oil obtained by steam distillation from the flowering herb *Thymus capitatus* Hoffm. et Link and various species of *Origanum*. It is a yellowish red to dark brownish red liquid having a pungent spicy odor suggestive of thyme oil. It is soluble in most fixed oils and in propylene glycol. It is soluble, with turbidity, in mineral oil, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum

under *Infrared Spectra of Essential Oils*, page 602, using the same test conditions as specified therein.

Assay Not less than 60% and not more than 75%, by volume, of phenols.
Angular Rotation Between −2° and +3°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.502 and 1.508 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.935 and 0.960.

TESTS

Assay Shake a suitable quantity of sample with about 2% of powdered tartaric acid, and filter. Proceed as directed under *Phenols*, page 502.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530. Occasionally the oil is too dark to read in a 100-mm tube.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml is soluble in 2 ml of 70% alcohol. The solution may become cloudy on dilution.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light. A precipitate may form in galvanized containers, and the oil darkens in iron drums.
Functional Use in Foods Flavoring agent.

Orris Root Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the peeled, dried, and aged rhizomes of *Iris pallida* Lam. (Fam. *Iridaceae*). At room temperature it is a light yellow to brown yellow mass, which melts between 38° and 50° to form a yellow to yellow brown liquid. It is soluble in most fixed oils, in mineral oil, and in propylene glycol. It is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 602, using the same test conditions as specified therein.

Assay Not less than 9.0% and not more than 20.0% of ketones, calculated as irone ($C_{14}H_{22}O$).
Acid Value Between 175 and 235.
Ester Value Between 4 and 35.
Heavy Metals (as Pb) Passes test.
Melting Range Between 38° and 50°.

TESTS

Assay Weigh accurately about 1 g, and proceed as directed under *Aldehydes*, page 499, using 103.2 as the equivalence factor (*e*) in the calculation. Allow the mixture to stand 1 h at room temperature before titrating.
Acid Value Determine as directed in the general method, page 499, using about 1 g, accurately weighed.
Ester Value Determine as directed in the general method, page 500, using about 1 g, accurately weighed.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Melting Range Determine as directed in the general procedure (*Class II*), page 520.

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or other suitably lined aluminum containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Oxystearin

DESCRIPTION

Oxystearin is a mixture of the glycerides of partially oxidized stearic and other fatty acids. It occurs as a tan to light brown, fatty or waxlike substance having a bland taste. It is soluble in ether, in solvent hexane, and in chloroform.

REQUIREMENTS

Acid Value Not more than 15.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Hydroxyl Value Between 30 and 45.
Iodine Value Not more than 15.
Refractive Index (butyro) Between 59 and 61 at 48° (equivalent to 1.465–1.467 on the Abbé scale.)
Saponification Value Between 225 and 240.
Unsaponifiable Matter Not more than 0.8%.

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504, using about 5 g, accurately weighed.

Iodine Value Determine by the *Wijs Method*, page 505.

Refractive Index, page 533 Melt the sample, filter through filter paper, and determine the refractive index at 48° with an Abbé or butyro refractometer.

Saponification Value Determine as directed in the general method, page 501, using about 3 g, accurately weighed.

Unsaponifiable Matter Determine as directed in the general method, page 509.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Crystallization inhibitor in salad and cooking oils; sequestrant; defoaming agent.

Palmarosa Oil

Geranium Oil, East Indian Type; Geranium Oil, Turkish Type

DESCRIPTION

The volatile oil obtained by steam distillation from the partially dried grass *Cymbopogon martini* Stapf. var. *motia*. It is a light yellow to yellow oil that is often hazy and brownish. It is soluble in most fixed oils and in propylene glycol. It is soluble, usually with opalescence or turbidity, in mineral oil. It is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 603, using the same test conditions as specified therein.

Assay for Alcohols Not less than 88.0% and not more than 94.0% of total alcohols, calculated as geraniol ($C_{10}H_{18}O$).

Assay for Esters Not less than 4.0% and not more than 18.0% of esters, calculated as geranyl acetate ($C_{12}H_{20}O_2$).

Angular Rotation Between −2° and +3°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.473 and 1.478 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.879 and 0.892.

TESTS

Assay for Alcohols Proceed as directed under *Total Alcohols*, page 499. Weigh accurately about 1 g of the acetylated oil for the saponification, and use 77.13 as the equivalence factor (*e*) in the calculation.

Assay for Esters Weigh accurately about 5 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method. One ml dissolves in 2 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Palmitic Acid

Hexadecanoic Acid

$C_{16}H_{32}O_2$ Mol wt 256.43

DESCRIPTION

A mixture of solid organic acids obtained from fats consisting chiefly of palmitic acid ($C_{16}H_{32}O_2$) with varying amounts of stearic acid ($C_{16}H_{36}O_2$). It occurs as a hard, white or faintly yellowish, somewhat glossy crystalline solid, or as a white or yellowish white powder. It has a slight characteristic odor and taste. Palmitic acid is practically insoluble in water. It is soluble in alcohol, in ether, and in chloroform. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Acid Value Between 204 and 220.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Iodine Value Not more than 2.0.

Residue on Ignition Not more than 0.1%.
Saponification Value Between 205 and 221.
Titer (Solidification Point) Between 53.3° and 62°.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.
Saponification Value Determine as directed in the general method, page 509, using about 3 g, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.
Unsaponifiable Matter, page 509 Determine as directed in the general method.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Component in the manufacture of other food-grade additives; defoaming agent.

DL-Panthenol

DL-Pantothenyl Alcohol; Racemic Pantothenyl Alcohol

$$HOCH_2C(CH_3)_2CH(OH)CONH(CH_2)_2CH_2OH$$

$C_9H_{19}NO_4$ Mol wt 205.25

DESCRIPTION

A racemic mixture of the dextrorotatory (active) and levorotatory (inactive) isomers of panthenol, the alcohol analogue of pantothenic acid. It occurs as a white to creamy white, crystalline powder having a slight, characteristic odor. Its solutions are neutral or alkaline to litmus. It is freely soluble in water, in alcohol, and in propylene glycol. It is soluble in chloroform and in ether, and is slightly soluble in glycerin. [NOTE: The physiological activity of DL-panthenol is one-half that of dexpanthenol (D-panthenol).]

REQUIREMENTS

Identification

It responds to the *Identification Tests* under *Dexpanthenol*, page 95.

Assay Not less than 99.0% and not more than 102.0% of $C_9H_{19}NO_4$ (DL-panthenol), calculated on the dried basis.
Aminopropanol Not more than 0.1%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Melting Range Between 64.5° and 68.5°.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 400 mg, accurately weighed, into a 300-ml reflux flask fitted with a standard-taper glass joint, add 50.0 ml of 0.1 *N* perchloric acid in glacial acetic acid, and reflux for 5 h. Cool, covering the condenser with foil to prevent contamination by moisture, and rinse the condenser with glacial acetic acid. Add 5 drops of crystal violet TS, and titrate with 0.1 *N* potassium acid phthalate in glacial acetic acid to a blue green endpoint. Perform a blank determination, and make any necessary correction (see page 2). Each ml of 0.1 *N* perchloric acid is equivalent to 20.53 mg of $C_9H_{19}NO_4$.
Aminopropanol Transfer about 10 g of the sample, accurately weighed, into a 50-ml flask, and dissolve in 25 ml of water. Add bromothymol blue TS, and titrate with 0.01 *N* sulfuric acid from a microburet to a yellow endpoint. Each ml of 0.01 *N* sulfuric acid is equivalent to 0.75 mg (750 μg) of aminopropanol.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 56° for 4 h in vacuum over phosphorus pentoxide.
Melting Range Determine as directed in the general procedure, page 519.
Residue on Ignition Ignite a 1-g sample as directed in the general method, page 533.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Paraffin, Synthetic

Fischer-Tropsch Paraffin

DESCRIPTION

A very hard, white, practically tasteless and odorless wax. It is synthesized by the Fischer-Tropsch process from carbon monoxide and hydrogen, which are catalytically converted to a mixture of paraffin hydrocarbons; the lower molecular weight fractions are removed by distillation, and the residue is hydrogenated and further treated by percolation through activated charcoal. It is soluble in hot hydrocarbon solvents.

REQUIREMENTS

Identification

Identify synthetic paraffin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 718. The sample is melted and prepared for analysis on a cesium bromide plate.

Absorptivity Less than 0.01 at 290 nm, in decahydronaphthalene at 88°C (190°F).

Arsenic (as As) Not more than 3 ppm.

Congealing Point Between 200° and 210°F (93.3° and 98.9°C).

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 3 ppm.

Oil Content Not more than 0.50%.

TESTS

Absorptivity Transfer about 100 mg of the sample, accurately weighed, into a 100-ml volumetric flask, dissolve in decahydronaphthalene at 88°C (190°F), dilute to volume at this temperature, and mix. Determine the absorbance of this solution in a 10-cm cell at 290 nm with a suitable spectrophotometer, the cell holders of which shall maintain the temperature of the sample cell and the reference cell at 88°C. Use decahydronaphthalene at 88°C in a matched cell as the blank. Cell lengths should be known to within ±0.5% or better of the nominal pathlength. Calculate the absorptivity (a) of the sample solution by the formula A/bc, in which A is the absorbance of the sample solution, corrected for the solvent blank; b is the exact pathlength of the sample cell, in cm; and c is the exact concentration of the sample solution, in g per l. [NOTE: A suitable spectrophotometer, as applied in this test, is an accurately calibrated instrument capable of measuring absorbance with a repeatability of ±0.1% or better from an average of 0.4 absorbance level at 290 nm; it has a spectral bandwidth of 2 nm or less, and wavelength measurements made with it shall be repeatable within ±0.2 nm.]

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Congealing Point

Definition The temperature at which the molten sample, when allowed to cool under the prescribed conditions, ceases to flow.

Thermometer Jacket Assembly Use an ASTM Congealing Point Thermometer having a range of 68° to 213°F and conforming to the requirements for an ASTM 54 F thermometer (see page 547). By means of a cork, fit the thermometer into a jacket consisting of a 1-oz glass vial, 25 mm in diameter and 55 mm in height, and adjust the thermometer so that the bottom of the bulb is 10 to 15 mm from the bottom of the vial.

Procedure Place a sample, of sufficient size to represent exactly the material under test, in a casserole or other suitable dish, and heat slowly in a water bath to a temperature approximately 15°F above the expected congealing point. Heat the *Thermometer Jacket Assembly* to approximately the same temperature as the prepared sample. When both the sample and the assembly have reached the required temperature, remove the assembly from the bath, then immediately remove the thermometer from its jacket, and immerse the thermometer bulb into the molten sample until the bulb is completely covered, taking care not to cover any part of the thermometer stem with the sample. As rapidly as possible, remove the thermometer and any adhering sample from the sample dish and place the thermometer in the jacket, holding both the thermometer and its jacket in a horizontal position during this operation. Rotate the thermometer in a horizontal position at the rate of approximately one revolution in 2 s, pausing momentarily at the completion of each revolution to inspect the drop of sample on the thermometer bulb. When the drop is observed to rotate with the bulb, read the thermometer, and record the reading as the congealing point, reported to the nearest 0.5°F.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Oil Content Determine as directed in the general method, page 525.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Parsley Herb Oil

DESCRIPTION

The oil obtained by steam distillation of the aboveground parts of the plant *Petroselinium sativum* Hoffm. (Fam. *Umbelliferae*), including the immature seed. It is a yellow to light brown liquid

having the odor of parsley herb. It is soluble in most fixed oils, in mineral oil, and in alcohol (with opalescence). It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 603, using the same test conditions as specified therein.

Acid Value Not more than 2.0.
Angular Rotation Between $+1°$ and $-9°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.503 and 1.530 at 20°.
Specific Gravity Between 0.908 and 0.940.

TESTS

Acid Value Determine as directed in the general method, page 499.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Parsley Seed Oil

DESCRIPTION

The oil obtained by steam distillation of the ripe seed of *Petroselinum sativum* Hoffm. (Fam. *Umbelliferae*). It is a yellow to light brown liquid having a rather harsh odor. It is soluble in most fixed oils and in mineral oil. It is slightly soluble in propylene glycol, but it is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 603, using the same test conditions as specified therein.

Acid Value Not more than 4.0.
Angular Rotation Between $-4°$ and $-10°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.513 and 1.522 at 20°.
Saponification Value Between 2 and 10.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.040 and 1.080.

TESTS

Acid Value Determine as directed in the general method, page 503.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 6 ml of 80% alcohol, occasionally with slight haziness.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, preferably glass, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Pectin

DESCRIPTION

Pectin is a purified carbohydrate polymer generally obtained by dilute-acid extraction of citrus albedo or apple pomace. It usually occurs as a practically odorless, yellowish white, coarse to fine powder having a mucilagenous taste. It dissolves in water, forming an opalescent colloidal solution. It is practically insoluble in alcohol.

The major part of the pectin chain is composed of $\alpha(1 \rightarrow 4)$-linked D-galacturonic acid units. Some of the carboxyl groups are esterified with methyl alcohol, while the remaining carboxylic units exist in the free acid form or as ammonium, potassium, or sodium salts.

Pectin is usually classified according to the degree of esterification. In *high-ester* pectin, a significant portion of the

carboxyl groups are in the form of methyl esters. Useful properties may differ with the degree of esterification and with the degree of polymerization. In *low-ester* pectin, a portion of the methyl esters may have been converted to primary amides. Both forms of pectin vary in their jellification properties, and therefore commercial pectin products are normally diluted with sugars for standardization to produce, for example, "150 jelly grade" *high-ester* pectin (see *FCC II*, pages 581–582 for test method) for use in jams and jellies, or "100 gel power" *low-ester* pectin for use in low-sugar gels. In addition to sugars, suitable food-grade buffer salts may be added for pH control and to achieve desirable setting characteristics.

NOTE: The following REQUIREMENTS and TESTS apply to the pectin as supplied, whether standardized or not, except for specifications covering the degrees of esterification and amide substitution, and the weight percent of total galacturonides in the pectin component, in which cases the test procedures provide for removing the sugars and soluble salts before analysis of the pectin component.

REQUIREMENTS

Identification

A. To a 1 in 100 solution of the sample in water add an equal volume of alcohol. A translucent, gelatinous precipitate is formed (difference from most gums).

B. To 10 ml of a 1 in 100 solution of the sample in water add 1 ml of thorium nitrate solution (1 in 10), stir, and allow to stand for 2 min. A stable precipitate or gel forms (difference from most gums).

C. To 5 ml of a 1 in 100 solution of the sample in water add 1 ml of sodium hydroxide TS, and allow to stand at room temperature for 15 min. A gel or semigel forms (difference from tragacanth and other gums).

D. Acidify the gel from *Identification Test C* with 1 ml of hydrochloric acid TS, and shake well. A voluminous, colorless, gelatinous precipitate forms, which, upon boiling, becomes white and flocculent (pectic acid).

Acid-Insoluble Ash Not more than 1%.

Arsenic (as As) Not more than 3 ppm.

Ash (Total) Not more than 10%.

Degree of Esterification of High-Ester Pectin Component Not less than 50%.

Degree of Esterification of Low-Ester Pectin Component Not more than 50%.

Degree of Amide Substitution of Low-Ester Pectin Component Not more than 40%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 12%.

Sodium Methyl Sulfate Not more than 0.1%.

Total Anhydrogalacturonides in Pectin Component Not less than 70%.

TESTS

Acid-Insoluble Ash Determine as directed in the general method, page 466.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Total) Determine as directed in the general method, page 466.

Degree of Esterification, Degree of Amide Substitution, and Total Anhydrogalacturonides in the Pectin Component Transfer 5.0 g of the pectin, as supplied, into a beaker, and stir for 10 min with a mixture of 5 ml of conc. hydrochloric acid and 100 ml of 60% isopropyl alcohol. Filter through a dry, coarse sintered-glass filter tube (30- to 60-ml capacity), and wash with six 15-ml portions of the acid-alcohol mixture, followed by 60% isopropyl alcohol until the filtrate is free from chloride. Finally, wash with 20 ml of anhydrous isopropyl alcohol, dry at 105° for 2.5 h, cool, and weigh.

Transfer 500.0 mg of the washed and dried sample into a 250-ml Erlenmeyer flask, and moisten with 2 ml of alcohol. Add 100 ml of carbon dioxide–free water, stopper, and swirl occasionally until the sample is completely hydrated. Add 5 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide, recording the volume required as V_1 (initial titer), in ml. Add 20.0 ml of 0.5 *N* sodium hydroxide, stopper, shake vigorously, and allow to stand for 15 min. Add 20.0 ml of 0.5 *N* hydrochloric acid, shake until the pink color disappears, then add 3 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a faint pink color that persists after vigorous shaking. Record the volume of 0.1 *N* sodium hydroxide required as V_2 (saponification titer), in ml.

Quantitatively transfer the contents of the flask into a 500-ml distillation flask fitted with a Kjeldahl trap and a water-cooled condenser, the delivery tube of which extends well beneath the surface of a mixture of 150 ml of carbon dioxide–free water and 20.0 ml of 0.1 *N* hydrochloric acid in a receiving flask. To the distillation flask add 20 ml of sodium hydroxide solution (1 in 10), seal the connections, and begin heating carefully to avoid excessive foaming. Continue heating until 80 to 120 ml of distillate has been collected. Add a few drops of methyl red TS to the receiving flask, and titrate the excess acid with 0.1 *N* sodium hydroxide, recording the volume required as *S*, in ml. Perform a blank determination on 20.0 ml of 0.1 *N* hydrochloric acid, and record the volume required as *B*, in ml. Record the amide titer ($B - S$) as V_3, and the total titer ($V_1 + V_2 + V_3$) as V_t. (NOTE: If the pectin is known to be of the non-amidated type, only V_1 and V_2 need be determined and V_3 may be regarded as zero.)

Calculate the degree of esterification by the formula $100 \times V_2/V_t$.

Calculate the degree of amide substitution by the formula $100 \times V_3/V_t$.

Calculate the weight percent of total anhydrogalacturonides by the formula $3.52V_1 + 3.80V_2 + 3.5V_3$.

Heavy Metals Prepare and test a 500-mg sample as directed

in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Sodium Methyl Sulfate

Barium Molybdate Dissolve 29.0 g of sodium molybdate, $Na_2MoO_4.2H_2O$, and 24.0 g of barium chloride, $BaCl_2.2H_2O$, in separate 1000-ml volumes of water, heat both solutions to 70°–80°, and then slowly add the barium chloride solution to the sodium molybdate solution while stirring. Allow the precipitate to settle, decant off the liquid, and wash the precipitate with three 100-ml portions of warm (70°) water, decanting each washing. Dissolve the precipitate in 200 ml of 2 *N* hydrochloric acid, dilute to about 1000 ml with water, add 1 ml of bromothymol blue TS, and mix. Heat to 70°–80°, add 150 ml of 2 *N* ammonia, with mixing, and titrate with 2 *N* ammonia to a green endpoint. Remove the liquid by decantation, and wash the precipitate with three 100-ml portions of water, decanting each washing through a Buchner funnel. Mix the precipitate with 100 ml of water, and pour the mixture into the funnel. Wash the filter cake with several portions of water, and dry at 110° overnight.

Buffer Solution Dissolve 31.0 g of boric acid and 8.55 g of sodium chloride in sufficient water to make 1000 ml.

Sodium Sulfate Solution Transfer 100.0 mg of anhydrous sodium sulfate into a 100-ml volumetric flask, dissolve in water, dilute to volume with water, and mix.

Standard Preparations Transfer 1.0, 2.0, 3.0, 4.0, and 5.0 ml of the *Sodium Sulfate Solution* into separate 100-ml volumetric flasks, add 1 ml of 70% perchloric acid and 2 to 3 drops of paranitrophenol solution (1 in 1000 in methanol) to each flask, and then add ammonium hydroxide, dropwise, to the first appearance of a yellow color. Add just sufficient dilute hydrochloric acid (1 in 50) to discharge the yellow color, then add 10.0 ml of the *Buffer Solution* and 50.0 ml of methanol, dilute to volume with water, and mix. Continue with each standard as directed under *Sample Preparation*, beginning with "Pipet 20.0 ml of this solution into a 125-ml Erlenmeyer flask. . . . "

Sample Preparation Transfer 5.00 g of the sample into a 100-ml beaker, mix well with about 5 g of powdered cellulose, and transfer the mixture into a 22- × 80-mm extraction thimble. Plug the thimble with cotton, and extract overnight on a steam bath in a suitable continuous-extraction apparatus, using 100 ml of a 3:1 mixture of methanol-chloroform as the solvent. Add 500 mg each of barium carbonate and sodium bicarbonate to the extract, and shake mechanically at medium agitation for 1 h. Filter through Whatman No. 40 or equivalent paper into a 125-ml long-neck boiling flask, and wash the flask and filter with two 10-ml portions of chloroform, taking precautions to ensure that any solids do not pass into the filtrate. If it is turbid, filter the filtrate again. Add a glass bead to the boiling flask, evaporate the filtrate to dryness on a steam bath, and cool. Rinse the sides of the flask with 10 ml of nitric acid and 1 ml of 70% perchloric acid, and evaporate at high heat until the dense white perchloric acid fumes just disappear from the bowl of the flask. Cool, rinse the sides of the flask with 10 ml of water, add 2 to 3 drops of paranitrophenol solution (1 in 1000 in methanol), and then add ammonium hydroxide, dropwise, to the first appearance of a yellow color. Add just sufficient dilute hydrochloric acid (1 in 50) to discharge the yellow color, and then transfer the solution into a 100-ml volumetric flask with the aid of 10.0 ml of the *Buffer Solution* and 10 ml of water. Add 50.0 ml of methanol to the volumetric flask, dilute to volume with water, and mix.

Pipet 20.0 ml of this solution into a 125-ml Erlenmeyer flask containing 200 mg of *Barium Molybdate*. Stopper, shake for 1 h, and filter through Whatman No. 40 or equivalent filter paper into a 50-ml Erlenmeyer flask. Pipet 10.0 ml of the filtrate into a 50-ml volumetric flask, and add 10 ml of water, 7 ml of hydrochloric acid, 3 ml of potassium thiocyanate solution (1 in 10), and 15 ml of acetone. Mix well, then heat at 60° to 70° in a water bath for 30 min, cool, dilute to volume with water, and mix.

Run a blank determination with 5 g of powdered cellulose in the same manner, using the same quantities of the same reagents as in the treatment of the sample.

Procedure Determine the absorbance of the five *Standard Preparations* and of the *Sample Preparation*, against the blank, in 1-cm cells at 460 nm, using a suitable spectrophotometer. Plot the absorbances of the *Standard Preparation* versus ml of *Sodium Sulfate Solution* used in preparing each standard, and then convert the volumes to percentages of sodium methyl sulfate, each 1.0 ml of *Sodium Sulfate Solution* being equivalent to 0.0188% of sodium methyl sulfate. From the absorbance of the *Sample Preparation*, determine the percentage of sodium methyl sulfate in the 5-g pectin sample taken for analysis by means of the standard curve.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Gelling agent; thickener; stabilizer; emulsifier.

Pennyroyal Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh or partly dried plant *Mentha pulegium* L. (Fam. *Labiatae*). It is a light yellow to yellow aromatic liquid having a mintlike odor. It is soluble in most fixed oils and in propylene glycol. It is soluble, with slight cloudiness, in mineral oil, but it is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths

(or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 603, using the same test conditions as specified therein.

Assay Not less than 88% and not more than 96%, by volume, of ketones.
Angular Rotation Between +18° and +25°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.483 and 1.488 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.928 and 0.940.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or suitably galvanized containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Pentaerythritol Ester of Partially Hydrogenated Wood Rosin

DESCRIPTION

A hard, amber-colored resin (color K or paler as determined by ASTM Designation D 509) produced by the esterification of partially hydrogenated wood rosin with pentaerythritol and purified by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify pentaerythritol ester of partially hydrogenated wood rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 718. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 7 and 18.
Arsenic (as As) Not more than 3 ppm.
Drop Softening Point Between 102° and 110°.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.

TESTS

Acid Value Determine as directed in the general procedure, page 533.
Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Drop Softening Point Determine as directed in the general procedure, page 534, using a bath temperature of 120°.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Masticatory substance in chewing gum base.

Pentaerythritol Ester of Wood Rosin

DESCRIPTION

A hard, pale, amber-colored resin (color M or paler as determined by ASTM Designation D 509) produced by the esterification of pale wood rosin with pentaerythritol and purified by steam stripping. It is soluble in acetone and in benzene, but is insoluble in water and in alcohol.

REQUIREMENTS

Identification

Identify pentaerythritol ester of wood rosin by comparing its infrared absorption spectrum with a typical spectrum as shown on page 718. The sample is melted and prepared for analysis on a cesium bromide plate.

Acid Value Between 6 and 16.
Arsenic (as As) Not more than 3 ppm.
Drop Softening Point Between 109° and 116°.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.

TESTS

Acid Value Determine as directed in the general procedure, page 533.

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Drop Softening Point Determine as directed in the general procedure, page 534, using a bath temperature of 125°.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Peppermint Oil

DESCRIPTION

An essential oil obtained by steam distillation from the fresh overground parts of the flowering plant of *Mentha piperita* L. (Fam. *Labiatae*); it may be rectified by distillation, but is neither partially nor wholly dementholized. It is a colorless or pale yellow liquid having a strong, penetrating odor of peppermint and a pungent taste, followed by a sensation of coldness when air is drawn into the mouth.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 604, using the same test conditions as specified therein.

B. Mix in a dry test tube 3 drops of the oil with 5 ml of a solution of nitric acid in glacial acetic acid (1 in 300), and place the tube in a beaker of boiling water. A blue color develops within 5 min, which, on continued heating, deepens and shows a copper-colored fluorescence, and then fades, leaving a golden yellow solution.

Assay for Total Esters Not less than 5.0% of esters, calculated as menthyl acetate ($C_{12}H_{22}O_2$).

Assay for Total Menthol Not less than 50.0% of menthol ($C_{10}H_{20}O$).

Angular Rotation Between −18° and −32°.

Dimethyl Sulfide Passes test (rectified); fails test (natural).

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.459 and 1.465 at 20°.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.896 and 0.908.

TESTS

Assay for Total Esters Weigh accurately about 10 g, and proceed as directed under *Ester Determination*, page 500, using 99.16 as the equivalence factor (*e*) in the calculation.

Assay for Total Menthol Proceed as directed under *Total Alcohols*, page 499, using a 2.5-g sample of the acetylized oil. Calculate the percentage of total menthol by the formula

$$7.814A(0.0021E)/(B - 0.021A),$$

in which A is the difference between the number of ml of 0.5 N hydrochloric acid required in the titration and the number of ml of 0.5 N hydrochloric acid required in the residual blank titration, B is the weight of the sample of the acetylized oil, and E is the percentage of total esters determined and calculated as menthyl acetate ($C_{12}H_{22}O_2$).

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Dimethyl Sulfide Distil 1 ml from a sample of 25 ml, and carefully superimpose the distillate on 5 ml of mercuric chloride TS in a test tube. A white film does not form at the zone of contact within 1 min if the sample is rectified.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 70% alcohol.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Labeling Label peppermint oil to indicate whether it is natural or rectified.

Functional Use in Foods Flavoring agent.

Perlite

Expanded Perlite

DESCRIPTION

In its natural state, perlite occurs as a dense, gray to brown, glassy volcanic rock consisting essentially of fused sodium potassium aluminum silicate plus 3% to 5% water. When

fractured and heated at high temperature (900° to 1100°) under proper conditions, it pops like popcorn (due to the presence of the occluded water), expanding to 20 or more times its original volume. The expanded material is crushed to yield a white, nonhygroscopic powder having a bulk density of 32 to 400 kg/m^3 (2 to 25 lb/ft^3) and a particle size ranging from less than one to several hundred μm. It is in this latter expanded and powdered state that perlite is used as a filter aid in food processing. The powder is slightly soluble in water and sparingly soluble in dilute acids and alkalies.

REQUIREMENTS

Identification

A. Mix about 1 g of the sample with 25 ml of diluted hydrochloric acid TS in a beaker, cover with a watch glass, heat on a steam bath for 15 min, and cool. Filter, and neutralize the filtrate to litmus paper with ammonia TS. The neutralized filtrate gives positive tests for *Aluminum*, page 515, for *Potassium*, page 517, and for *Sodium*, page 517.

B. Prepare a bead by fusing a few crystals of sodium ammonium phosphate on a platinum loop in the flame of a burner. Place the hot, transparent bead in contact with a sample, and again fuse. Silica floats about in the bead, producing, upon cooling, an opaque bead with a weblike structure.

Arsenic (as As) Not more than 10 ppm.
Lead Not more than 10 ppm.
Loss on Drying Not more than 3% (powdered form).
Loss on Ignition Not more than 7% (glassy form).
pH Between 5 and 9 (filtrate from a 10% suspension).

TESTS

Arsenic Transfer 10.0 g of the sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat at 70° for 15 min. Cool, and decant through a Whatman No. 3 or equivalent filter paper into a 100-ml volumetric flask. Wash the slurry with three 10-ml portions of hot water and the filter paper with 15 ml of hot water, dilute the solution to volume with water, and mix. A 3.0-ml portion of this solution meets the requirements of the *Arsenic Test*, page 464.

Lead A 10.0-ml portion of the solution prepared in the *Arsenic Test* meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry the powder at 105° for 2 h.

Loss on Iguition Ignite a 250-mg crushed sample of the glassy form to constant weight at 1000°.

pH, page 531 Boil 10 g with 100 ml of water for 30 min, make up to 100 ml with water, and filter through a fine-pore sintered-glass funnel. Use the resulting filtrate for the determination of pH.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Filter aid in food processing.

Petitgrain Oil, Paraguay Type

DESCRIPTION

The volatile oil obtained by steam distillation from the leaves and small twigs of the bitter orange tree, *Citrus aurantium* L. subspecies *amara*. It is a yellow to brownish yellow liquid having a somewhat harsh, bitter-sweet, floral odor. It is soluble in most fixed oils and is soluble, with opalescence or turbidity, in mineral oil and in propylene glycol. It is relatively insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 604, using the same test conditions as specified therein.

Assay Not less than 45.0% and not more than 60% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −4° and +1°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.455 and 1.462 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.878 and 0.889.

TESTS

Assay Weigh accurately about 2 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 70% alcohol. The solution usually develops opalescence or turbidity upon further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or aluminum containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Petrolatum

White Petrolatum; Yellow Petrolatum

DESCRIPTION

A purified mixture of semisolid hydrocarbons obtained from petroleum, occurring as an unctuous mass, and varying in color from white to yellowish or light amber. It is transparent in thin layers, and has not more than a slight fluorescence, even after being melted. It is insoluble in water, and is almost insoluble in cold or hot alcohol and in cold absolute alcohol. It is soluble in ether, in solvent hexane, and in most fixed and volatile oils, and is freely soluble in benzene, in carbon disulfide, in chloroform, and in turpentine oil. It may contain any antioxidant permitted by the federal Food and Drug Administration, in an amount not greater than that required to produce its intended effect.

REQUIREMENTS

Acidity or Alkalinity Passes test.
Color Passes test.
Consistency Passes test (between 100 and 275).
Fixed Oils, Fats, and Rosin Passes test.
Melting Range Between 38° and 60°.
Organic Acids Passes test.
Residue on Ignition Passes test.
Specific Gravity Between 0.815 and 0.880 at 60°.
Ultraviolet Absorption (polynuclear hydrocarbons) Passes test.

TESTS

Acidity or Alkalinity Introduce 35 g of the sample into a 250-ml separator, add 100 ml of boiling water, and shake vigorously for 5 min. After the petrolatum and water have separated, draw off the water into a casserole, wash the sample in the separator with two 50-ml portions of boiling water, and add the washings to the casserole. To the accumulated 200 ml of water add 1 drop of phenolphthalein TS, and boil. The solution does not acquire a pink color. If the addition of phenolphthalein produces no pink color, add 0.1 ml of methyl orange TS. No red or pink color is produced.

Color Melt about 10 g on a steam bath, and pour about 5 ml of the liquid into a 150- × 50-mm clear-glass bacteriological test tube, keeping the sample melted. The petrolatum is not darker than a solution made by mixing 3.8 ml of ferric chloride CS and 1.2 ml of cobaltous chloride CS in a similar tube, the comparison of the two being made in reflected light against a white background, holding the sample tube directly against the background at such an angle that there is no fluorescence.

Consistency

Apparatus Determine the consistency of petrolatum by means of a penetrometer fitted with a polished cone-shaped metal plunger weighing 150 g, having a detachable steel tip of the following dimensions: the tip of the cone has an angle of 30°, the point being truncated to a diameter of 0.38 ± 0.03 mm, the base of the tip is 8.38 ± 0.05 mm in diameter, and the length of the tip is 15 ± 0.25 mm. The remaining portion of the cone has an angle of 90°, is 28.2 mm in height, and has a maximum diameter at the base of 65.1 mm. The containers for the test are flat-bottomed metal or glass cylinders that are 102 ± 6 mm in diameter and not less than 60 mm in height.

Procedure Melt a quantity of the sample at 82° ± 2.5°, and pour into one or more of the containers, filling to within 6 mm of the rim. Cool at 25° ± 2.5° over a period of not less than 16 h, protecting from drafts. Two h before the test, place the containers in a water bath at 25° ± 0.5°. If the room temperature is below 23.5° or above 26.5°, adjust the temperature of the cone to 25° ± 0.5° by placing it in the water bath.

Without disturbing the surface of the sample, place the container on the penetrometer table, and lower the cone until the tip just touches the top surface of the sample at a spot 25 to 38 mm from the edge of the container. Adjust the zero setting, and quickly release the plunger, then hold it free for 5 s. Secure the plunger, and read the total penetration from the scale. Make 3 or more trials, each so spaced that there is no overlapping of the areas of penetration. When the penetration exceeds 20 mm, use a separate container of the sample for each trial. Read the penetration to the nearest 0.1 mm. Calculate the average of the three or more readings, and conduct further trials to a total of 10 if the individual results differ from the average by more than ± 3%. The final average of the trials is not less than 10.0 mm and not more than 27.5 mm, indicating a consistency value between 100 and 275.

Fixed Oils, Fats, and Rosin Digest 10 g of the sample at 100° with 10 g of sodium hydroxide and 50 ml of water for 30 min. Separate the water layer, and add to it an excess of diluted sulfuric acid TS. No oily or solid matter separates.

Melting Range, page 520 Determine as directed in *Procedure for Class III.*

Organic Acids Weigh 20 g of the sample, add 50 ml of alcohol, previously neutralized to phenolphthalein TS with sodium hydroxide, and 50 ml of water, agitate thoroughly, and heat to boiling. Add 1 ml of phenolphthalein TS, and titrate rapidly, with vigorous agitation, to the production of a sharp pink endpoint, noting the change in the alcohol-water layer. Not more than 0.4 ml of 0.1 *N* sodium hydroxide is required.

Residue on Ignition Heat 4 g of the sample in an open porcelain or platinum dish over a Bunsen flame. It volatilizes without emitting any acrid odor, and on ignition yields not more than 0.05% of residue.

Specific Gravity Determine by any reliable method (see page 3).

Ultraviolet Absorption It meets the ultraviolet absorbance specifications required by the federal Food and Drug Administration for petrolatum.

Packaging and Storage Store in tight containers.
Functional Use in Foods Defoaming agent; lubricant; protective coating; release agent.

Petroleum Wax

Refined Paraffin Wax; Microcrystalline Wax

DESCRIPTION

A refined mixture of solid hydrocarbons, paraffinic in nature, obtained from petroleum. It occurs as a translucent, tasteless, and odorless wax. It may be prepared as "refined paraffin wax" or as "microcrystalline wax." The refined wax is usually obtained from a lower molecular weight fraction of petroleum and has lower viscosities when molten than the microcrystalline wax. The microcrystalline wax is usually higher in molecular weight, in flash point, and in melting point than the refined wax. These waxes are graded and sold according to melting point, which ranges from about 120° to 200°F (48° to 93°C), and color, which varies from amber to almost white. They exhibit a low order of solubility in organic solvents, but are most soluble in aromatic hydrocarbons and least soluble in ketones, esters, and alcohols.

REQUIREMENTS

Identification

Identify refined petroleum wax and microcrystalline wax by comparing their infrared absorption spectra with the respective typical spectra as shown on page 719. The samples are melted and prepared for analysis on cesium bromide plates.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 3 ppm.
Ultraviolet Absorbance (polynuclear hydrocarbons) 280–289 nm, not more than 0.15; 290–299 nm, not more than 0.12; 300–359 nm, not more than 0.08; 360–400 nm, not more than 0.02.

The following additional specifications, where applicable, should conform to the representations of the vendor: **Color**, **Melting Point**, and **Odor**.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Color Determine by any suitable procedure, such as ASTM D 1500–58 T.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) as the control (*Solution A*).
Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Melting Point Determine by any suitable procedure, such as ASTM D 127–49.
Odor Determine by any suitable procedure, such as ASTM D 1833.
Ultraviolet Absorbance Determine as directed in the federal food additive regulation for Petroleum Wax (21 CFR 121.1156).

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Masticatory substance in chewing gum base; protective coating; defoaming agent.

Petroleum Wax, Synthetic

Synthetic Wax (Ethylene Polymer)

DESCRIPTION

A refined mixture of solid hydrocarbons, paraffinic in nature, prepared by the catalytic polymerization of ethylene. Synthetic petroleum wax ranges in melting point from about 185° to 240°F (85° to 116°C). The color of this wax varies from amber to almost white. It is most soluble in aromatic hydrocarbons and least soluble in ketones, esters, and alcohols.

REQUIREMENTS

Identification

Identify synthetic petroleum wax by comparing its infrared absorption spectrum with a typical spectrum as shown on page 719. The sample is melted and prepared for analysis on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 3 ppm.
Molecular Weight (average) Between 500 and 1200.
Ultraviolet Absorbance (polynuclear hydrocarbons) 280–289 nm, not more than 0.15; 290–299 nm, not more than 0.12; 300–359 nm, not more than 0.08; 360–400 nm, not more than 0.02.

The following additional specifications, where applicable, should conform to the representations of the vendor: **Color**, **Melting Point**, and **Odor**.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.
Color Determine by any suitable procedure, such as ASTM D 1500–58 T.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Melting Point Determine by any suitable procedure, such as ASTM D 127–49.

Molecular Weight

Apparatus Use a suitable vapor pressure osmometer, such as the Hewlett-Packard Model 302A or equivalent, equipped with dual thermistor beads.

Calibration Standards Dissolve accurately weighed amounts of benzil ($C_6H_5COCOC_6H_5$) in *o*-dichlorobenzene to produce solutions containing approximately 3, 7, 10, and 15 mg of benzil, respectively, per g of solution, and heat to 100° on a steam bath.

Sample Preparations Dissolve accurately weighed amounts of the sample in *o*-dichlorobenzene to produce solutions containing approximately 10, 20, 35, and 50 mg of sample, respectively, per g of solution, and heat to 100° on a steam bath. (Other suitable concentrations that give ΔR readings between 5 and 25 may be used in the *Procedure* below.)

Procedure Following the manufacturer's instructions, balance the osmometer to zero with *o*-dichlorobenzene on both thermistor beads, and establish the calibration constant K_S at 100°, using the four *Calibration Standards*. When the temperature within the osmometer has re-equilibrated to 100°, place an aliquot of the most concentrated *Sample Preparation* on the sample thermistor bead. After 4.0 min, balance the instrument to zero with the potentiometer, and record the ΔR value. Repeat this procedure with the same *Sample Preparation* two or three times, and average the ΔR values for that concentration. In a similar manner, obtain the average ΔR values for each of the other three concentrations of the *Sample Preparation*. Plot the four average ΔR values for the *Sample Preparations* as a function of ΔR/concentration, and extrapolate the line to zero to obtain the constant K_U for the sample. Divide K_S by K_U to obtain the molecular weight of the sample tested.

Odor Determine by any suitable procedure, such as ASTM D 1833.

Ultraviolet Absorbance Determine as directed in the federal food additive regulation for Petroleum Wax (21 CFR 121.1156).

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base; protective coating; defoaming agent.

DL-Phenylalanine

DL-α-Amino-β-phenylpropionic Acid

$C_6H_5CH_2CH(NH_2)COOH$

$C_9H_{11}NO_2$ Mol wt 165.19

DESCRIPTION

White, odorless, crystalline platelets. It is soluble in water, in dilute mineral acids, and in solutions of alkali hydroxides. It is very slightly soluble in alcohol. It is optically inactive.

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS. A reddish purple color is produced.
B. Heat 5 ml of a 1 in 100 solution with a few drops of potassium dichromate TS. A characteristic odor is evolved.
C. To a 10-mg sample add 500 mg of potassium nitrate and 2 ml of sulfuric acid, and heat the mixture on a water bath for 20 min. Cool, add 2 ml of hydroxylamine TS, immerse in ice water for 10 min, and then add 10 ml of sodium hydroxide TS. A reddish violet color is produced.

Assay Not less than 98.0% and not more than 102.0% of $C_9H_{11}NO_2$ after drying.

Ammonium Salts (as NH_3) Not more than 0.03%.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.02%.

Heavy Metals (as Pb) Not more than 0.002%.

Iron Not more than 0.005%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Nitrogen (Total) Between 8.3% and 8.65%.

Residue on Ignition Not more than 0.3%.

Sulfate Not more than 0.04%.

TESTS

Assay Transfer about 500 mg, previously dried at 105° for 2 h and accurately weighed, into a 250-ml flask. Dissolve the sample in 75 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

Ammonium Salts

Ammonium Chloride Standard Solution Dissolve 78.5 mg of ammonium chloride in sufficient water to make 250.0 ml, and dilute 10.0 ml of this solution to 100.0 ml with water. The dilute solution contains the equivalent of 10 µg of NH_3 in each ml.

Procedure Place 5 g of sodium hydroxide pellets and 300 ml of water in a 500-ml distillation flask. Remove ammonia

from the solution by distilling until 25 ml of the distillate gives no color with 0.5 ml of alkaline mercuric-potassium iodide TS. Allow the solution in the distillation flask to cool, and add 50 mg of phenylalanine. Distil, collecting two 25-ml fractions in 50-ml Nessler tubes. Add 0.5 ml of alkaline mercuric-potassium iodide TS to the distillates and to a series of standards, prepared by dilution of the *Ammonium Chloride Standard Solution*, containing the equivalent of 0, 5, 10, 15, and 20 μg of ammonia in 25 ml of solution. After allowing 10 min for color development, determine the amounts of ammonia present in the distillates by comparing their color intensities with those of the standards. If the second 25 ml of distillate from the phenylalanine sample contains appreciable ammonia, distil and collect additional 25-ml fractions and determine their ammonia content.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 100-mg sample does not exceed that shown in a control containing 20 μg of chloride ion (Cl).

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Iron To the ash obtained in the test for *Residue on Ignition* add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, and dilute with water to 50 ml. Dilute 10 ml of this solution to 40 ml with water, and add 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 μg Fe) in an equal volume of a solution containing the quantities of the reagents used in the test.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Nitrogen (Total) Determine as directed under *Nitrogen Determination*, page 521, using about 300 mg of a sample, previously dried and accurately weighed.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Sulfate, page 471 Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 200 μg of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Phenylalanine

L-α-Amino-β-phenylpropionic Acid

$C_6H_5CH_2CH(NH_2)COOH$

$C_9H_{11}NO_2$ Mol wt 165.19

DESCRIPTION

Colorless or white platelike crystals or a white crystalline powder having a slight characteristic odor and a slightly bitter taste. One g is soluble in about 35 ml of water. It is slightly soluble in alcohol, in dilute mineral acids, and in alkali hydroxide solutions. It melts with decomposition at about 283°. The pH of a 1 in 100 solution is between 5.4 and 6.0.

REQUIREMENTS

Identification

A. Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS. A reddish purple color is produced.

B. Heat 5 ml of a 1 in 100 solution with a few drops of potassium dichromate TS. A characteristic odor is evolved.

C. To a 10 mg sample add 500 mg of potassium nitrate and 2 ml of sulfuric acid, and heat the mixture on a water bath for 20 min. Cool, add 2 ml of hydroxylamine TS, immerse in ice water for 10 min, and then add 10 ml of sodium hydroxide TS. A reddish violet color is produced.

Assay Not less than 98.5% and not more than the equivalent of 102.0% of $C_9H_{11}NO_2$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]_D^{20}$: Between −33.0° and −35.2°, on the dried basis.

TESTS

Assay Dissolve about 300 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Each ml of 0.1 *N* perchloric acid is equivalent to 16.52 mg of $C_9H_{11}NO_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 2 g of previously dried sample in sufficient water to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Phosphoric Acid

Orthophosphoric Acid

H_3PO_4 Mol wt 98.00

DESCRIPTION

A colorless, odorless solution of H_3PO_4, usually available in concentrations ranging from 75.0% to 85.0%. It is miscible with water and with alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Phosphate*, page 517.

Assay Not less than the minimum or within the range of percentage claimed by the vendor.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Weigh accurately about 1.5 g in a tared, glass-stoppered flask, and dilute to 120 ml with water. Add 0.5 ml of thymolphthalein TS, mix, and titrate with 1 *N* sodium hydroxide to the first appearance of a blue color. Each ml of 1 *N* sodium hydroxide is equivalent to 49.00 mg of H_3PO_4.
Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.
Fluoride Determine on a 1-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.1 ml of *Fluoride Standard Solution*.
Heavy Metals A solution of 2 g in 10 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in tight containers.
Functional Use in Foods Acid; sequestrant.

Pimenta Leaf Oil

Pimento Leaf Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the leaves of the evergreen shrub *Pimenta officinalis* Lindl. (Fam. *Myrtaceae*). It is a pale yellow to light brownish yellow liquid when freshly distilled, becoming darker with age. In contact with iron, it acquires a blue shade, turning to dark brown on extended contact. It has a spicy odor. It is soluble in propylene glycol, and it is soluble, with slight opalescence, in most fixed oils. It is relatively insoluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 604, using the same test conditions as specified therein.

Assay Not less than 80% and not more than 91%, by volume, of phenols.
Angular Rotation Between −2° and +0.5°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.531 and 1.536 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.037 and 1.050.

TESTS

Assay Shake a suitable quantity of the oil with about 2% of powdered tartaric acid for about 2 min, then filter. Using a sample of the filtered oil, proceed as directed under *Phenols*, page 502, modified by heating the flask on a boiling water bath for 10 min and cooling, after shaking the mixture of oil and 1 *N* potassium hydroxide.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol; a slight opalescence may occur when additional solvent is added.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, stainless steel, or tin-lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Pimenta Oil

Pimenta Berries Oil; Pimento Oil; Allspice Oil

DESCRIPTION

The volatile oil distilled from the fruit of *Pimenta officinalis*, Lindley (Fam. *Myrtaceae*). It is a colorless, yellow, or reddish yellow liquid, which becomes darker with age. It has the characteristic odor and taste of allspice. It is affected by light.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 604, using the same test conditions as specified therein.

Assay Not less than 65%, by volume, of phenols.
Angular Rotation Between −4° and 0°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.527 and 1.540 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.018 and 1.048.

TESTS

Assay Proceed as directed under *Phenols*, page 502, modified by heating on a steam bath for 10 min, after shaking for 5 min. Then cool and let stand overnight, or until the liquids are clear.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 70% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Pine Needle Oil, Dwarf

Pine Needle Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh leaves of *Pinus mugo* Turra var. *pumilio* (Haenke) Zenari (Fam. *Pinaceae*). It is a colorless or yellow liquid having a pleasant aromatic odor and a bitter, pungent taste.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 611, using the same test conditions as specified therein.

Assay Not less than 3.0% and not more than 10.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between −5° and −15°.
Distillation Range Not more than 10% distils below 165°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.475 and 1.480 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.853 and 0.871.

TESTS

Assay Weigh accurately about 10 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Distillation Range Proceed as directed in the general method, page 478.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 10 ml of 90% alcohol, often with turbidity.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Pine Needle Oil, Scotch Type

DESCRIPTION

A volatile oil obtained by steam distillation from the needles of *Pinus sylvestris* L. (Fam. *Pinaceae*). It is a colorless or yellowish liquid with an aromatic, turpentinelike odor. It is soluble in most fixed oils, soluble, with faint opalescence, in mineral oil, and slightly soluble in propylene glycol. It is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 611, using the same test conditions as specified therein.

Assay Not less than 1.5% and not more than 5.0% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Between $-4°$ and $+10°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.473 and 1.479 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.857 and 0.885.

TESTS

Assay Weigh accurately about 10 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 6 ml of 90% alcohol, occasionally with slight opalescence.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Poloxamer 331

α-Hydro-*omega*-hydroxy-poly(oxyethylene)-poly(oxypropylene)(51–57 moles)poly(oxyethylene) Block Copolymer, Avg mol wt 3800

DESCRIPTION

Poloxamer 331 is a block copolymer condensate of ethylene oxide and propylene oxide having an average molecular weight of 3800. It occurs as a practically colorless liquid having a specific gravity of about 1.02 and a refractive index of about 1.452. It is very slightly soluble in water at 25°, but is freely soluble at 0°; it is freely soluble in alcohol, but is insoluble in propylene glycol and in ethylene glycol.

REQUIREMENTS

Arsenic (as As) Not more than 3 ppm.
Cloud Point of a 1 in 10 Solution Between 9° and 12°.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 5 ppm.
Hydroxyl Value Between 27.2 and 32.1.
Molecular Weight Between 3500 and 4125.
pH of a 2.5% Solution Between 6.0 and 7.4.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Cloud Point Prepare a 10% solution of the sample in water at a temperature below the expected cloud point, and transfer about 100 ml of this solution into a 50- × 120-mm test tube. Immerse the tube in a water bath, previously cooled to at least 10° below the expected cloud point, so that the water level is a few mm above that of the test solution. Place a suitable thermometer (see page 547) in the test solution, and position it so that the immersion line will be at the surface of the liquid. Stir the solution slowly with a mechanical stirrer (about 200 rpm), and heat gradually so that the test solution is heated at a rate of about 1° per min. Do not allow the temperature of the water bath to rise more than 10° above that of the test solution at any time. Continue heating in this manner, and record the temperature (cloud point) at which the test solution becomes cloudy.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals Prepare and test a 4-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value

Distilled Pyridine Distil pyridine over phthalic anhydride (about 60 g for each 1000 ml), discarding the first 25 ml and the last 50 ml of distillate from each 1000 ml distilled.

Phenolphthalein Indicator Prepare a 1% solution of phenolphthalein in undistilled pyridine.

Phthalation Reagent Dissolve 14.4 g of phthalic anhydride in sufficient *Distilled Pyridine* to make 100 ml, mix vigorously, and store in a brown bottle. Allow to stand at least 2 h before use. Determine the suitability of the reagent as follows: Mix 10.0 ml of the reagent with 25 ml of undistilled pyridine and 50 ml of water, allow to stand for 15 min, then add a few drops of *Phenolphthalein Indicator*, and titrate with 0.5 *N* sodium hydroxide. Multiply the volume, in ml, of the alkali solution required by its exact normality; if the result is not within the range 18.8 to 20.0, adjust the concentration of the reagent accordingly.

Procedure Transfer about 15 g of the sample, accurately weighed, into a 250-ml hydroxyl flask, and add 25.0 ml of *Phthalation Reagent* to the flask, using a pipet previously rinsed with the reagent and touching the tip of the pipet against the protrusion of the flask approximately 15 s after the pipet has drained. In the same manner, transfer the same volume of the reagent into a second flask to serve as the blank. Add a few glass beads to each flask, swirl to dissolve the sample, and reflux for 1 h. Cool the flasks to room temperature, and wash each condenser with two 10-ml portions of undistilled pyridine. Disconnect the condensers, add 10 ml of water to each flask, stopper, swirl, and allow to stand for 10 min. To each flask add 50.0 ml of approximately 0.66 *N* sodium hydroxide, then add 0.5 ml of *Phenolphthalein Indicator*, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for at least 15 s. Calculate the uncorrected hydroxyl value, *h*, by the formula

$$(B - S) \times (N \times 56.1/W),$$

in which *B* is the volume, in ml, of 0.5 *N* sodium hydroxide required for the blank, *S* is the volume required for the sample, *N* is the exact normality of the sodium hydroxide solution, and *W* is the weight of the sample, in g. Correct the results, if the sample contains significant acidity or alkalinity, as follows: Dissolve approximately the same amount of the sample, accurately weighed, as used above in 40 ml of undistilled pyridine, and add 60 ml of water and 0.5 ml of *Phenolphthalein Indicator*. If the solution is colorless, titrate with 0.1 *N* sodium hydroxide to a light pink endpoint, recording the volume, in ml, required as *v*. If the solution is pink, titrate with 0.1 *N* hydrochloric acid to the disappearance of the pink color, recording the volume, in ml, required as *v'*. Calculate the acidity correction factor, *A*, by the formula

$$v \times n \times 56.1/w,$$

in which *n* is the exact normality of the sodium hydroxide solution, and *w* is the weight of the sample, in g. Calculate the alkalinity correction factor, *A'*, by the formula

$$v' \times n' \times 56.1/w,$$

in which *n'* is the exact normality of the hydrochloric acid solution. Finally, calculate the corrected hydroxyl value, *H*, by the formula $h + A$, or $h - A'$, whichever is appropriate.

Molecular Weight Determine the *Hydroxyl Value, H*, and then calculate the molecular weight by the formula $56{,}100 \times (2/H)$.

pH Prepare a 2.5% solution of the sample in a 1:1 mixture of methanol-water, and determine the pH by the *Potentiometric Method*, page 531.

Packaging and Storage Store in tight containers.

Functional Use in Foods Solubilizing and stabilizing agent in flavor concentrates.

Poloxamer 407

α-Hydro-*omega*-hydroxy-poly(oxyethylene)-poly(oxypropylene)(63–71 moles)poly(oxyethylene) Block Copolymer, Avg mol wt 12,500

DESCRIPTION

Poloxamer 407 is a copolymer condensate of ethylene oxide and propylene oxide having an average molecular weight of 12,500. It occurs as a white solid having a melting range of about 52° to 56°. It is freely soluble in alcohol and in water, but is insoluble in propylene glycol and in ethylene glycol.

REQUIREMENTS

Arsenic (as As) Not more than 3 ppm.
Cloud Point of a 1 in 10 Solution Above 100°.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 5 ppm.
Hydroxyl Value Between 8.5 and 11.5.
Molecular Weight Between 9760 and 13,200.
pH of a 2.5% Solution Between 6.0 and 7.4.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Cloud Point Dissolve about 5 g of the sample in 50 ml of water in a test tube, place the tube in a boiling water bath, and heat to 100°. The solution does not become cloudy.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals Prepare and test a 4-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value

Distilled Pyridine, Phenolphthalein Indicator, and *Phthalation Reagent* Prepare as directed in the monograph for *Poloxamer 331*, page 228.

Procedure Proceed as directed for *Procedure* under *Hydroxyl Value* in the monograph for *Poloxamer 331*, page 228, using a sample of about 45 g accurately weighed, and adding 25 ml of *Distilled Pyridine* to both the sample flask and the blank flask before refluxing.

Molecular Weight Determine the *Hydroxyl Value, H*, and then calculate the molecular weight by the formula $56{,}100 \times (2/H)$.

pH Prepare a 2.5% solution of the sample in a 1:1 mixture of methanol-water, and determine the pH by the *Potentiometric Method*, page 531.

Packaging and Storage Store in tight containers.

Functional Use in Foods Solubilizing and stabilizing agent in flavor concentrates.

Polyethylene

DESCRIPTION

A white, translucent, partially crystalline and partially amorphous resin produced by the direct polymerization of ethylene at high temperatures and high pressure. Various grades and types, differing from one another in molecular weight, molecular weight distribution, degree of chain branching, and extent of crystallinity, are available. It is soluble in hot benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify polyethylene by comparing its infrared absorption spectrum with a typical spectrum as shown on page 720. Prepare the sample by dissolving it in hot toluene and evaporating on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Molecular Weight Between 2000 and 21,000.
Volatiles Not more than 0.5%.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Molecular Weight Determine as directed in the general method, page 468.

Volatiles Dry a 4-g sample for 45 min at 105° as directed under *Loss on Drying*, page 518.

Caution: To reduce explosion hazard, pass carbon dioxide or nitrogen into the lower part of the drying oven at a rate of about 100 ml per min.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Polyethylene Glycols

PEG

DESCRIPTION

Polyethylene glycols are addition polymers of ethylene oxide and water, ranging in molecular weight from about 200 to about 9500 and having the general formula $HOCH_2(CH_2OCH_2)_nCH_2OH$, in which n represents the average number of oxyethylene groups. Commercially available polyethylene glycols are usually designated by a number that roughly corresponds to the nominal molecular weight. Polyethylene glycols having a nominal molecular weight of 600 or below occur as clear to slightly hazy, colorless or practically colorless, viscous, slightly hygroscopic liquids that are miscible with water. Polyethylene glycols having a nominal molecular weight of 1000 or above are freely soluble in water and occur as creamy white, waxy solids or as flakes resembling paraffin. The polyethylene glycols are soluble in many organic solvents, including aliphatic ketones and alcohols, chloroform, glycol ethers, esters, and aromatic hydrocarbons; they are insoluble in ether and in most aliphatic hydrocarbons. As their molecular weight increases, water solubility, vapor pressure, hygroscopicity, and solubility in organic solvents decrease, while solidification point, specific gravity, flash point, and viscosity increase. They may contain a suitable antioxidant.

REQUIREMENTS

Arsenic (as As) Not more than 3 ppm.
Completeness and Color of Solution Passes test.
1,4-Dioxane Passes test.
Ethylene Glycol and Diethylene Glycol Not more than 0.25%, individually or combined.
Ethylene Oxide Not more than 0.02%.
Heavy Metals (as Pb) Not more than 5 ppm.

Nominal Molecular Weight PEG's having nominal molecular weights below 1000: not less than 95.0% and not more than 105.0% of the labeled value; PEG's having nominal molecular weights between 1000 and 7000: not less than 90.0% and not more than 110.0% of the labeled value; PEG's having nominal molecular weights above 7000: not less than 87.5% and not more than 112.5% of the labeled value.

pH Between 4.5 and 7.5.

Residue on Ignition Not more than 0.1%.

Viscosity Passes test.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Completeness and Color of Solution A solution of 5 g of the sample in 50 ml of water is colorless. It is clear for liquid grades and not more than slightly hazy for solid grades.

1,4-Dioxane Determine as directed in the general method, page 477.

Ethylene Glycol and Diethylene Glycol

POLYETHYLENE GLYCOLS HAVING NOMINAL MOLECULAR WEIGHTS BELOW 450

Apparatus Use a suitable gas chromatograph (see page 475) equipped with a hydrogen flame ionization detector (Varian Aerograph 600D or equivalent), containing a 1.5-m × 3-mm (id) stainless steel column packed with sorbitol 12%, by weight, on 60/80-mesh nonacid-washed diatomaceous earth (Chromosorb W, or equivalent).

Operating Conditions The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: *column temperature*, 165°; *inlet temperature*, 260°; *carrier gas*, nitrogen (or other suitable inert gas), flowing at a rate of 70 ml per min; *recorder*, −0.5 to +1.05 mV, full span, 1-s full-response time; *hydrogen and air flow to burner*, optimize to give maximum sensitivity.

Standard Solutions Prepare chromatographic standards by dissolving accurately weighed amounts of commercial ethylene glycol and diethylene glycol, previously purified by distillation if necessary, in water. Suitable concentrations range from 0.2 to 1 mg of each glycol per ml.

Sample Preparation Transfer about 4 g of the sample, accurately weighed, into a 10-ml volumetric flask, dilute to volume with water, and mix.

Procedure Inject a 2-µl portion of each of the *Standard Solutions* into the chromatograph, and obtain the chromatogram for each solution. Under the stated conditions, the elution time is approximately 2.0 min for ethylene glycol and 6.5 min for diethylene glycol. Measure the peak heights, and record the values as follows: A = height, in mm, of the ethylene glycol peak; B = weight, in mg, of ethylene glycol per ml of the *Standard Solution*; C = height, in mm, of the diethylene glycol peak; and D = weight, in mg, of diethylene glycol per ml of the *Standard Solution*.

Similarly, inject a 2-µl portion of the *Sample Preparation* into the chromatograph, and obtain the chromatogram, recording the height of the ethylene glycol peak as E and that of the diethylene glycol peak as F. Calculate the percentage of ethylene glycol in the sample by the formula

$$(E \times B)/(A \times \text{sample weight, in g});$$

calculate the percentage of diethylene glycol in the sample by the formula

$$(F \times D)/(C \times \text{sample weight, in g}).$$

Not more than 0.2% of total ethylene and diethylene glycols is found.

POLYETHYLENE GLYCOLS HAVING NOMINAL MOLECULAR WEIGHTS OF 450 OR HIGHER

Sample Preparation Dissolve 50.0 g of the sample in 75 ml of diphenyl ether in a 250-ml distillation flask. Warm the mixture, if necessary, just enough to melt the crystals. Slowly distil at a pressure of 1 to 2 mm of Hg into a receiver graduated to 100 ml in 1-ml subdivisions, until 25 ml of distillate has been collected. Add 20.0 ml of water to the distillate, shake vigorously, and allow the layers to separate. Cool in an ice bath to solidify the diphenyl ether and facilitate its removal. Filter the water layer, wash the diphenyl ether with 5.0 ml of ice-cold water, pass the washings through the filter, and collect the filtrate and washings in a 25-ml volumetric flask. Warm to room temperature, dilute to volume with water, if necessary, and mix. Mix this solution with 25.0 ml of freshly distilled acetonitrile in a 125-ml glass-stoppered flask.

Standard Preparation Transfer 62.5 mg of diethylene glycol to a 25-ml volumetric flask, dilute to volume with a 1:1 mixture of freshly distilled acetonitrile and water, and mix.

Procedure Transfer 10.0 ml each of the *Sample Preparation* and of the *Standard Preparation* into separate 50-ml flasks each containing 15 ml of ceric ammonium nitrate TS, and mix. Within 2 to 5 min, determine the absorbance of each solution in a 1-cm cell at the wavelength of maximum absorbance occurring between 400 and 600 nm, with a suitable spectrophotometer, using a blank consisting of 15 ml of ceric ammonium nitrate TS and 10 ml of a 1:1 mixture of acetonitrile and water. The absorbance of the solution from the *Sample Preparation* does not exceed that from the *Standard Preparation*.

Ethylene Oxide

Morpholine Solution Mix 1 part of redistilled morpholine with 9 parts of anhydrous methanol.

Mixed Indicator Weigh 50 mg of 4,4'-bis(amino-1-naphthylazo-2,2'-stilbenedisulfonic acid) and 10 mg of brilliant yellow into a 60-ml vial, pipet 1.5 ml of 0.1 N sodium hydroxide into the vial, and mix. Add 3.5 ml of water, mix, then transfer to a storage bottle with the aid of 45 ml of methanol, and mix.

Standard Methanolic Hydrochloric Acid Mix 8.5 ml of conc. hydrochloric acid and 1000 ml of anhydrous methanol, and standardize by titrating 9.00 ml with 0.1 N sodium hydroxide, using phenolphthalein TS as indicator. Restandardize if this solution is used more than 48 h after standardization.

Procedure Place 50 ml of anhydrous methanol in a 250-ml conical flask, add 4 to 6 drops of *Mixed Indicator*, and titrate with *Standard Methanolic Hydrochloric Acid* to a clear

blue color. (NOTE: Up to 20 drops of the indicator may be required in some cases.) For the sample blank, transfer about 25 g of the PEG sample, accurately weighed, to the flask, and dissolve it by swirling. Titrate with *Standard Methanolic Hydrochloric Acid* to a clear blue color, approaching the endpoint carefully, using small increments of titrant.

Transfer 50-ml portions of *Morpholine Solution* to two heat-resistant pressure bottles, the second of which is used as the reagent blank. To the first bottle add about 25 g of the PEG sample, accurately weighed, and dissolve it by swirling. Cap the bottles, wrap them securely in cloth bags, place them close together in a water bath maintained at 98° ± 2°, and heat for 30 min, keeping the water level in the bath just above the liquid level in the bottles. Remove the bottles from the bath, allow them to cool in air to room temperature, then loosen the wrappers, uncap to release any pressure, and remove the wrappers. Slowly add 20 ml of acetic anhydride to each bottle, swirl to effect complete solution, and allow to stand at room temperature for 15 min. If the bottles are still warm, cool them to room temperature. To each bottle add 4 to 6 drops (or more, see above) of *Mixed Indicator*, and titrate with *Standard Methanolic Hydrochloric Acid* to a clear blue color, adding very small increments when approaching the endpoint. Calculate the percentage of ethylene oxide by the formula

$$4.4N[(A - B)/W_1] - (C/W_2),$$

in which N is the exact normality of the *Standard Methanolic Hydrochloric Acid*; A, B, and C are the volumes, in ml, required in the titration of the sample, the reagent blank, and the sample blank, respectively; and W_1 and W_2 are the weights, in g, of the PEG sample taken for the reaction and for the sample blank, respectively.

Heavy Metals A solution of 4 g of the sample mixed with 5.0 ml of 0.1 *N* hydrochloric acid, diluted with water to 25 ml, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nominal Molecular Weight

Phthalic Anhydride Solution Place 49.0 g of phthalic anhydride in an amber bottle and dissolve it in 300 ml of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow to stand overnight before using.

Sample Preparation for Liquid Polyethylene Glycols Carefully introduce 25.0 ml of the *Phthalic Anhydride Solution* into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample equivalent to its expected average molecular weight divided by 160. (Thus, a sample of about 1.3 g would be taken for PEG 200, or about 3.8 g for PEG 600.) Stopper the bottle, and wrap it securely in a fabric bag.

Sample Preparation for Solid Polyethylene Glycols Carefully introduce 25.0 ml of the *Phthalic Anhydride Solution* into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample, previously melted, equivalent to its expected molecular weight divided by 160; because of limited solubility, however, do not use more than 25 g of any sample. Add 25 ml of pyridine, freshly distilled over phthalic anhydride, swirl to effect solution, stopper the bottle, and wrap it securely in a fabric bag.

Procedure Immerse the sample bottle in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat in the water bath for 30 to 60 min, using 60 min for PEG's having molecular weights of 3000 or higher, then remove the bottle from the bath and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1 in 100 solution of phenolphthalein in pyridine, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for 15 s, recording the volume, in ml, of 0.5 *N* sodium hydroxide required as *S*. Perform a blank determination on 25.0 ml on the *Phthalic Anhydride Solution* plus any additional pyridine added to the sample bottle, and record the volume, in ml, of 0.5 *N* sodium hydroxide required as *B*. Calculate the nominal molecular weight of the sample by the formula $2000W/(B - S)N$, in which W is the weight of the sample, in g, $(B - S)$ is the difference between the volume of 0.5 *N* sodium hydroxide consumed by the blank and by the sample, and N is the exact normality of the sodium hydroxide solution.

pH Determine potentiometrically on a solution prepared by dissolving 5 g of the sample in 100 ml of carbon dioxide–free water and adding 0.3 ml of saturated potassium chloride solution.

Residue on Ignition Ignite 25 g as directed in *Method I*, page 533.

Viscosity Determine as directed under *Viscosity of Dimethylpolysiloxane*, page 549, maintaining the constant-temperature bath at 100° ± 0.3° and using a capillary viscometer having a flow time of at least 200 s for the sample being tested. The viscosity is within the limits specified in the table below. (For PEG's not listed in the table, calculate the limits by interpolation.)

Nominal Average Mol Wt	Viscosity Range (centistokes)	Nominal Average Mol Wt	Viscosity Range (centistokes)
200	4.1–4.8	2400	49–65
300	5.4–6.4	2500	51–70
400	6.8–8.0	2600	54–74
500	8.3–9.6	2700	57–78
600	9.9–11.3	2800	60–83
700	11.5–13.0	2900	64–88
800	12.5–14.5	3000	67–93
900	15.0–17.0	3250	73–105
1000	16.0–19.0	3350	76–110
1100	18.0–22.0	3500	87–123
1200	20.0–24.5	3750	99–140
1300	22.0–27.5	4000	110–158
1400	24–30	4250	123–177
1450	25–32	4500	140–200
1500	26–33	4750	155–228
1600	28–36	5000	170–250
1700	31–39	5500	206–315
1800	33–42	6000	250–390
1900	35–45	6500	295–480
2000	38–49	7000	350–590
2100	40–53	7500	405–735
2200	43–56	8000	470–900
2300	46–60		

Packaging and Storage Store in tight containers.

Labeling Label to indicate the nominal average molecular weight.

Functional Use in Foods Dispersing, coating, binding, plasticizing agent; lubricant; flavoring adjuvant.

Polyglycerol Esters of Fatty Acids

DESCRIPTION

Polyglycerol esters of fatty acids are mixed partial esters formed by reacting polymerized glycerols with edible fats, oils, or fatty acids. Minor amounts of mono-, di-, and triglycerides, free glycerol and polyglycerols, free fatty acids, and sodium salts of fatty acids may be present. The polyglycerols vary in degree of polymerization, which is specified by a number (such as tri-, penta-, deca-, etc.) that is related to the average number of glycerol residues per polyglycerol molecule. A specified polyglycerol consists of a distribution of molecular species characteristic of its nominal degree of polymerization. By varying the proportions as well as the nature of the fats or fatty acids to be reacted with the polyglycerols, a large and diverse class of products may be obtained. They include light yellow to amber, oily to very viscous liquids; light tan to medium brown, plastic or soft solids; and light tan to brown, hard, waxy solids. The esters range from very hydrophilic to very lipophilic, but as a class tend to be dispersible in water and soluble in organic solvents and oils. They conform to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

The following specifications should conform to the representations of the vendor: **Acid Value, Hydroxyl Value, Iodine Value, Residue on Ignition, Saponification Value,** and **Sodium Salts of Fatty Acids.**

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Transfer to a 300-ml Erlenmeyer flask an accurately weighed sample approximately equivalent to 561 divided by the expected hydroxyl value, ±10%. Mix 9 volumes of pyridine with 1 volume of acetic anhydride, pipet 25.0 ml of this solution into the sample flask, and pipet 25.0 ml into a separate 300-ml Erlenmeyer flask to serve as the blank. Add boiling stones to each flask, and fit the flasks with air condensers, lubricating the joints only with a few drops of pyridine. Reflux the sample solution gently by heating on a hot plate, confining the vapors in the lower portion of the condenser, and continue refluxing for 45 min. Do not heat the blank. Cool the sample flask to room temperature, and rinse the condenser, the condenser tip, and the sides of the flask with 25 ml of pyridine. Add about 50 ml of 0.55 *N* sodium hydroxide to each flask, mix by swirling for about 45 s, then add 1 ml of phenolphthalein TS and 75 ml of isopropanol, and continue the titration with stirring to the first pink color that persists for at least 30 s. Calculate the hydroxyl value by the formula

$$AV + [(56.1)(B - S)(N/W)],$$

in which *AV* is the *Acid Value*, determined as directed above; $(B - S)$ is the difference between the volume, in ml, of 0.55 *N* sodium hydroxide required for the blank and for the sample, respectively; *N* is the exact normality of the sodium hydroxide solution; and *W* is the weight of the sample, in g.

Iodine Value Determine by the *Wijs Method*, page 505.

Residue on Ignition Determine as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509, using an accurately weighed amount of the sample approximately equivalent to 700 divided by the expected saponification value.

Sodium Salts of Fatty Acids Dissolve about 5 g of the sample, accurately weighed, in 75 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to an emerald green endpoint. Perform a blank determination and make any necessary correction, recording the net volume of perchloric acid consumed as *V*. Calculate the number of mg of potassium hydroxide equivalent to the sodium salts per g of the sample by the formula $56.1 \times (VN/W)$, in which *N* is the exact normality of the perchloric acid, and *W* is the weight of the sample, in g.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier.

Polyisobutylene

DESCRIPTION

A synthetic polymer produced by the low-temperature polymerization of isobutylene in liquid ethylene, methyl chloride, or hexane, using an aluminum chloride catalyst. After completion

of polymerization, volatile components are removed by raising the temperature of the reaction mixture. Low-molecular-weight grades are soft and gummy; high-molecular-weight grades are tough and elastic. All grades are light in color, odorless, and tasteless, and are soluble in benzene and in diisobutylene but insoluble in water.

REQUIREMENTS

Identification

Identify polyisobutylene by comparing its infrared absorption spectrum with a typical spectrum as shown on page 720. Prepare the sample by dissolving it in hot toluene and evaporating on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Molecular Weight (Flory) Not less than 37,000.
Volatiles Not more than 0.3%.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Molecular Weight Determine as directed in the general method, page 469.

Volatiles Dry a 5-g sample for 2 h at 105° as directed under *Loss on Drying*, page 518.

Caution: To reduce explosion hazard, pass carbon dioxide or nitrogen into the lower part of the drying oven at a rate of about 100 ml per min.

Packaging and Storage Store low-molecular-weight grades in metal drums; store high-molecular-weight grades in talc-coated cartons, or wrap in polyethylene film.

Functional Use in Foods Masticatory substance in chewing gum base.

Polypropylene Glycol

DESCRIPTION

Polypropylene glycol is an addition polymer of propylene glycol and water, represented by the formula $HO(C_3H_6O)_nC_3H_6OH$, in which n represents the average number of oxypropylene groups. It is a clear, colorless or practically colorless, viscous liquid. It is soluble in water and in such organic solvents as aliphatic ketones and alcohols, but it is insoluble in ether and in most aliphatic hydrocarbons.

REQUIREMENTS

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 5 ppm.
Nominal Molecular Weight Not less than 90.0% and not more than 110.0% of the labeled value.
pH Between 6.0 and 9.0.
Propylene Oxide Not more than 0.02%.
Residue on Ignition Not more than 0.01%.
Viscosity Passes test.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 4 g of the sample mixed with 5.0 ml of 0.1 *N* hydrochloric acid, diluted with water to 25 ml, meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Nominal Molecular Weight

Phthalic Anhydride Solution Place 49.0 g of phthalic anhydride in an amber bottle, and dissolve it in 300 ml of pyridine that has been freshly distilled over phthalic anhydride. Shake the bottle vigorously until solution is effected, and allow to stand overnight before using.

Test Preparation Carefully introduce 25.0 ml of the *Phthalic Anhydride Solution* into a clean, dry, heat-resistant pressure bottle. To the bottle add an accurately weighed amount of the sample equivalent to its expected nominal molecular weight divided by 160.

Procedure Immerse the sample bottle in a water bath, maintained between 96° and 100°, to the same depth as that of the mixture in the bottle. Heat the water bath for 30 min, then remove the bottle from the bath, and allow it to cool to room temperature. Uncap the bottle carefully to release any pressure, remove the bottle from the fabric bag, add 5 drops of a 1 in 100 solution of phenolphthalein in pyridine, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for 15 s, recording the volume, in ml, of 0.5 *N* sodium hydroxide required as *S*. Perform a blank determination on 25.0 ml of the *Phthalic Anhydride Solution* plus any additional pyridine added to the sample bottle, and record the volume, in ml, of 0.5 *N* sodium hydroxide required as *B*. Calculate the nominal molecular weight of the sample by the formula

$$2000W/N(B - S),$$

in which W is the weight of the sample, in g, $(B - S)$ is the difference between the volume of 0.5 *N* sodium hydroxide consumed by the blank and by the sample, and N is the exact normality of the sodium hydroxide solution.

pH Determine potentiometrically on a solution prepared by

dissolving 10 ml of the sample in 100 ml of methanol neutralized with either 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide.

Propylene Oxide

Magnesium Chloride Solution Add 100 ml of 10 *N* hydrochloric acid to 950 g of magnesium chloride, $MgCl_2.6H_2O$, dissolve in water, dilute to 1000 ml with water, and mix. Carefully add 400 ml of anhydrous methanol to 100 ml of the stock solution, and allow the mixture to come to room temperature before using.

Indicator Dissolve 100 mg of bromocresol green in 100 ml of anhydrous methanol.

Procedure Place 150 ml of anhydrous methanol into each of two 500-ml glass-stoppered conical flasks, the second of which is used as the reagent blank. Into each flask, pressure-pipet 25.0 ml of the *Magnesium Chloride Solution*, allowing the same drainage time for each transfer, and mix thoroughly. To the first flask add about 50 g of the sample, accurately weighed, and dissolve by swirling. Add about 1 ml of the *Indicator* to each flask, and titrate with 0.1 *N* alcoholic potassium hydroxide to a brilliant blue endpoint, recording the volume required as *S*, in ml. Titrate the reagent blank flask in the same manner, and record the volume required as *B*.

To correct for the alkalinity in the sample, place 150 ml of anhydrous methanol in a 500-ml conical flask, add about 50 g of the sample, accurately weighed, and swirl to effect solution. Add 1 ml of the *Indicator*, and titrate with 0.1 *N* hydrochloric acid to a yellow endpoint, recording the volume required as *C*, in ml. Calculate the percentage of propylene oxide in the sample by the formula

$$5.81 \times \left[\frac{(B-S)N_1}{W_1} - \frac{CN_2}{W_2}\right],$$

in which N_1 is the exact normality of the potassium hydroxide, N_2 is the exact normality of the hydrochloric acid, and W_1 and W_2 are the weights, in g, of the sample taken for the reaction and the alkalinity correction, respectively.

Residue on Ignition Ignite 10 g as directed in the general method, page 533.

Viscosity Determine as directed under *Viscosity of Dimethylpolysiloxane*, page 549, maintaining the constant-temperature bath at 37.8° ± 0.2° and using a capillary viscometer having a flow time of at least 200 s for the sample being tested. The viscosity of a sample having a nominal molecular weight of 1000 is between 85 and 97 centistokes, and that of a sample having a nominal molecular weight of 2000 is between 150 and 175 centistokes.

Packaging and Storage Store in tight containers.

Functional Use in Foods Defoaming agent.

Polysorbate 20

Polyoxyethylene (20) Sorbitan Monolaurate

DESCRIPTION

Polysorbate 20 is a mixture of laurate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is a lemon- to amber-colored liquid having a faint, characteristic odor and a warm, somewhat bitter taste. It is soluble in water, in alcohol, in ethyl acetate, in methanol, and in dioxane, but is insoluble in mineral oil and in mineral spirits.

REQUIREMENTS

Identification

To 5 ml of a 1 in 20 solution add 5 ml of sodium hydroxide TS, boil for a few min, cool, and acidify with diluted hydrochloric acid TS. The solution is strongly opalescent.

Assay for Oxyethylene Content Not less than 70.0% and not more than 74.0% of oxyethylene groups (—C_2H_4O—), equivalent to between 97.3% and 103.0% of polysorbate 20, calculated on the anhydrous basis.

Acid Value Not more than 2.

Arsenic (as As) Not more than 3 ppm.

1,4-Dioxane Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.

Hydroxyl Value Between 96 and 108.

Lauric Acid Between 15 and 17 g per 100 g of sample.

Residue on Ignition Not more than 0.25%.

Saponification Value Between 40 and 50.

Water Not more than 3%.

TESTS

Assay for Oxyethylene Content Weigh accurately a 65-mg sample, and proceed as directed in the general method, page 507.

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Lauric Acid Transfer about 25 g of the sample, accurately weighed, into a 500-ml round-bottom boiling flask, add 250 ml of alcohol and 7.5 g of potassium hydroxide, and mix. Connect a suitable condenser to the flask, reflux the mixture

for 1 to 2 h, then transfer to an 800-ml beaker, rinsing the flask with about 100 ml of water and adding it to the beaker. Heat on a steam bath to evaporate the alcohol, adding water occasionally to replace the alcohol, and evaporate until the odor of alcohol can no longer be detected. Adjust the final volume to about 250 ml with hot water. Neutralize the soap solution with dilute sulfuric acid (1 in 2), add 10% in excess, and heat, while stirring, until the fatty acid layer separates. Transfer the fatty acids into a 500-ml separator, wash with three or four 20-ml portions of hot water, and combine the washings with the original aqueous layer from the saponification. Extract the combined aqueous layer with three 50-ml portions of petroleum ether, add the extracts to the fatty acid layer, evaporate to dryness in a tared dish, cool, and weigh. The lauric acid so obtained has an *Acid Value* between 250 and 275 (*Method I*, page 503).

Residue on Ignition Ignite 5 g as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509, using about 8 g, accurately weighed.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Emulsifier; stabilizer.

Polysorbate 60

Polyoxyethylene (20) Sorbitan Monostearate

DESCRIPTION

Polysorbate 60 is a mixture of stearate and palmitate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is a lemon- to orange-colored, oily liquid or semigel having a faint characteristic odor and a warm, somewhat bitter taste. It is soluble in water, in aniline, in ethyl acetate, and in toluene, but is insoluble in mineral oil and in vegetable oils. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 20 solution add 5 ml of sodium hydroxide TS, boil for a few min, cool, and acidify with diluted hydrochloric acid TS. The solution is strongly opalescent.

B. A mixture of 60 volumes of polysorbate 60 with 40 volumes of water at 25° or below yields a gelatinous mass.

Assay for Oxyethylene Content Not less than 65.0% and not more than 69.5% of oxyethylene groups (—C_2H_4O—), equivalent to between 97.0% and 103.0% of polysorbate 60, calculated on the anhydrous basis.

Acid Value Not more than 2.

Arsenic (as As) Not more than 3 ppm.

1,4-Dioxane Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.

Hydroxyl Value Between 81 and 96.

Residue on Ignition Not more than 0.25%.

Saponification Value Between 45 and 55.

Stearic and Palmitic Acids Between 24 and 26 g per 100 g of sample.

Water Not more than 3%.

TESTS

Assay for Oxyethylene Content Weigh accurately a 65-mg sample, and proceed as directed in the general method, page 507.

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Residue on Ignition Ignite 5 g as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509, using about 8 g, accurately weighed.

Stearic and Palmitic Acids Isolate the fatty acids as directed in the test for *Lauric Acid* under *Polysorbate 20*, page 234, and determine the weight of the acids. The product so obtained has an *Acid Value* between 200 and 212 (*Method I*, page 503) and a *Solidification Point*, page 538, not below 52°.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Emulsifier; stabilizer.

Polysorbate 65

Polyoxyethylene (20) Sorbitan Tristearate

DESCRIPTION

Polysorbate 65 is a mixture of stearate and palmitate partial esters of sorbitol and its anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is a tan, waxy solid having a faint, characteristic odor and a warm, somewhat bitter

taste. It is soluble in mineral oil and in vegetable oils, mineral spirits, acetone, ether, dioxane, alcohol, and methanol, and it is dispersible in water and in carbon tetrachloride. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

To 5 ml of a 1 in 20 solution add 5 ml of sodium hydroxide TS, boil for a few min, cool, and acidify with diluted hydrochloric acid TS. The solution is strongly opalescent.

Assay for Oxyethylene Content Not less than 46.0% and not more than 50.0% of oxyethylene groups (—C_2H_4O—), equivalent to between 96.0% and 104.0% of polysorbate 65, calculated on the anhydrous basis.
Acid Value Not more than 2.
Arsenic (as As) Not more than 3 ppm.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Hydroxyl Value Between 44 and 60.
Residue on Ignition Not more than 0.25%.
Saponification Value Between 88 and 98.
Stearic and Palmitic Acids Between 42 and 44 g per 100 g of sample.
Water Not more than 3%.

TESTS

Assay for Oxyethylene Content Weigh accurately a 90-mg sample, and proceed as directed in the general method, page 507.
Acid Value Determine as directed under *Method II* in the general procedure, page 504.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
1,4-Dioxane Determine as directed in the general method, page 477.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.
Residue on Ignition Ignite 5 g as directed in the general method, page 533.
Saponification Value Determine as directed in the general method, page 509, using about 6 g, accurately weighed.
Stearic and Palmitic Acids Isolate the fatty acids as directed in the test for *Lauric Acid* under *Polysorbate 20*, page 234, and determine the weight of the acids. The product so obtained has an *Acid Value* between 200 and 212 (*Method I*, page 503) and a *Solidification Point*, page 538, not below 52°.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Emulsifier; stabilizer.

Polysorbate 80

Polyoxyethylene (20) Sorbitan Monooleate

DESCRIPTION

Polysorbate 80 is a mixture of oleate partial esters of sorbitol and sorbitol anhydrides condensed with approximately 20 moles of ethylene oxide (C_2H_4O) for each mole of sorbitol and its mono- and dianhydrides. It is a yellow- to orange-colored, oily liquid having a faint, characteristic odor and a warm, somewhat bitter taste. It is very soluble in water, producing an odorless, nearly colorless solution, and is soluble in alcohol, in fixed oils, in ethyl acetate, and in toluene. It is insoluble in mineral oil. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 20 solution add 5 ml of sodium hydroxide TS, boil for a few min, cool, and acidify with diluted hydrochloric acid TS. The solution is strongly opalescent.
B. To a 1 in 20 solution add bromine TS, dropwise. The bromine is decolorized.
C. A mixture of 60 volumes of polysorbate 80 with 40 volumes of water at 25° or below yields a gelatinous mass.

Assay for Oxyethylene Content Not less than 65.0% and not more than 69.5% of oxyethylene groups (—C_2H_4O—), equivalent to between 96.5% and 103.5% of polysorbate 80, calculated on the anhydrous basis.
Acid Value Not more than 2.
Arsenic (as As) Not more than 3 ppm.
1,4-Dioxane Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Hydroxyl Value Between 65 and 80.
Oleic Acid Between 22 and 24 g per 100 g of sample.
Residue on Ignition Not more than 0.25%.
Saponification Value Between 45 and 55.
Water Not more than 3%.

TESTS

Assay for Oxyethylene Content Weigh accurately a 65-mg sample, and proceed as directed in the general method, page 507.
Acid Value Determine as directed under *Method II* in the general procedure, page 503.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

1,4-Dioxane Determine as directed in the general method, page 477.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Oleic Acid Isolate the fatty acids as directed in the test for *Lauric Acid* under *Polysorbate 20*, page 234, and determine the weight of the acid. The product so obtained has an *Acid Value* between 196 and 206 (*Method I*, page 503) and an *Iodine Value* between 80 and 92 (*Wijs Method*, page 505).

Residue on Ignition Ignite 5 g as directed in the general method, page 533.

Saponification Value Determine as directed in the general method, page 509, using about 8 g, accurately weighed.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Emulsifier; stabilizer.

Polyvinyl Acetate

DESCRIPTION

A clear, water-white to pale yellow solid resin prepared by the polymerization of vinyl acetate. After completion of polymerization, the resin is freed of traces of residual catalyst (usually a peroxide), monomer, and/or solvent by vacuum drying, steam sparging, washing, or any combination of these treatments. The resin is soluble in benzene and in acetone, but is insoluble in water.

REQUIREMENTS

Identification

Identify polyvinyl acetate by comparing its infrared absorption spectrum with a typical spectrum as shown on page 721. The sample is melted and prepared for analysis on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.

Free Acetic Acid Not more than 0.05%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 3 ppm.

Molecular Weight Not less than 2000.

Volatiles Not more than 1%.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Free Acetic Acid Transfer 10.0 g of the sample into a 250-ml glass-stoppered Erlenmeyer flask, dissolve in 75 ml of benzene, add 60 ml of alcohol, and mix. Add phenolphthalein TS, and titrate with 0.02 *N* methanolic potassium hydroxide to a faint pink endpoint. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.02 *N* methanolic potassium hydroxide is equivalent to 1.201 mg of $C_2H_4O_2$.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Molecular Weight Determine as directed in the general method, page 469.

Volatiles Dry a sample of about 1.5-g at 100° for 2 h in vacuum as directed under *Loss on Drying*, page 518.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Polyvinylpolypyrrolidone

Crospovidone; PVPP; 1-Vinyl-2-pyrrolidone Crosslinked Insoluble Polymer

DESCRIPTION

A crosslinked homopolymer of purified vinylpyrrolidone, produced catalytically. It is insoluble in water and in other common solvents. It occurs as a white to off-white, hygroscopic free-flowing powder having a faint bland odor.

REQUIREMENTS

Identification

Add 0.1 ml of iodine TS to a suspension of 1 g of the sample in 10 ml of water. The reagent is discolored after the mixture is shaken for 30 s (distinction from PVP, which produces a red color). When 1 ml of starch TS is added and the mixture is shaken, no blue color is formed.

Extractable Substances Not more than 0.005% (when used as a clarifying agent in beverages and vinegar).

Heavy Metals (as Pb) Not more than 10 ppm.

Nitrogen Not less than 11.0% and not more than 12.8%.

pH of a 1 in 100 Suspension Between 5.0 and 11.0.
Residue on Ignition Not more than 0.4%.
Unsaturation (as vinylpyrrolidone) Not more than 0.1%.
Water Not more than 6%.

TESTS

Extractable Substances Slurry 60 g of the sample with 600 ml of water in a 1000-ml beaker, then cover the beaker, and digest on a steam bath or hot plate at 90° to 100° for 3 h. Cool to room temperature, centrifuge at 2500 rpm for 1 h, and decant the supernatant liquid. Add water to make up the 600-ml original volume, and slurry a 100-ml aliquot of the mixture with 4 g of filter aid composed of equal parts, by weight, of Hyflo Super-Cel and Dicalcite 115, or equivalent materials. Filter the mixture through a 0.5-in. bed of the same filter aid mixture in a 70- to 100-mm Buchner funnel. Transfer 50 ml of the filtrate into a 150-ml beaker, add 10 ml of 71% perchloric acid, and mix by stirring with a glass rod. Determine the haze reading within 1 min after addition of the acid with a Coleman Nepho-Colorimeter Model No. 9, or equivalent instrument. Perform a blank on the filter aid mixture under the same conditions, beginning with " . . . slurry a 100-ml aliquot. . . . " Transfer 50 ml of the blank filtrate to a 150-ml beaker, and add 0.25 ml of a 0.1% solution of PVP (average mol wt 40,000), followed by 10 ml of 71% perchloric acid. Stir the solution with a glass rod, and read the haze within 1 min after addition of the acid. The haze reading on the test sample is no greater than that of the blank.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nitrogen Determine as directed in *Method II* under *Nitrogen Determination*, page 522, using a 100-mg sample. In the wet-digestion step, repeat the addition of hydrogen peroxide (usually three to six times) until a clear, light green solution is obtained, then heat for an additional 4 h, and continue as directed, beginning with "Cautiously add 2 ml of water. . . . "

pH of a 1 in 100 Suspension Determine as directed in the general method, page 531, using a 1-g sample suspended in 100 ml of water.

Residue on Ignition Ignite a 2-g sample as directed in the general method, page 533.

Unsaturation (as vinylpyrrolidone) Suspend a 4-g sample in 30 ml of water, stir for 15 min, and filter through a sintered-glass filter having a porosity between 9 and 15 µm, collecting the filtrate in a 250-ml flask. Wash the residue with 100 ml of water, add 500 mg of sodium acetate to the combined filtrates, and titrate with 0.1 *N* iodine until the color of iodine no longer fades. Add an additional 3.0 ml of 0.1 *N* iodine, allow to stand for 10 min, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding 3 ml of starch TS as the endpoint is approached. Perform a blank determination (see page 2), and make any necessary correction. Not more than 0.72 ml is consumed.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Clarifying agent.

Potassium Acid Tartrate

Potassium Bitartrate; Cream of Tartar

KOOCCH(OH)CH(OH)COOH

$C_4H_5KO_6$ Mol wt 188.18

DESCRIPTION

Potassium acid tartrate is a salt of L(+)-tartaric acid. It occurs as colorless or slightly opaque crystals, or as a white, crystalline powder, having a pleasant, acid taste. A saturated solution is acid to litmus. One g dissolves in 165 ml of water at 25°, in 16 ml of boiling water, and in 8820 ml of alcohol.

REQUIREMENTS

Identification

A. When sufficiently heated, it chars and emits flammable vapors having an odor resembling that of burning sugar. At a higher temperature and with free access to air, the carbon of the black residue is consumed, and there remains a white, fused mass of potassium carbonate that imparts a reddish purple color to a nonluminous flame.
B. A saturated solution yields a yellowish orange precipitate with sodium cobaltinitrite TS.
C. Neutralize a saturated solution with sodium hydroxide TS in a test tube, add silver nitrate TS, then just sufficient ammonia TS to dissolve the white precipitate, and boil the solution. Silver is deposited on the inner surface of the tube, forming a mirror.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $C_4H_5KO_6$ after drying.
Ammonia Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Insoluble Matter Passes test.
Lead Not more than 10 ppm.

TESTS

Assay Weigh accurately about 6 g, previously dried at 105° for 3 h, dissolve it in 100 ml of boiling water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 188.2 mg of $C_4H_5KO_6$.

Ammonia Heat 500 mg with 5 ml of sodium hydroxide TS. No odor of ammonia is detected.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Insoluble Matter Agitate 500 mg with 3 ml of ammonia TS. No undissolved residue remains.
Lead Dissolve 1 g in 3 ml of dilute nitric acid (1 in 2), boil for 1 min, cool, and dilute to 20 ml with water. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.
Functional Use in Foods Acid; buffer.

Potassium Alginate

Algin

$(C_6H_7O_6K)_n$ Equiv wt, *Calculated*, 214.22
Equiv wt, *Actual* (Avg), 238.00

DESCRIPTION

The potassium salt of alginic acid (see *Alginic Acid*, page 13) occurs as a white to yellowish, fibrous or granular powder. It is nearly odorless and tasteless. It dissolves in water to form a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than 30% by weight. It is insoluble in chloroform, in ether, and in acids having a pH lower than about 3.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 100 solution add 1 ml of calcium chloride TS. A voluminous, gelatinous precipitate is formed.
B. To 10 ml of a 1 in 100 solution add 1 ml of diluted sulfuric acid TS. A heavy gelatinous precipitate is formed.
C. Potassium alginate meets the requirements of *Identification Test C* under *Alginic Acid*, page 13.
D. Extract the *Ash* from the potassium alginate with diluted hydrochloric acid TS, and filter. The filtrate gives positive tests for *Potassium*, page 517.

Assay It yields not less than 16.5% and not more than 19.5% of carbon dioxide (CO_2), corresponding to between 89.2% and 105.5% of potassium alginate (equiv wt 238.00).
Ash Between 22% and 33% after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 15%.

TESTS

Assay Proceed as directed in the *Alginates Assay*, page 463. Each ml of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 28.75 mg of potassium alginate (equiv wt 238.00).
Ash Determine as directed under *Ash* in the monograph on *Alginic Acid*, page 14.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Determine as directed in the test for *Heavy Metals* under *Alginic Acid*, page 14.
Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Potassium Bicarbonate

$KHCO_3$ Mol wt 100.12

DESCRIPTION

Colorless, transparent, monoclinic prisms or a white, granular powder. It is odorless and stable in air. Its solutions are neutral or alkaline to phenolphthalein TS. One g dissolves in 2.8 ml of water. It is almost insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Potassium*, page 517, and for *Bicarbonate*, page 516.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $KHCO_3$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.25%.
Normal Carbonate Passes test.

TESTS

Assay Dissolve about 4 g, accurately weighed, in 25 ml of water, add methyl orange TS, and titrate with 1 *N* sulfuric acid. Each ml of 1 *N* sulfuric acid is equivalent to 100.1 mg of $KHCO_3$.
Arsenic A solution of 1 g in 4 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Dissolve 2 g in 5 ml of water and 8 ml of diluted hydrochloric acid TS, boil gently for 1 min, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry over silica gel for 4 h.
Normal Carbonate Dissolve 1 g of the sample without agitation in 20 ml of water at a temperature not above 5°, and add 2 ml of 0.1 *N* hydrochloric acid and 2 drops of

phenolphthalein TS. The solution does not assume more than a faint pink color immediately.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Alkali; leavening agent.

Potassium Bromate

$KBrO_3$ Mol wt 167.00

DESCRIPTION

White crystals or a granular powder. It is soluble in water and slightly soluble in alcohol. The pH of a 1 in 20 solution is between 5 and 9.

REQUIREMENTS

Identification

A. A 1 in 20 solution imparts a violet color to a nonluminous flame.
B. To a 1 in 20 solution add sulfurous acid dropwise. A yellow color is produced that disappears upon the addition of an excess of sulfurous acid.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $KBrO_3$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.5%.

TESTS

Assay Dissolve about 100 mg, previously dried to constant weight over a suitable desiccant and accurately weighed, in 50 ml of water contained in a 250-ml glass-stoppered Erlenmeyer flask. Add 3 g of potassium iodide, followed by 3 ml of hydrochloric acid. Allow the mixture to stand for 5 min, add 100 ml of cold water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the endpoint is approached. Perform a blank determination (see page 2). Each ml of 0.1 *N* sodium thiosulfate consumed is equivalent to 2.783 mg of $KBrO_3$.

Arsenic Dissolve 1 g in a mixture of 5 ml of hydrochloric acid and 5 ml of water, and evaporate the solution until crystals appear. Cool, dissolve the residue in water, and dilute to 35 ml. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 2 g in 10 ml of water, add 10 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 10 ml of hydrochloric acid, again evaporate to dryness, and then dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry over a suitable desiccant to constant weight.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Maturing agent; dough conditioner.

Potassium Carbonate

K_2CO_3 Mol wt 138.21

DESCRIPTION

Potassium carbonate is anhydrous or contains 1.5 molecules of water of crystallization. The anhydrous form occurs as a white, granular powder and the hydrate form as small, white, translucent crystals or granules. It is odorless, has a strongly alkaline taste, is very deliquescent, and its solutions are alkaline. One g dissolves in 1 ml of water at 25°, and in about 0.7 ml of boiling water. It is insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Potassium*, page 517, and for *Carbonate*, page 516.

Assay Not less than 99.0% of K_2CO_3 after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Insoluble Substances Passes test.
Lead Not more than 10 ppm.
Loss on Drying K_2CO_3 (anhydrous): not more than 1%; $K_2CO_3 \cdot 1\text{-}1/2H_2O$ (hydrated): between 10% and 16.5%.

TESTS

Assay Weigh accurately, in a stoppered weighing bottle, about 1 g of the dried sample obtained in the test for *Loss on Drying*, dissolve it in 50.0 ml of 1 *N* sulfuric acid, add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each ml of 1 *N* sulfuric acid is equivalent to 69.11 mg of K_2CO_3.

Arsenic A solution of 1 g cautiously dissolved in diluted hydrochloric acid TS (about 5 ml) meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals To 1 g add 2 ml of water and 6 ml of diluted hydrochloric acid TS, boil for 1 min, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances No residue is left on dissolving 1 g in 20 ml of water.

Lead A solution of 1 g cautiously dissolved in diluted hydrochloric acid TS (about 5 ml) meets the requirements of

the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry about 3 g, accurately weighed, at 180° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Alkali.

Potassium Carbonate Solution

DESCRIPTION

A clear or slightly turbid, colorless, alkaline solution that absorbs carbon dioxide when exposed to the air, forming potassium bicarbonate.

REQUIREMENTS

Identification

It gives positive tests for *Potassium*, page 517, and for *Carbonate*, page 516.

Assay Not less than 97.0% and not more than 103.0%, by weight, of the labeled amount of K_2CO_3.

Arsenic (as As) Not more than 3 ppm, calculated on the basis of K_2CO_3 determined in the *Assay*.

Heavy Metals (as Pb) Not more than 0.002%, calculated on the basis of K_2CO_3 determined in the *Assay*.

Lead Not more than 10 ppm, calculated on the basis of K_2CO_3 determined in the *Assay*.

TESTS

Assay Based on the stated or labeled percentage of K_2CO_3, weigh accurately a volume of the solution equivalent to about 1 g of potassium carbonate, and add it to 50.0 ml of 1 *N* sulfuric acid. Add methyl orange TS, and titrate the excess acid with 1 *N* sodium hydroxide. Each ml of 1 *N* sulfuric acid is equivalent to 69.11 mg of K_2CO_3.

Arsenic Dilute the equivalent of 1 g of K_2CO_3, calculated on the basis of the *Assay*, to 10 ml with water, and cautiously neutralize to litmus with diluted hydrochloric acid TS. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dilute the equivalent of 1 g of K_2CO_3, calculated on the basis of the *Assay*, with a mixture of 5 ml of water and 6 ml of diluted hydrochloric acid TS, and heat to boiling. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dilute the equivalent of 1 g of K_2CO_3, calculated on the basis of the *Assay*, with a mixture of 6 ml of diluted hydrochloric acid TS and 5 ml of water. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.

Solutions Usually Available A concentration, weight in weight, of about 50.0%.

Functional Use in Foods Alkali.

Potassium Chloride

KCl Mol wt 74.55

DESCRIPTION

Colorless, elongated, prismatic, or cubical crystals, or a white, granular powder. It is odorless, has a saline taste, and is stable in air. Its solutions are neutral to litmus. One g dissolves in 2.8 ml of water at 25°, and in about 2 ml of boiling water. It is insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Potassium*, page 517, and for *Chloride*, page 516.

Assay Not less than 99.0% of KCl after drying.

Acidity or Alkalinity Passes test.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Iodide or Bromide Passes test.

Loss on Drying Not more than 1%.

Sodium Passes test.

TESTS

Assay Dry about 250 mg at 105° for 2 h, weigh accurately, and dissolve in 50 ml of water in a glass-stoppered flask. Add, while agitating, 50.0 ml of 0.1 *N* silver nitrate, 3 ml of nitric acid, and 5 ml of nitrobenzene, shake vigorously, add 2 ml of ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each ml of 0.1 *N* silver nitrate is equivalent to 7.455 mg of KCl.

Acidity or Alkalinity To a solution of 5 g in 50 ml of recently boiled and cooled water add 3 drops of phenolphthalein TS. No pink color is produced. Then add 0.3 ml of 0.02 *N* sodium hydroxide. A pink color is produced.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodide or Bromide Dissolve 2 g of the sample in 6 ml of water, add 1 ml of chloroform, and then add, dropwise and with constant agitation, 5 ml of a mixture of equal parts of chlorine TS and water. The chloroform is free from even a transient violet or permanent orange color.

Loss on Drying, page 518 Dry at 105° for 2 h.

Sodium A 1 in 20 solution, tested on a platinum wire, does not impart a pronounced yellow color to a nonluminous flame.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement; gelling agent; salt substitute; yeast food.

Potassium Citrate

$CH_2(COOK)C(OH)(COOK)CH_2COOK.H_2O$

$C_6H_5K_3O_7.H_2O$ Mol wt 324.41

DESCRIPTION

Transparent crystals or a white, granular powder. It is odorless, has a cooling, saline taste, and is deliquescent when exposed to moist air. One g dissolves in about 0.5 ml of water. It is almost insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Potassium*, page 517, and for *Citrate*, page 516.

Assay Not less than 99.0% of $C_6H_5K_3O_7$ after drying.
Alkalinity Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Between 3% and 6%.

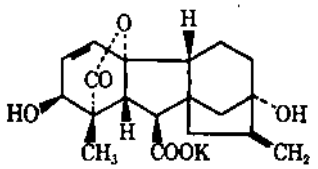
TESTS

Assay Dissolve about 250 mg, previously dried at 180° for 4 h and accurately weighed, in 40 ml of glacial acetic acid, warming slightly to effect solution. Cool the solution to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 10.213 mg of $C_6H_5K_3O_7$.
Alkalinity A 1 in 20 solution is alkaline to litmus, but after the addition of 0.2 ml of 0.1 *N* sulfuric acid to 10 ml of this solution no pink color is produced by the addition of 1 drop of phenolphthalein TS.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals A solution of 2 g in 25 ml meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 180° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Miscellaneous and general purpose; buffer; sequestrant.

Potassium Gibberellate



$C_{19}H_{21}KO_6$ Mol wt 384.47

DESCRIPTION

A white to slightly off-white, odorless or practically odorless, crystalline powder. It is soluble in water, in alcohol, and in acetone. The pH of a 1 in 20 solution is about 6. It is deliquescent.

REQUIREMENTS

Identification

A. Dissolve a few mg of the sample in 2 ml of sulfuric acid. A reddish solution having a green fluorescence is formed.
B. A 1 in 10 solution of the sample gives positive tests for *Potassium*, page 517.

Assay Not less than 80.0% and not more than 87.0% of $C_{19}H_{21}KO_6$, equivalent to between 72.1% and 78.4% of $C_{19}H_{22}O_6$ (gibberellic acid).
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm
Loss on Drying Between 5% and 13%.
Residue on Ignition Between 19% and 23%.
Specific Rotation $[\alpha]_D^{20°}$: Between +43.0° and +60.0°.

TESTS

Assay

Standard Preparation Transfer an accurately weighed quantity of FCC Gibberellic Acid Reference Standard, equivalent to about 25 mg of pure gibberellic acid (corrected for phase purity and volatiles content), into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Transfer 10.0 ml of this solution into a second 50-ml volumetric flask, dilute to volume with methanol, and mix.

Assay Preparation Transfer about 65 mg of the sample, accurately weighed, into a 50-ml volumetric flask, dissolve in methanol, dilute to volume with methanol, and mix. Transfer 10.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with methanol, and mix.

Procedure Transfer 5.0 ml of the *Assay Preparation* into a

25- × 200-mm glass-stoppered tube, and transfer 4.0-ml and 5.0-ml portions of the *Standard Preparation* into separate similar tubes. Place the tubes in a boiling water bath, evaporate to dryness, and then dry in an oven at 90° for 10 min. Remove the tubes from the oven, stopper, and allow to cool to room temperature. Dissolve the residue in each tube in 10.0 ml of dilute sulfuric acid (8 in 10), heat in a boiling water bath for 10 min, and then cool in a 10° water bath for 5 min. Determine the absorbance of the solutions in 1-cm cells at 535 nm with a suitable spectrophotometer, using the dilute sulfuric acid as the blank. Record the absorbance of the solution from the *Assay Preparation* as A_U. Note the absorbance of the two solutions prepared from the 4.0-ml and 5.0-ml aliquots of the *Standard Preparation*, and record the absorbance of the final solution giving the value nearest to that of the sample as A_S; record as V the volume of the aliquot used in preparing this solution. Calculate the quantity, in mg, of $C_{19}H_{21}KO_6$ in the sample taken by the formula

$$500 \times (C/0.8983) \times (V/5) \times (A_U/A_S),$$

in which C is the exact concentration, in mg per ml, of the *Standard Preparation*, and 0.8983 is the ratio of the molecular weight of potassium gibberellate to that of gibberellic acid.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 100° in vacuum for 4 h.

Residue on Ignition Ignite a 1-g sample as directed in the general method, page 533.

Specific Rotation, page 530. Determine in a solution containing 50 mg in each ml.

Packaging and Storage Store in tight containers protected from light.

Functional Use in Foods Enzyme activator.

Potassium Gluconate

D-Gluconic Acid, Monopotassium Salt; Monopotassium D-Gluconate

$$CH_2OH(CHOH)_4COOK$$

$C_6H_{11}KO_7$ Mol wt 234.25

DESCRIPTION

White or yellowish white crystalline powder or granules. It is odorless and has a slightly bitter taste. It is freely soluble in water and in glycerin, slightly soluble in alcohol, and insoluble in ether.

REQUIREMENTS

Identification

A. It responds to the flame test for *Potassium*, page 517.

B. To 5 ml of a warm solution (1 in 10) add 0.7 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, heat on a steam bath for 30 min, and cool. Induce crystallization by scratching the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

Assay Not less than 98.0% of $C_6H_{11}KO_7$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 3%.

Reducing Substances Not more than 0.5%.

TESTS

Assay Transfer about 175 mg, accurately weighed, into a clean, dry 200-ml Erlenmeyer flask, add 75 ml of glacial acetic acid, and dissolve by heating on a hot plate. Cool, add quinaldine red TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid, using a 10-ml microburet, to a colorless endpoint. Each ml of 0.1 *N* perchloric acid is equivalent to 23.42 mg of $C_6H_{11}KO_7$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 25 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Reducing Substances Determine as directed in the test for *Reducing Substances* under *Copper Gluconate*, page 90.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement; sequestrant.

Potassium Glycerophosphate

$C_3H_7K_2O_6P.3H_2O$ Mol wt 302.30

DESCRIPTION

Potassium glycerophosphate is a pale yellow, syrupy liquid containing three molecules of water of hydration, or it is prepared as a colorless to pale yellow, syrupy solution having a concentration of 50% to 75%. It is very soluble in water, and its solutions are alkaline to litmus paper.

REQUIREMENTS

Identification

A. A 1 in 10 solution gives positive tests for *Potassium*, page 517.

B. Heat a mixture of 100 mg of the sample with 500 mg of potassium bisulfate. Pungent vapors of acrolein are evolved.

Assay $C_3H_7K_2O_6P.3H_2O$: not less than 80.0% of $C_3H_7K_2O_6P$; potassium glycerophosphate solutions: not less than 95.0% and not more than 105.0% of the labeled concentration of $C_3H_7K_2O_6P$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 5 ppm.

TESTS

Assay Weigh accurately a portion of the sample equivalent to about 4 g of $C_3H_7K_2O_6P$, dissolve it in 30 ml of water, add methyl orange TS, and titrate with 0.5 *N* hydrochloric acid. Each ml of 0.5 *N* hydrochloric acid is equivalent to 124.13 mg of $C_3H_7K_2O_6P$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutrient; dietary supplement.

Potassium Hydroxide

Caustic Potash

KOH Mol wt 56.11

DESCRIPTION

White or nearly white pellets, flakes, sticks, fused masses, or other forms. Upon exposure to air, it readily absorbs carbon dioxide and moisture, and deliquesces. One g dissolves in 1 ml of water, in about 3 ml of alcohol, and in about 2.5 ml of glycerin. It is very soluble in boiling alcohol.

REQUIREMENTS

Identification

A 1 in 25 solution gives positive tests for *Potassium*, page 517.

Assay Not less than 85.0% of total alkali, calculated as KOH.
Arsenic (as As) Not more than 3 ppm.
Carbonate (as K_2CO_3) Not more than 3.5%.
Heavy Metals (as Pb) Not more than 0.003%.
Insoluble Substances Passes test.
Lead Not more than 10 ppm.
Mercury Not more than 0.1 ppm.

TESTS

Assay Dissolve about 1.5 g, accurately weighed, in 40 ml of recently boiled and cooled water, cool to 15°, add phenolphthalein TS, and titrate with 1 *N* sulfuric acid. At the discharge of the pink color, record the volume of acid required, then add methyl orange TS and continue the titration until a persistent pink color is produced. Record the total volume of acid required for the titration. Each ml of 1 *N* sulfuric acid is equivalent to 56.11 mg of total alkali, calculated as KOH.

Arsenic Dissolve 1 g in about 10 ml of water, cautiously neutralize to litmus paper with sulfuric acid, and cool. This solution meets the requirements of the *Arsenic Test*, page 464.

Carbonate Each ml of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* is equivalent to 138.2 mg of K_2CO_3.

Heavy Metals Dissolve 670 mg in a mixture of 5 ml of water and 5 ml of diluted hydrochloric acid TS. Heat to boiling, cool, dilute to 25 ml with water, and filter. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances A 1 in 20 solution is complete, clear, and colorless.

Lead Dissolve 1 g in a mixture of 5 ml of water and 11 ml of diluted hydrochloric acid TS, and cool. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Mercury Determine as directed under *Mercury Limit Test*, page 520, preparing the *Standard Preparation* and the *Sample Preparation* as follows:

Standard Preparation Prepare the stock solution and the dilutions to obtain a solution containing 1 µg of mercury per ml, as directed on page 520. Transfer 1.0 ml of the final solution (1 µg of mercury) to a 50-ml beaker, and add 20 ml of water, 1 ml of dilute sulfuric acid solution (1 in 5), and 1 ml of potassium permanganate solution (1 in 25). Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Transfer 10.0 g of the sample into a 100-ml beaker, dissolve in 15 ml of water, add 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with dilute hydrochloric acid solution (1 in 2). Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.
Functional Use in Foods Alkali.

Potassium Hydroxide Solution

DESCRIPTION

A clear or slightly turbid, colorless or slightly colored, strongly caustic, hygroscopic solution that absorbs carbon dioxide when exposed to the air, forming potassium carbonate.

REQUIREMENTS

Identification

It gives positive tests for *Potassium*, page 517.

Assay Not less than 97.0% and not more than 103.0%, by weight, of the labeled amount of KOH calculated as total alkali.
Arsenic (as As) Not more than 3 ppm, calculated on the basis of KOH determined in the *Assay*.
Carbonate (as K_2CO_3) Not more than 3.5% of the KOH determined in the *Assay*.
Heavy Metals (as Pb) Not more than 0.003%, calculated on the basis of KOH determined in the *Assay*.
Lead Not more than 10 ppm, calculated on the basis of KOH determined in the *Assay*.
Mercury Not more than 1 ppm, calculated on the basis of KOH determined in the *Assay*.

TESTS

Assay Based on the stated or labeled percentage of KOH, weigh accurately a volume of the solution equivalent to about 1.5 g of potassium hydroxide, and dilute it to 40 ml with recently boiled and cooled water. Continue as directed in the *Assay* under *Potassium Hydroxide*, page 244, beginning with " . . . cool to 15°. . . . "
Arsenic Dilute the equivalent of 1 g of KOH, calculated on the basis of the *Assay*, to 10 ml with water, cautiously neutralize to litmus paper with sulfuric acid, and cool. This solution meets the requirements of the *Arsenic Test*, page 464.
Carbonate Each ml of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* is equivalent to 138.2 mg of K_2CO_3.
Heavy Metals Dilute the equivalent of 670 mg of KOH, calculated on the basis of the *Assay*, with a mixture of 5 ml of water and 5 ml of diluted hydrochloric acid TS, and heat to boiling. Cool, dilute to 25 ml with water, and filter. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead Dilute the equivalent of 1 g of KOH, calculated on the basis of the *Assay*, with a mixture of 5 ml of water and 11 ml of diluted hydrochloric acid TS. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Mercury Determine as directed under *Mercury Limit Test*, page 520, using the following as the *Sample Preparation*: Transfer an accurately weighed amount of the sample, equivalent to 2.0 g of KOH, into a 50-ml beaker, add 10 ml of water and 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with dilute hydrochloric acid solution (1 in 2). Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.
Solutions Usually Available A nominal concentration, weight in weight, of 50%.
Functional Use in Foods Alkali.

Potassium Iodate

KIO_3 Mol wt 214.00

DESCRIPTION

A white, odorless, crystalline powder. One g dissolves in about 15 ml of water. It is insoluble in alcohol. The pH of a 1 in 20 solution is between 5 and 8.

REQUIREMENTS

Identification

To 1 ml of a 1 in 10 solution of the sample add 1 drop of starch TS and a few drops of 20% hypophosphorous acid. A transient blue color appears.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of KIO_3 after drying.
Arsenic (as As) Not more than 3 ppm.
Chlorate Passes test (limit about 0.01%).
Heavy Metals (as Pb) Not more than 10 ppm.
Iodide Passes test (limit about 0.002%).
Loss on Drying Not more than 0.5%.

TESTS

Assay Weigh accurately about 1.2 g, previously dried at 105° for 3 h, dissolve it in about 50 ml of water in a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 10.0 ml into a 250-ml glass-stoppered flask, add 40 ml of water, 3 g of potassium iodide, and 10 ml of dilute hydrochloric acid (3 in 10), and stopper the flask. Allow to stand for 5 min, add 100 ml of cold water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 3.567 mg of KIO_3.

Arsenic Dissolve 1 g in a mixture of 2 ml of hydrochloric acid and 1 ml of sulfuric acid, and evaporate to fumes of sulfur trioxide. Add 1 ml of hydrochloric acid, again evaporate to fumes, and dissolve the residue in 10 ml of water. Heat on a steam bath, discharge any yellow color remaining with hydrazine sulfate, cool, and dilute with water to 35 ml. This solution meets the requirements of the *Arsenic Test*, page 464.

Chlorate To 2 g in a beaker add 2 ml of sulfuric acid. The sample remains white, and no odor or gas is evolved.

Heavy Metals, page 512 Mix 2 g of the sample with 10 ml of hydrochloric acid (*Solution B*). Prepare a standard (*Solution A*) by adding a few mg of potassium chloride to 10 ml of hydrochloric acid. Cautiously evaporate both solutions to dryness, then repeat the acid treatment on both the sample and standard residues twice, using 5-ml portions of hydrochloric acid and evaporating to dryness each time. Dissolve the residues in 10 ml of water, heat on a steam bath, and discharge any yellow color remaining in the sample solution with hydrazine sulfate. Cool each solution and neutralize to phenolphthalein with 0.1 *N* sodium hydroxide. Transfer the solutions to 50-ml Nessler tubes, add 2.0 ml of *Standard Lead Solution* (20 µg Pb) to the standard, and dilute both solutions with water to 25 ml. To each tube add 6 ml of diluted acetic acid TS and 10 ml of freshly prepared hydrogen sulfide TS, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is no darker than that of *Solution A*.

Iodide Dissolve 1 g in 10 ml of water and add 1 ml of diluted sulfuric acid TS and 1 drop of starch TS. No blue color is formed.

Loss on Drying, page 518 Dry at 105° for 3 h.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Maturing agent; dough conditioner.

Potassium Iodide

KI Mol wt 166.00

DESCRIPTION

Hexahedral crystals, either transparent and colorless or somewhat opaque and white, or a white, granular powder. It is stable in dry air but slightly hygroscopic in moist air. One g is soluble in 0.7 ml of water at 25°, in 0.5 ml of boiling water, in 2 ml of glycerin, and in 22 ml of alcohol. The pH of a 1 in 20 solution is between 6 and 10.

REQUIREMENTS

Identification

A 1 in 10 solution responds to the tests for *Potassium*, page 517, and for *Iodide*, page 516.

Assay Not less than 99.0% and not more than the equivalent of 101.5% of KI after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodate Not more than 4 ppm.
Loss on Drying Not more than 1%.
Nitrate, Nitrite, and Ammonia Passes test.
Thiosulfate and Barium Passes test.

TESTS

Assay Dissolve about 500 mg, previously dried at 105° for 4 h and accurately weighed, in about 10 ml of water, and add 35 ml of hydrochloric acid and 5 ml of chloroform. Titrate with 0.05 *M* potassium iodate until the purple color of iodine disappears from the chloroform. Add the last portions of the iodate solution dropwise, agitating vigorously and continuously. After the chloroform has been decolorized, allow the mixture to stand for 5 min. If the chloroform develops a purple color, titrate further with the iodate solution. Each ml of 0.05 *M* potassium iodate is equivalent to 16.60 mg of KI.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodate Dissolve 1.1 g of the sample in sufficient ammonia– and carbon dioxide–free water to make 10 ml of solution, and transfer to a color-comparison tube. Add 1 ml of starch TS and 0.25 ml of 1 *N* sulfuric acid, mix, and compare the color with that of a control containing, in each 10 ml, 100 mg of potassium iodide, 1 ml of standard iodate solution (prepared by diluting 1 ml of a 1 in 2500 solution of potassium iodate to 100 ml with water), 1 ml of starch TS, and 0.25 ml of 1 *N* sulfuric acid. Any color produced in the solution of the sample does not exceed that in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Nitrate, Nitrite, and Ammonia Dissolve 1 g of the sample in 5

ml of water in a 40-ml test tube, and add 5 ml of sodium hydroxide TS and about 200 mg of aluminum wire. Insert a plug of cotton in the upper portion of the tube, and place a piece of moistened red litmus paper over the mouth of the tube. Heat in a steam bath for about 15 min. No blue coloration of the paper is discernible.

Thiosulfate and Barium Dissolve 500 mg of the sample in 10 ml of ammonia– and carbon dioxide–free water, and add 2 drops of diluted sulfuric acid. No turbidity develops within 1 min.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Potassium Metabisulfite

Potassium Pyrosulfite

$K_2S_2O_5$ Mol wt 222.31

DESCRIPTION

White or colorless, free-flowing crystals, crystalline powder, or granules, usually having an odor of sulfur dioxide. It gradually oxidizes in air to the sulfate. It is soluble in water, and is insoluble in alcohol. Its solutions are acid to litmus.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Potassium*, page 517, and for *Sulfite*, page 517.

Assay Not less than 90.0% of $K_2S_2O_5$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Iron Not more than 10 ppm.

Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 250 mg, add it to exactly 50 ml of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 5.558 mg of $K_2S_2O_5$.

Arsenic Dissolve 1 g of the sample in 10 ml of water in a 150-ml beaker, cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur trioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool, repeat the washing and fuming procedure, and cool again. This solution meets the requirements of the *Arsenic Test*, page 464, omitting the addition of 20 ml of dilute sulfuric acid (1 in 5).

Heavy Metals Dissolve 2 g in 20 ml of water, add 5 ml of hydrochloric acid, evaporate to about 1 ml on a steam bath, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron To 1 g of the sample add 2 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 2 ml of hydrochloric acid and 20 ml of water, add a few drops of bromine TS, and boil the solution to remove the bromine. Cool, dilute with water to 25 ml, then add 50 mg of ammonium persulfate and 5 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced in a control containing 1.0 ml of *Iron Standard Solution* (10 µg Fe).

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 100 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in well-filled, tight containers, and avoid exposure to excessive heat.

Functional Use in Foods Preservative; antioxidant.

Potassium Nitrate

KNO_3 Mol wt 101.10

DESCRIPTION

Colorless, transparent prisms, white granules, or white crystalline powder. It is odorless, has a salty taste, and produces a cooling sensation in the mouth. It is slightly hygroscopic in moist air. Its solutions are neutral to litmus. One g dissolves in 3 ml of water at 25°, in 0.5 ml of boiling water, and in about 620 ml of alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Potassium*, page 517, and for *Nitrite*, page 517.

Assay Not less than 99.0% of KNO_3 after drying.

Arsenic (as As) Not more than 3 ppm.

Chlorate Passes test.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 1%.

TESTS

Assay Weigh accurately about 400 mg, previously dried at 105° for 4 h, dissolve in 10 ml of hydrochloric acid in a small

beaker or porcelain dish, and evaporate to dryness on a steam bath. Dissolve the residue in 10 ml of hydrochloric acid, and again evaporate to dryness, continuing the heat until the residue, when dissolved in water, is neutral to litmus. Transfer the residue with the aid of 25 ml of water to a glass-stoppered flask, add exactly 50 ml of 0.1 *N* silver nitrate, then add 3 ml of nitric acid and 3 ml of nitrobenzene, and shake vigorously. Add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each ml of 0.1 *N* silver nitrate is equivalent to 10.11 mg of KNO_3.

Arsenic Dissolve 1 g in 3 ml of water, add 2 ml of sulfuric acid, and evaporate to strong fumes of sulfur trioxide. Cool, wash down the sides of the container with water, and heat again to strong fuming. Repeat the washing and fuming three more times, then cool and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Chlorate Sprinkle about 100 mg of a dry sample upon 1 ml of sulfuric acid. The mixture does not become yellow.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 1 g of the sample in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Antimicrobial agent; preservative.

Potassium Nitrite

KNO_2 Mol wt 85.10

DESCRIPTION

Small, white or yellowish, deliquescent granules or cylindrical sticks. It is very soluble in water, but is sparingly soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution is alkaline to litmus and gives positive tests for *Potassium*, page 517, and for *Nitrite*, page 517.

Assay Not less than 90.0% of KNO_2.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.

TESTS

Assay Transfer about 1.2 g, accurately weighed, into a 100-ml volumetric flask, dissolve in water, dilute to volume, and mix. Pipet 10 ml of this solution into a mixture of 50.0 ml of 0.1 *N* potassium permanganate, 100 ml of water, and 5 ml of sulfuric acid, keeping the tip of the pipet well below the surface of the liquid. Warm the solution to 40°, allow it to stand for 5 min, and add 25.0 ml of 0.1 *N* oxalic acid. Heat the mixture to about 80°, and titrate with 0.1 *N* potassium permanganate. Each ml of 0.1 *N* potassium permanganate is equivalent to 4.255 mg of KNO_2.

Arsenic Dissolve 1 g in 10 ml of diluted sulfuric acid TS, boil gently for 1 min, cool, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 1 g in 15 ml of diluted hydrochloric acid TS, and evaporate to dryness on a steam bath. To the residue add 2 ml of hydrochloric acid, again evaporate to dryness, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.

Functional Use in Foods Color fixative in meat and meat products; antimicrobial agent.

Potassium Phosphate, Dibasic

Dipotassium Monophosphate; Dipotassium Phosphate

K_2HPO_4 Mol wt 174.18

DESCRIPTION

A colorless or white, granular salt that is deliquescent when exposed to moist air. One g is soluble in about 3 ml of water. It is insoluble in alcohol. The pH of a 1% solution is about 9.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Potassium*, page 517, and for *Phosphate*, page 517.

Assay Not less than 98.0% of K_2HPO_4 after drying.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Insoluble Substances Not more than 0.2%.
Lead Not more than 5 ppm.
Loss on Drying Not more than 2%.

TESTS

Assay Transfer about 6.5 g of the sample, previously dried at 105° for 4 h and accurately weighed, into a 250-ml beaker, add 50.0 ml of 1 *N* hydrochloric acid and 50 ml of water, and

stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and titrate the excess acid with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*) of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 to pH 8.8). When *A* is equal to or less than *B*, each ml of the volume *A* of 1 *N* hydrochloric acid is equivalent to 174.2 mg of K_2HPO_4. When *A* is greater than *B*, each ml of the volume $2B - A$ of 1 *N* sodium hydroxide is equivalent to 174.2 mg of K_2HPO_4.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*), using glacial acetic acid to adjust the pH of the sample solution.

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; sequestrant; yeast food.

Potassium Phosphate, Monobasic

Potassium Biphosphate; Potassium Dihydrogen Phosphate; Monopotassium Phosphate

KH_2PO_4 Mol wt 136.09

DESCRIPTION

Colorless crystals or a white granular or crystalline powder. It is odorless, and is stable in air. It is freely soluble in water, but is insoluble in alcohol. The pH of a 1 in 100 solution is between 4.2 and 4.7.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Potassium*, page 517, and for *Phosphate*, page 517.

Assay Not less than 98.0% of KH_2PO_4 after drying.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Insoluble Substances Not more than 0.2%.

Lead Not more than 5 ppm.

Loss on Drying Not more than 1%.

TESTS

Assay Transfer about 5 g of the sample, previously dried at 105° for 4 h and accurately weighed, into a 250-ml beaker, add 100 ml of water and 5.0 ml of 1 *N* hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*), if any, of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 and pH 8.8). Each ml of the volume $B - A$ of 1 *N* sodium hydroxide is equivalent to 136.1 mg of KH_2PO_4.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; sequestrant; yeast food.

Potassium Phosphate, Tribasic

Tripotassium Phosphate

K_3PO_4 Mol wt 212.27

DESCRIPTION

Tribasic potassium phosphate is anhydrous or may contain one molecule of water of hydration. It occurs as white, odorless, hygroscopic crystals or granules. It is freely soluble in water,

but is insoluble in alcohol. The pH of a 1 in 100 solution is about 11.5.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Potassium*, page 517, and for *Phosphate*, page 517.

Assay Not less than 97.0% of K_3PO_4, calculated on the ignited basis.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Insoluble Substances Not more than 0.2%.
Lead Not more than 5 ppm.
Loss on Ignition K_3PO_4 (anhydrous): not more than 3%; $K_3PO_4.H_2O$ (monohydrate): between 8% and 20%.

TESTS

Assay Dissolve an accurately weighed quantity of the sample, equivalent to about 8 g of anhydrous K_3PO_4, in 40 ml of water in a 400-ml beaker, and add 100.0 ml of 1 *N* hydrochloric acid. Pass a stream of carbon dioxide–free air, in fine bubbles, through the solution for 30 min to expel carbon dioxide, covering the beaker loosely to prevent any loss by spraying. Wash the cover and sides of the beaker with a few ml of water, and place the electrodes of a suitable pH meter in the solution. Titrate the solution with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4, then calculate the volume (*A*) of 1 *N* hydrochloric acid consumed. Protect the solution from absorbing carbon dioxide, and continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached. Calculate the volume (*B*) of 1 *N* sodium hydroxide consumed in this titration. When *A* is equal to or greater than 2*B*, each ml of the volume *B* of 1 *N* sodium hydroxide is equivalent to 212.3 mg of K_3PO_4. When *A* is less than 2*B*, each ml of the volume $A - B$ of 1 *N* sodium hydroxide is equivalent to 212.3 mg of K_3PO_4.
Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.
Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.
Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.
Loss on Ignition, page 518 Ignite at about 800° for 30 min.
Packaging and Storage Store in tight containers.
Functional Use in Foods Emulsifier.

Potassium Polymetaphosphate

Potassium Metaphosphate; Potassium Kurrol's Salt

$(KPO_3)x$

DESCRIPTION

Potassium polymetaphosphate is a straight-chain polyphosphate having a high degree of polymerization. It occurs as a white, odorless powder. It is insoluble in water, but is soluble in dilute solutions of sodium salts.

REQUIREMENTS

Identification

A. Finely powder about 1 g of the sample, and add it slowly to 100 ml of a 1 in 50 solution of sodium chloride while stirring vigorously. A gelatinous mass is formed.
B. Mix 500 mg with 10 ml of nitric acid and 50 ml of water, boil for about 30 min, and cool. The resulting solution gives positive tests for *Potassium*, page 517, and for *Phosphate*, page 517.

Assay Not less than 59.0% and not more than 61.0% of P_2O_5.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 5 ppm.
Viscosity Between 6.5 and 15 centipoises.

TESTS

Assay Transfer about 1 g of the sample, accurately weighed, into a 500-ml volumetric flask, add 100 ml of water and 25 ml of nitric acid, and boil for 10 min on a hot plate. Cool, dilute to volume with water, and mix. Pipet 20.0 ml of this solution into a 500-ml Erlenmeyer flask, add 100 ml of water, and heat just to boiling. Add with stirring 50 ml of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared Gooch crucible (or fritted-glass crucible of medium porosity), and wash with five 25-ml portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 µg of P_2O_5.
Arsenic A solution of 1 g in 15 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.
Fluoride Place 5 g of the sample, 25 ml of water, 50 ml of sulfuric acid, 5 drops of silver nitrate solution (1 in 2), and a few glass beads in a 250-ml distilling flask connected with a

condenser and carrying a thermometer and a capillary tube, both of which must extend into the liquid. Connect a small dropping funnel, filled with water, or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole that exposes about one third of the flask to the flame. Distil into a 250-ml flask until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary to maintain the temperature between 135° and 140°. Continue the distillation until 225 to 240 ml has been collected, then dilute to 250 ml with water, and mix.

Place a 50-ml aliquot of this solution in a 100-ml Nessler tube. In another similar Nessler tube place 50 ml of water as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarinsulfonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4000), and mix well. Add, dropwise, and with stirring, 0.05 *N* sodium hydroxide to the tube containing the distillate until its color just matches that of the control, which is faintly pink. Then add to each tube exactly 1.0 ml of 0.1 *N* hydrochloric acid, and mix well. From a buret, graduated in 0.05-ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the color of the liquid just changes to a faint pink. Note the volume of the solution added, add exactly the same volume to the control, and mix. Now add to the control sodium fluoride TS (10 μg F per ml) from a buret to make the colors of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final color comparison. Check the endpoint by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct color change should take place. Note the volume of sodium fluoride TS added. The volume of sodium fluoride TS required for the control solution should not exceed 1.0 ml.

Heavy Metals Warm 1 g with 10 ml of diluted hydrochloric acid TS until no more dissolves, dilute with water to 25 ml, and filter. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 5 μg of lead ion (Pb) in the control.

Viscosity Dissolve 300 mg of the sample in 200 ml of a solution of sodium pyrophosphate (3.5 g of $Na_4P_2O_7$ dissolved in 1000 ml of water), using a magnetic stirrer. When solution is complete, or after 30 min, whichever occurs first, transfer 10 ml into an Ostwald-Fenske viscometer, and determine the time, *T*, in seconds, required for the liquid to flow from the upper to the lower mark in the capillary tube. Calculate the viscosity, in centipoises, by the formula *Tv/dt*, in which *t* is the time, in seconds, required for a glycerin-water mixture of known viscosity, *v*, and specific gravity, *d*, to flow from the upper to the lower mark of the capillary tube during calibration of the viscometer under similar conditions.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Fat emulsifier; moisture-retaining agent.

Potassium Pyrophosphate

Tetrapotassium Pyrophosphate

$K_4P_2O_7$ Mol wt 330.34

DESCRIPTION

Colorless crystals, or a white, granular solid. It is hygroscopic. It is very soluble in water, but is insoluble in alcohol. The pH of a 1 in 100 solution is about 10.5.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Potassium*, page 517.

B. Dissolve 100 mg of the sample in 100 ml of diluted nitric acid TS. Add 0.5 ml of this solution to 30 ml of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 ml of the solution to 30 ml of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 95.0% of $K_4P_2O_7$, calculated on the ignited basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Insoluble Substances Not more than 0.1%.

Lead Not more than 5 ppm.

Loss on Ignition Not more than 0.5%.

TESTS

Assay Dissolve about 600 mg, accurately weighed, in 100 ml of water in a 400-ml beaker, and adjust the pH of the solution to exactly 3.8 with hydrochloric acid, using a pH meter. Add 50 ml of a 1 in 8 solution of zinc sulfate (125 g of $ZnSO_4.7H_2O$ dissolved in water, diluted to 1000 ml, filtered, and adjusted to pH 3.8), and allow to stand for 2 min. Titrate the liberated acid with 0.1 *N* sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the endpoint, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 *N* sodium hydroxide is equivalent to 16.52 mg of $K_4P_2O_7$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the

insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Ignition Ignite at about 800° for 30 min.

Packaging and Storage Store in tight containers.

Functional Use in Foods Emulsifier; texturizer.

Potassium Sorbate

2,4-Hexadienoic Acid, Potassium Salt

$$CH_3CH=CHCH=COOK$$

$C_6H_7KO_2$ Mol wt 150.22

DESCRIPTION

White crystals, crystalline powder, or pellets. It melts at about 270° with decomposition. It is soluble in alcohol and freely soluble in water.

REQUIREMENTS

Identification

A. A 1 in 10 solution gives positive tests for *Potassium*, page 517.

B. To 2 ml of a 1 in 10 solution add a few drops of bromine TS. The color is discharged.

Assay Not less than 98.0% and not more than the equivalent of 101.0% of $C_6H_7KO_2$, calculated on the dried basis.

Acidity (as sorbic acid) Passes test (about 1%).

Alkalinity (as K_2CO_3) Passes test (about 1%).

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 1%.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 40 ml of glacial acetic acid in a 250-ml glass-stoppered Erlenmeyer flask, warming if necessary to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid to a blue green endpoint that persists for at least 30 s. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 15.02 mg of $C_6H_7KO_2$.

Acidity or Alkalinity Dissolve 1.1 g in 20 ml of water and add 3 drops of phenolphthalein TS. If the solution is colorless, titrate with 0.1 *N* sodium hydroxide to a pink color that persists for 15 s. Not more than 1.1 ml is required. If the solution is pink in color, titrate with 0.1 *N* hydrochloric acid. Not more than 0.8 ml is required to discharge the pink color.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 3 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Preservative.

Potassium Sulfate

K_2SO_4 Mol wt 174.25

DESCRIPTION

Colorless or white crystals or crystalline powder having a bitter, saline taste. One g dissolves in about 8.5 ml of water. It is insoluble in alcohol. The pH of a 1 in 20 solution is between 5.5 and 8.5.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Potassium*, page 517.

Assay Not less than 99.0% of K_2SO_4.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Selenium Not more than 30 ppm.

TESTS

Assay Dissolve about 500 mg, accurately weighed, in 200 ml of water, add 1 ml of hydrochloric acid, and heat to boiling. Gradually add, in small portions and while stirring constantly, an excess of hot barium chloride TS (about 8 or 9 ml), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.7466, indicates its equivalent of K_2SO_4.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 3 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 1 g of the sample in the control (*Solution A*).

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Water corrective; miscellaneous and general purpose.

Potassium Sulfite

K_2SO_3 Mol wt 158.25

DESCRIPTION

A white, odorless, granular powder. It undergoes oxidation in air. One g dissolves in about 3.5 ml of water. It is slightly soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Potassium*, page 517, and for *Sulfite*, page 517.

Assay Not less than 90.0% of K_2SO_3.
Alkalinity (as K_2CO_3) Between 0.25% and 0.45%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Selenium Not more than 30 ppm.

TESTS

Assay Dissolve about 750 mg, accurately weighed, in a mixture of 100.0 ml of 0.1 *N* iodine and 5 ml of diluted hydrochloric acid TS, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 7.912 mg of K_2SO_3.

Alkalinity Dissolve 1 g in 20 ml of water, add 25 ml of 3% hydrogen peroxide, previously neutralized to methyl red TS, mix thoroughly, cool to room temperature, and titrate with 0.02 *N* hydrochloric acid. Perform a blank determination (see page 2) using 25 ml of the neutralized hydrogen peroxide solution. Each ml of 0.02 *N* hydrochloric acid is equivalent to 1.38 mg of K_2CO_3.

Arsenic Dissolve 1 g of the sample in 10 ml of water in a 150-ml beaker, cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur trioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool, repeat the washing and fuming procedure, and cool again. This solution meets the requirements of the *Arsenic Test*, page 464, omitting the addition of 20 ml of dilute sulfuric acid (1 in 5).

Heavy Metals Dissolve 2 g in 10 ml of water, add 4 ml of hydrochloric acid, and evaporate to dryness on a steam bath. To the residue add 5 ml of hot water and 1 ml of hydrochloric acid, and again evaporate to dryness. Dissolve the residue in water and dilute to 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in tight containers.
Functional Use in Foods Preservative; antioxidant.

Potassium Tripolyphosphate

Pentapotassium Triphosphate; Potassium Triphosphate

$K_5P_3O_{10}$ Mol wt 448.41

DESCRIPTION

White granules or a white powder. It is hygroscopic and is very soluble in water. The pH of a 1 in 100 solution is between 9.2 and 10.1.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Potassium*, page 517.
B. To 1 ml of a 1 in 100 solution add a few drops of silver nitrate TS. A white precipitate is formed that is soluble in diluted nitric acid TS.

Assay Not less than 85.0% of $K_5P_3O_{10}$.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 10 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Insoluble Substances Not more than 2%.
Lead Not more than 5 ppm.
Loss on Drying Not more than 0.7%.

TESTS

Assay Proceed as directed in the *Assay* under *Sodium Tripolyphosphate*, page 305. Calculate the quantity, in mg, of $K_5P_3O_{10}$ in the sample taken by the formula $0.650 \times 25V$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

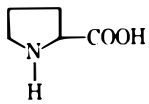
Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at about 105° for 1 h.

Packaging and Storage Store in tight containers.
Functional Use in Foods Texturizer.

L-Proline

L-2-Pyrrolidinecarboxylic Acid



$C_5H_9NO_2$ Mol wt 115.13

DESCRIPTION

White crystals or a crystalline powder. It is odorless and has a slightly sweet taste. It is very soluble in water and in alcohol, but is insoluble in ether.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution of the sample add 1 ml of triketohydrindene hydrate TS. A yellow color is produced.

Assay Not less than 98.5% and mot more than the equivalent of 101.0% of $C_5H_9NO_2$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20}$: Between $-84.0°$ and $-86.0°$, on the dried basis.

TESTS

Assay Dissolve about 220 mg of the sample, previously dried at 105° for 3 h and accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Each ml of 0.1 *N* perchloric acid is equivalent to 11.51 mg of $C_5H_9NO_2$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 4 g of a previously dried sample in sufficient water to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

Propionic Acid

CH_3CH_2COOH

$C_3H_6O_2$ Mol wt 74.08

DESCRIPTION

An oily liquid having a slightly pungent, rancid odor. It is miscible with water and with alcohol and various other organic solvents.

REQUIREMENTS

Assay Not less than 99.5% of $C_3H_6O_2$.
Aldehydes (as propionaldehyde) Passes test (limit about 0.05%).
Arsenic (as As) Not more than 3 ppm.
Distillation Range Between 138.5° and 142.5°.
Heavy Metals (as Pb) Not more than 10 ppm.
Nonvolatile Residue Not more than 0.01%.
Readily Oxidizable Substances (as formic acid) Passes test (limit about 0.05%).
Specific Gravity Between 0.993 and 0.997 at 20°/20°.
Water Not more than 0.15%.

TESTS

Assay Mix about 1.5 g, accurately weighed, with 100 ml of recently boiled and cooled water in a 250-ml Erlenmeyer flask, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first appearance of a faint pink endpoint that persists for at least 30 s. Each ml of 0.5 *N* sodium hydroxide is equivalent to 37.04 mg of $C_3H_6O_2$.
Aldehydes Transfer 10.0 ml of the sample into a 250-ml glass-stoppered Erlenmeyer flask containing 50 ml of water and 10.0 ml of a 1 in 8 solution of sodium bisulfite, stopper the flask, and shake vigorously. Allow the mixture to stand for 30 min, then titrate with 0.1 *N* iodine to the same brownish yellow endpoint obtained with a blank treated with the same quantities of the same reagents (see page 2). The difference between the volume of 0.1 *N* iodine required for the blank and that required for the sample is not more than 1.75 ml.
Arsenic A *Sample Solution* prepared as directed for organic

compounds meets the requirements of the *Arsenic Test*, page 464.

Distillation Range Determine as directed in the general procedure, page 478.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Transfer 100 ml into a tared 125-ml platinum evaporating dish, previously heated at 105° to constant weight, and evaporate the sample to dryness on a steam bath. Heat the dish at 105° for 30 min or to constant weight, cool in a desiccator, and weigh.

Readily Oxidizable Substances Dissolve 15 g of sodium hydroxide in 50 ml of water, cool, add 6 ml of bromine, stirring to effect complete solution, and dilute to 2000 ml with water. Transfer 25.0 ml of this solution into a 250-ml glass-stoppered Erlenmeyer flask containing 100 ml of water, and add 10 ml of a 1 in 5 solution of sodium acetate and 10.0 ml of the sample. Allow to stand for 15 min, add 5 ml of a 1 in 4 solution of potassium iodide and 10 ml of hydrochloric acid, and titrate with 0.1 *N* sodium thiosulfate just to the disappearance of the brown color. Perform a blank determination (see page 2). The difference between the volume of 0.1 *N* sodium thiosulfate required for the blank and that required for the sample is not more than 2.2 ml.

Specific Gravity Determine by any reliable method (see page 3).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Preservative; mold and rope inhibitor.

Propylene Glycol

1,2-Propanediol; 1,2-Dihydroxypropane; Methyl Glycol

$$CH_3CH(OH)CH_2OH$$

$C_3H_8O_2$ Mol wt 76.10

DESCRIPTION

A clear, colorless, viscous liquid having a slight, characteristic taste. It is practically odorless. It absorbs moisture when exposed to moist air. It is miscible with water, acetone, and chloroform in all proportions. It is soluble in ether and will dissolve many essential oils, but is immiscible with fixed oils.

REQUIREMENTS

Identification

A. Mix 500 mg with 3.6 g of triphenylchloromethane and 5 ml of pyridine, and heat under a reflux condenser on a steam bath for 1 h. Cool, dissolve the mixture in 100 ml of warm acetone, and stir well with 100 mg of activated charcoal. Filter, evaporate the filtrate to about 50 ml, and allow to stand overnight in a refrigerator. Filter off the crystals, recrystallize until free from pyridine (three times), and dry in a current of air. The crystals so obtained melt at about 176° (see page 519).

B. Heat gently 1 ml with 500 mg of potassium bisulfate. A fruity odor is evolved, and when the solution is heated to dryness no sharp, acrid odor of acrolein is perceptible.

Assay Not less than 99.5%, by weight, of $C_3H_8O_2$.

Acidity Passes test.

Arsenic (as As) Not more than 3 ppm.

Distillation Range Between 185° and 189°.

Heavy Metals (as Pb) Not more than 10 ppm.

Residue on Ignition Not more than 0.007%.

Specific Gravity Between 1.035 and 1.037.

Water Not more than 0.2%.

TESTS

Assay Inject a 10-µl portion of the sample into a suitable gas chromatograph in which the detector is the thermal conductivity type and the column is 1-m × 1/4-in. stainless steel tubing packed with 4% Carbowax compound 20 M on 40/60-mesh Chromosorb T, or equivalent materials. The carrier is helium flowing at 75 ml per min. The injection port temperature is 240°; the column temperature is 120° to 200°, programmed at a rate of 5° per min; and the block temperature is 250°. Under the conditions described, the approximate retention time for propylene glycol is 5.7 min, and for the three isomers of dipropylene glycol, 8.2, 9.0, and 10.2 min. Measure the area under all peaks by any convenient means, and calculate the area percentage of propylene glycol and report as weight percentage.

Acidity Add 1 ml of phenolphthalein TS to 50 ml of water, then add 0.1 *N* sodium hydroxide until the solution remains pink for 30 s. To this solution add 10 ml of the sample, accurately measured, and titrate with 0.1 *N* sodium hydroxide until the original pink color returns and remains for 30 s. Not more than 0.2 ml of 0.1 *N* sodium hydroxide is required.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Distillation Range Determine as directed in the general procedure, page 478.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Residue on Ignition, page 533 Heat a 50-g sample in a tared 100-ml shallow dish until it ignites, and allow it to burn without further application of heat in a place free from drafts. Cool, moisten the residue with 5 ml of sulfuric acid, and ignite at about 800° for 15 min.

Specific Gravity Determine by any reliable method (see page 3).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.
Functional Use in Foods Solvent; wetting agent; humectant.

Propylene Glycol Alginate

Hydroxypropyl Alginate; Algin Derivative

$(C_9H_{14}O_7)_n$ Equiv wt, *Calculated*, 234.20

DESCRIPTION

The propylene glycol ester of alginic acid (see *Alginic Acid*, page 14) varies in composition according to its degree of esterification and the percentages of free and neutralized carboxyl groups in the molecule. It occurs as a white to yellowish, fibrous or granular powder. It is practically odorless and tasteless. It dissolves in water, in solutions of dilute organic acids, and, depending upon the degree of esterification, in hydroalcoholic mixtures containing up to 60% by weight of alcohol to form stable, viscous colloidal solutions at a pH of 3.

REQUIREMENTS

Identification

Transfer 20 ml of the saponified solution obtained in the determination of *Esterified Carboxyl Groups* into a 250-ml Erlenmeyer flask, add 50 ml of 0.1 *M* periodic acid, swirl, and allow to stand for 30 min. Add 2 g of potassium iodide, titrate with 0.1 *N* sodium thiosulfate to a faint yellow color, and then dilute the mixture to 200 ml with water. To 10 ml of this solution add 5 ml of hydrochloric acid and 10 ml of modified Schiff's reagent. A blue to blue violet color develops in about 20 min (*formaldehyde*). To another 10-ml portion of the solution add 1 ml of a saturated solution of piperazine hydrate and 0.5 ml of sodium nitroferricyanide TS. A green color develops (*acetaldehyde*). (NOTE: Oxidation of propylene glycol alginate yields formaldehyde and acetaldehyde.)

Assay It yields not less than 16% and not more than 20% of carbon dioxide (CO_2), calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Ash Not more than 10% after drying.
Esterified Carboxyl Groups Between 40% and 85%.
Free Carboxyl Groups Not more than 35%, calculated on the dried basis.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 20%.
Neutralized Carboxyl Groups Between 10% and 45%.

TESTS

Assay Proceed as directed in the *Alginates Assay*, page 463.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Ash Determine as directed under *Ash* in the monograph on *Alginic Acid*, page 14.
Esterified Carboxyl Groups Transfer quantitatively the solution obtained in the determination of *Free Carboxyl Groups* into a 1-L Erlenmeyer flask, add a few drops of phenolphthalein TS and 50.0 ml of 0.1 *N* sodium hydroxide. Stopper the flask, swirl the solution, and then allow it to stand for 30 min at room temperature. Titrate the excess sodium hydroxide to a faint pink endpoint with 0.1 *N* hydrochloric acid. Transfer the solution to a 600-ml beaker and complete the titration to a pH of 7.0, determining the endpoint potentiometrically. Calculate the percentage of esterified carboxyl groups by the formula

$$\frac{(\text{No. ml } 0.1\ N\ \text{NaOH consumed}) \times 44}{\%\ CO_2 \times \text{wt of sample in g}},$$

in which % CO_2 is the percentage of carbon dioxide as determined in the *Assay*.
Free Carboxyl Groups Transfer about 1 g, accurately weighed, into a 600-ml beaker. Dissolve the sample in 200 ml of water, stirring mechanically for a minimum of 30 min, and titrate with 0.1 *N* sodium hydroxide to a pH of 7.0, determining the endpoint potentiometrically. Calculate the percentage of free carboxyl groups by the formula

$$(V \times 44)/(\%\ CO_2 \times W),$$

in which V is the volume of 0.1 *N* sodium hydroxide consumed, in ml; % CO_2 is the percentage of carbon dioxide in the sample as determined by the *Assay*; and W is the weight of the sample taken, in g, calculated on the dried basis.
Heavy Metals Determine as directed in the test for *Heavy Metals* under *Alginic Acid*, page 14.
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 4 h.
Neutralized Carboxyl Groups Calculate the percentage of neutralized carboxyl groups by subtracting the sum of the percentage of *Free Carboxyl Groups* and the percentage of *Esterified Carboxyl Groups* from 100%.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Propylene Glycol Mono- and Diesters

Propylene Glycol Mono- and Diesters of Fatty Acids;
Propylene Glycol Monostearate (or other appropriate ester)

DESCRIPTION

A mixture of propylene glycol mono- and diesters of fats and/or fatty acids. It has a bland odor and taste and occurs as a clear liquid or as white to yellowish white beads, flakes, or other solid material. It is insoluble in water, but is soluble in alcohol, in ethyl acetate, and in chloroform and other chlorinated hydrocarbons.

REQUIREMENTS

Acid Value Not more than 4.
Arsenic(as As) Not more than 3 ppm.
Free Propylene Glycol Not more than 1.5%.
Heavy Metals(as Pb) Not more than 10 ppm.
Hydroxyl Value, Iodine Value, and Saponification Value Not greater than the values stated or within the range claimed by the vendor.
Residue on Ignition Not more than 0.5%.
Soap (as potassium stearate) Not more than 7%.
Total Monoester Content Not less than the minimum percentage claimed by the vendor.

TESTS

Acid Value Determine as directed in *Method II* under *Acid Value*, page 14.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Free Propylene Glycol Determine as directed under *Free Glycerin or Propylene Glycol*, page 504.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504, using about 2 g, accurately weighed.
Iodine Value Determine by the *Wijs Method*, page 505.
Residue on Ignition Ignite 5 g as directed in the general method, page 533.
Saponification Value Weigh accurately about 4 g, and proceed as directed in the general method, page 509.
Soap Weigh accurately about 5 g, and proceed as directed in the general method, page 509, using 31.0 as the equivalence factor (*e*) in the calculation.
Total Monoester Content Determine the percentage of free propylene glycol (*G*) in the sample as directed under *Free Glycerin or Propylene Glycol*, page 504, and determine the *Hydroxyl Value* (*H*) as directed in *Method II* of the general procedure, page 504. Calculate the hydroxyl equivalent of free propylene glycol (*F*) by the formula 561.1*G*/38.

Separate the fatty acids as described in the test for *Lauric Acid* under *Polysorbate 20*, page 234, and determine the *Acid Value* (*AV*) of the acids as directed in *Method I* of the general procedure, page 503.

Calculate the average molecular weight (*mol wt*) of the monoester by the formula

$$(56{,}109/AV) + 76.10 - 18.02.$$

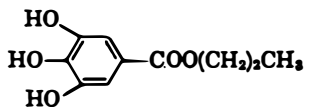
Finally, calculate the percentage of total monoester in the original sample by the formula

$$(H - F) \times mol\ wt/561.$$

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Emulsifier; stabilizer.

Propyl Gallate

Gallic Acid, Propyl Ester



$C_{10}H_{12}O_5$ Mol wt 212.20

DESCRIPTION

A fine, white to nearly white, odorless powder having a slightly bitter taste. It is slightly soluble in water and freely soluble in alcohol and in ether.

REQUIREMENTS

Identification

Place about 5 g of the sample and several boiling chips in a 500-ml round-bottom flask, connect a water-cooled condenser to the flask, and introduce a steady stream of nitrogen into the flask, maintaining the flow of nitrogen at all times during the remainder of the procedure. Pour 100 ml of 1 *N* sodium hydroxide through the top of the condenser, heat the solution to boiling, boil for 30 min, and cool. Place the reaction flask in an ice bath, and slowly, with occasional swirling, add dilute sulfuric acid (10%) until a pH of 2 to 3 is obtained (using pH paper). Filter the precipitate through a sintered-glass crucible, wash with a minimum amount of water, and dry at 110° for 2 h. The gallic acid so obtained melts at about 240° with decomposition (see page 519).

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{10}H_{12}O_5$ after drying.
Arsenic(as As) Not more than 3 ppm.
Heavy Metals(as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Melting Range Between 146° and 150°.
Residue on Ignition Not more than 0.1%.

TESTS

Assay Transfer about 200 mg, previously dried at 110° for 4 h and accurately weighed, to a 400-ml beaker, dissolve it in 150 ml of water, and heat to boiling. With constant and vigorous stirring, add 50 ml of bismuth nitrate TS, continue stirring and heating until precipitation is complete, and cool. Filter the yellow precipitate on a tared sintered-glass crucible, wash it with cold dilute nitric acid (1 in 300), and dry at 110° to constant weight. The weight of the precipitate so obtained, multiplied by 0.4866, represents its equivalent of $C_{10}H_{12}O_5$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 110° for 4 h.

Melting Range Determine as directed in the general procedure, page 519, after drying at 110° for 4 h.

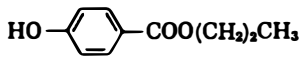
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Antioxidant.

Propylparaben

Propyl *p*-Hydroxybenzoate



$C_{10}H_{12}O_3$ Mol wt 180.20

DESCRIPTION

Small, colorless crystals or a white powder. One g dissolves in about 2500 ml of water at 25°, in about 400 ml of boiling water, in about 1.5 ml of alcohol, and in about 3 ml of ether.

REQUIREMENTS

Identification

Dissolve about 500 mg in 10 ml of sodium hydroxide TS, and boil for 30 min, allowing the solution to evaporate to a volume of about 5 ml. Cool the mixture, and carefully acidify with diluted sulfuric acid TS. Collect the precipitate on a filter when cool, wash it several times with small portions of water, and dry in a desiccator over silica gel. The liberated *p*-hydroxybenzoic acid melts between 212° and 217° (see page 519).

Assay Not less than 99.0% of $C_{10}H_{12}O_3$, calculated on the dried basis.

Acidity Passes test.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

Melting Range Between 95° and 98°.

Residue on Ignition Not more than 0.05%.

TESTS

Assay Place in a flask about 2 g, accurately weighed, add 40.0 ml of 1 *N* sodium hydroxide, and rinse the sides of the flask with water. Cover with a watch glass, boil gently for 1 h, cool, and titrate the excess sodium hydroxide with 1 *N* sulfuric acid to pH 6.5. Perform a blank determination with the same quantities of the same reagents in the same manner, and make any necessary correction (see page 2). Each ml of 1 *N* sodium hydroxide is equivalent to 180.2 mg of $C_{10}H_{12}O_3$, calculated on the dried basis.

Acidity Heat 750 mg with 15 ml of water at 80° for 1 min, cool, and filter. The filtrate is acid or neutral to litmus. To 10 ml of the filtrate add 0.2 ml of 0.1 *N* sodium hydroxide and 2 drops of methyl red TS. The solution is yellow, without even a light cast of pink.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals, page 512 Dissolve 2 g in 23 ml of acetone, and add 2 ml of diluted acetic acid TS, 2 ml of water, and 10 ml of hydrogen sulfide TS. Any color does not exceed that produced in a control made with 23 ml of acetone, 2 ml of diluted acetic acid TS, 2 ml of *Standard Lead Solution* (20 µg Pb ion), and 10 ml of hydrogen sulfide TS.

Loss on Drying, page 518 Dry over silica gel for 5 h.

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition Ignite 4 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Preservative; antimicrobial agent.

PVP

Polyvinylpyrrolidone; Povidone; Poly[1-(2-oxo-1-pyrrolidinyl)ethylene]

DESCRIPTION

PVP is a polymer of purified 1-vinyl-2-pyrrolidone produced catalytically. It occurs as a white to tan powder, free from objectionable odor. It is soluble in water, in alcohol, and in chloroform, and is insoluble in ether. The pH of a 1 in 20 solution is between 3 and 7.

REQUIREMENTS

Identification

A. To 10 ml of a 1 in 50 solution of the sample add 20 ml of 1 *N* hydrochloric acid and 5 ml of potassium dichromate TS. An orange yellow precipitate is produced.
B. Add 5 ml of a 1 in 50 solution of the sample to 75 mg of cobalt nitrate and 300 mg of ammonium thiocyanate dissolved in 2 ml of water, mix, and then make the resulting solution acid with diluted hydrochloric acid TS. A pale blue precipitate forms.
C. To 5 ml of a 1 in 200 solution of the sample add a few drops of iodine TS. A deep red color is produced.

Aldehydes (as acetaldehyde) Not more than 0.5%.
Arsenic (as As) Not more than 1 ppm.
Ash (Total) Not more than 0.02%.
Heavy Metals (as Pb) Not more than 10 ppm.
Nitrogen Not less than 11.5% and not more than 12.8%, calculated on the anhydrous basis.
Relative Viscosity of a 1 in 100 Solution Between 1.188 and 1.325, equivalent to an average molecular weight of 40,000 (determined by viscosimetric method). [NOTE: The relative viscosity of a 1 in 100 solution of PVP used as a clarifying agent in beer production is between 3.225 and 5.652, equivalent to an average molecular weight of 360,000 (determined by osmometric method).]
Unsaturation (as vinylpyrrolidone) Not more than 1.0%.
Water Not more than 5%.

TESTS

Aldehydes Transfer about 10 g of the sample, accurately weighed and dissolved in 300 ml of water, into a 1000-ml round-bottom flask containing 80 ml of 25% sulfuric acid, reflux for about 45 min under a water-cooled condenser, and then distil about 100 ml into a receiver containing 20.0 ml of 1 *N* hydroxylamine hydrochloride previously adjusted to pH 3.1. Titrate the contents of the receiver with 0.1 *N* sodium hydroxide to a pH of 3.1, using a pH meter. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* sodium hydroxide is equivalent to 4.405 mg of C_2H_4O.

Arsenic A *Sample Solution* prepared with 3 g of the sample as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Total) Weigh accurately about 10 g, and proceed as directed in the general method, page 466.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nitrogen Determine as directed in *Method II* under *Nitrogen Determination*, page 522, using a 100-mg sample. In the wet-digestion step, repeat the addition of hydrogen peroxide (usually three to six times) until a clear, light green solution is obtained, then heat for an additional 4 h, and continue as directed, beginning with "Cautiously add 2 ml of water. . . . "

Relative Viscosity Transfer an accurately weighed portion of the sample, equivalent to 1 g of anhydrous PVP, into a 250-ml Erlenmeyer flask, and calculate the amount of water to be added to make a 1.0% solution. Allow the mixture to stand at room temperature, with occasional swirling, until solution is complete, and then allow to stand for 1 h longer. Filter through a dry sintered-glass filter funnel of coarse porosity, then pipet 10.0 ml of the filtrate into a Cannon-Fenske viscometer, and place the viscometer in a water bath maintained at 25° ± 0.05°. After allowing the sample solution and pipet to warm in the water bath for 10 min, draw the solution by means of very gentle suction up through the capillary until the meniscus is from 3 to 4 mm above the upper etched mark. Release the vacuum, and, when the meniscus reaches the upper etched mark, begin timing the flow through the capillary. Record the exact time when the meniscus reaches the lower etched mark, and calculate the flow time to the nearest 0.1 s. Repeat this operation until at least three readings are obtained. The readings must agree within 0.3 s; if not, repeat the determination with additional 10-ml portions of the sample solution after recleaning the viscosity pipet with sulfuric acid–dichromate cleaning solution. Calculate the average flow time for the sample solution, and then obtain the flow time in a similar manner for 10 ml of filtered water for the same viscosity pipet. Calculate the relative viscosity, *z*, of the sample by dividing the average flow time of the sample solution by that of the water sample. (NOTE: The K-value may be calculated by the formula

$$\frac{\sqrt{300c(\log z) + [c + 1.5c(\log z)]^2} + 1.5c(\log z) - c}{0.15c + 0.003c^2}$$

in which *c* is the weight, in g, on the anhydrous basis, of the sample in each 100.0 g of solution, and *z* is as defined above.)

Unsaturation Dissolve about 4 g of the sample, accurately weighed, in 30 ml of water in a 125-ml round-bottom flask, add 500 mg of sodium acetate, mix, and begin titrating with 0.1 *N* iodine. When the iodine color no longer fades, add 3 additional ml of the titrant, and allow the solution to stand for 5 to 10 min. Add starch TS, and titrate the excess iodine with 0.1 N sodium thiosulfate. Perform a blank determination (see page 2), using the same volume of 0.1 *N* iodine, accurately measured, as was used for the sample. Each ml of 0.1 *N* iodine is equivalent to 5.556 mg of vinylpyrrolidone.

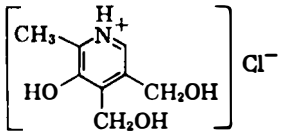
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Clarifying agent; stabilizer; bodying agent; tableting aid; dispersant.

Pyridoxine Hydrochloride

5-Hydroxy-6-methyl-3,4-pyridinedimethanol Hydrochloride; Vitamin B_6 Hydrochloride; Vitamin B_6



$C_8H_{11}NO_3.HCl$ Mol wt 205.64

DESCRIPTION

Colorless or white crystals or a white, crystalline powder. It is stable in air, but is slowly affected by sunlight. Its solutions are acid to litmus, having a pH of about 3. One g dissolves in 5 ml of water and in about 100 ml of alcohol. It is insoluble in ether. It melts at about 206° with some decomposition.

REQUIREMENTS

Identification

A. Place 1 ml of a solution containing about 100 µg in each ml into each of two test tubes marked *A* and *B*, and add to each tube 2 ml of a 1 in 5 sodium acetate solution. To tube *A* add 1 ml of water, and to tube *B* add 1 ml of a 1 in 25 boric acid solution, and mix. Cool both tubes to about 20°, and rapidly add to each tube 1 ml of a 1 in 200 solution of 2,6-dichloroquinonechlorimide in alcohol. A blue color is produced in *A*, which fades rapidly and becomes red in a few minutes, but no blue color is produced in *B*.

B. To 2 ml of a 1 in 200 solution add 0.5 ml of phosphotungstic acid TS. A white precipitate is formed.

C. It gives positive tests for *Chloride*, page 516.

Assay Not less than 98.0% of $C_8H_{11}NO_3.HCl$, calculated on the dried basis.

Chloride Content Not less than 16.9% and not more than 17.6% of Cl, calculated on the dried basis.

Heavy Metals (as Pb) Not more than 0.003%.

Loss on Drying Not more than 0.5%.

Residue on Ignition Not more than 0.1%.

TESTS

Assay Dissolve about 400 mg, accurately weighed, in a mixture of 10 ml of glacial acetic acid and 10 ml of mercuric acetate TS, warming slightly to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 20.56 mg of $C_8H_{11}NO_3.HCl$.

Chloride Content Dissolve about 500 mg of the sample, accurately weighed, in 50 ml of methanol in a glass-stoppered flask. Add 5 ml of glacial acetic acid and 2 to 3 drops of eosin Y TS, and titrate with 0.1 *N* silver nitrate. Each ml of 0.1 *N* silver nitrate is equivalent to 3.545 mg of Cl.

Heavy Metals Prepare and test a 670-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

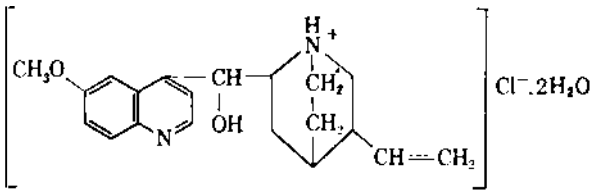
Loss on Drying, page 518 Dry in a vacuum over silica gel for 4 h.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

Packaging and Storage Store in tight, light-resistant containers, and avoid exposure to sunlight.

Functional Use in Foods Nutrient; dietary supplement.

Quinine Hydrochloride



$C_{20}H_{24}N_2O_2.HCl.2H_2O$ Mol wt 396.92

DESCRIPTION

White, silky, glistening needles. It is odorless, has a very bitter taste, and effloresces when exposed to warm air. Its solutions are neutral or alkaline to litmus. One g dissolves in 16 ml of water, in 1 ml of alcohol, in about 7 ml of glycerin, and in about 1 ml of chloroform. It is very slightly soluble in ether.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 1000 solution of the sample add 1 or 2 drops of bromine TS followed by 1 ml of ammonia TS. The liquid acquires an emerald green color due to the formation of thalleioquin.

B. A 1 in 100 solution is levorotatory (see page 530).

C. It gives positive tests for *Chloride*, page 516.

Assay Not less than 99.0% and not more than 101.5% of $C_{20}H_{24}N_2O_2.HCl$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Barium Passes test.

Chloroform-Alcohol Insoluble Substances Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 10%.

Other Cinchona Alkaloids Passes test.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.15%.

Specific Rotation $[\alpha]_D^{25}$: Between −247° and −252°.

Sulfate Not more than 0.05%.

TESTS

Assay Dissolve about 150 mg of the sample, accurately weighed, in 20 ml of acetic anhydride, add 2 drops of malachite green TS and 5.5 ml of mercuric acetate TS, and titrate with 0.1 *N* perchloric acid from a microburet to a yellow endpoint. Perform a blank determination (see page 2). Each ml of 0.1 *N* perchloric acid is equivalent to 17.99 mg of $C_{20}H_{24}N_2O_2.HCl$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Barium To 10 ml of a hot solution of the sample (1 in 20) add 1 ml of diluted sulfuric acid TS. No turbidity is produced.

Chloroform-Alcohol Insoluble Substances One g dissolves completely in 7 ml of a mixture of 2 volumes of chloroform and 1 volume of absolute alcohol.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 120° for 3 h.

Other Cinchona Alkaloids Dissolve about 2.5 g in 60 ml of water in a separator, add 10 ml of ammonia TS, extract the mixture successively with 30 ml and 20 ml of chloroform, and evaporate the combined chloroform extracts to dryness on a steam bath. Dissolve 1.5 g of the residue in 25 ml of alcohol, dilute the solution with 50 ml of hot water, add 1 *N* sulfuric acid (about 5 ml) until the solution is acid, using 2 drops of methyl red TS as the indicator, and neutralize the excess of acid with 1 *N* sodium hydroxide. Evaporate the solution to dryness on a steam bath, powder the residue, and agitate it in a test tube with 20 ml of water at 65° for 30 min. Cool the mixture to 15°, macerate it at this temperature for 2 h with occasional shaking, and then filter it through a filter paper (8 to 10 cm). Transfer 5 ml of the filtrate, at a temperature of 15°, to a test tube, and mix it gently, with shaking, with 6 ml of ammonia TS (which must contain between 10% and 10.2% of NH_3, have a temperature of 15°, and be added at once). A clear liquid is produced.

Readily Carbonizable Substances, page 532 Dissolve 100 mg in 2 ml of sulfuric acid TS. The solution is no darker than *Matching Fluid M*.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

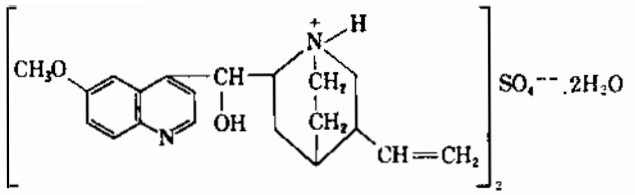
Specific Rotation, page 530 Determine in a solution containing 200 mg in 10 ml of 0.1 *N* hydrochloric acid.

Sulfate, page 471 Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 250 µg of sulfate (SO_4).

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Flavoring agent.

Quinine Sulfate



$(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$ Mol wt 782.96

DESCRIPTION

Fine, white, needlelike crystals, usually lusterless, making a light and readily compressible mass. It is odorless and has a persistent, very bitter taste. It darkens on exposure to light. Its saturated solution is neutral or alkaline to litmus. One g dissolves in about 500 ml of water and in about 120 ml of alcohol at 25°, in about 35 ml of water at 100°, and in about 10 ml of alcohol at 80°. It is slightly soluble in chloroform and in ether, but is freely soluble in a mixture of 2 volumes of chloroform and 1 volume of absolute alcohol.

REQUIREMENTS

Identification

A. Acidify a saturated solution of the sample with diluted sulfuric acid TS. The resulting solution has a vivid blue fluorescence and is levorotatory (see page 530).

B. To 5 ml of a 1 in 1000 solution add 1 or 2 drops of bromine TS followed by 1 ml of ammonia TS. The liquid acquires an emerald green color due to the formation of thalleioquin.

C. A 1 in 50 solution made with the aid of a few drops of hydrochloric acid gives positive tests for *Sulfate*, page 517.

Assay Not less than 99.0% and not more than 101.0% of $(C_{20}H_{24}N_2O_2)_2.H_2SO_4$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Chloroform-Alcohol Insoluble Substances Not more than 0.1%.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 5%.

Other Cinchona Alkaloids Passes test.

Readily Carbonizable Substances Passes test.

Residue on Ignition Not more than 0.05%.

Specific Rotation $[\alpha]_D^{25°}$: Between −240° and −244°.

TESTS

Assay Dissolve about 200 mg of the sample, accurately weighed, in 20 ml of acetic anhydride, add 2 drops of malachite green TS, and titrate with 0.1 *N* perchloric acid from a microburet to a yellow endpoint. Perform a blank determination (see page 2). Each ml of 0.1 *N* perchloric acid is equivalent to 24.90 mg of $(C_{20}H_{24}N_4O_2)_2.H_2SO_4$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloroform-Alcohol Insoluble Substances Warm 2 g of the sample with 15 ml of a mixture of 2 volumes of chloroform and 1 volume of absolute alcohol at 50° for 10 min. Filter through a tared, sintered-glass filter, using gentle suction, and wash the filter with five 10-ml portions of the chloroform-alcohol mixture. Dry at 105° for 1 h, cool, and weigh.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 120° for 3 h.

Other Cinchona Alkaloids Agitate 1.8 g, previously dried at 50° for 2 h, with 20 ml of water at 65° for 30 min. Cool the mixture to 15°, macerate it at this temperature for 2 h with occasional shaking, and then filter it through a filter paper (8 to 10 cm). Transfer 5 ml of the filtrate, at a temperature of 15°, to a test tube, and mix it gently, without shaking, with 6 ml of ammonia TS (which must contain between 10% and 10.2% of NH_3, have a temperature of 15°, and be added at once). A clear liquid is produced.

Readily Carbonizable Substances, page 532 Dissolve 200 mg in 5 ml of sulfuric acid TS. The solution is no darker than *Matching Fluid M*.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

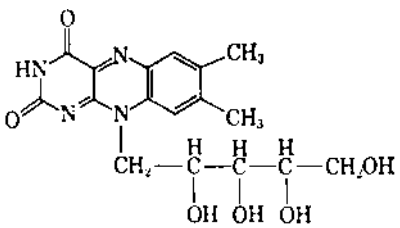
Specific Rotation, page 530 Determine in a solution containing 200 mg in 10 ml of 0.1 *N* hydrochloric acid.

Packaging and Storage Store in well-closed, light-resistant containers.

Functional Use in Foods Flavoring agent.

Riboflavin

Vitamin B_2



$C_{17}H_{20}N_4O_6$ Mol wt 376.37

DESCRIPTION

A yellow to orange yellow, crystalline powder having a slight odor. It melts at about 280° with decomposition, and its saturated solution is neutral to litmus. When dry, it is not affected by diffused light, but when in solution, light induces deterioration. One g dissolves in from 3000 to about 20,000 ml of water, the variations being due to differences in the internal crystalline structure. It is less soluble in alcohol than in water. It is insoluble in ether and in chloroform, but is very soluble in dilute solutions of alkalies. In aqueous sodium hydroxide solutions it is levorotatory, and in aqueous hydrochloric acid it is dextrorotatory.

REQUIREMENTS

Identification

A solution of 1 mg in 100 ml of water is pale greenish yellow by transmitted light and has an intense yellowish green fluorescence that disappears upon the addition of mineral acids or alkalies.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{17}H_{20}N_4O_6$, calculated on the dried basis.

Loss on Drying Not more than 1.5%.

Lumiflavin Passes test.

Residue on Ignition Not more than 0.3%.

TESTS

Assay (NOTE: Conduct this assay so that the solutions are protected from direct sunlight at all stages.) Place about 50 mg, accurately weighed, in a 1000-ml volumetric flask containing about 50 ml of water. Add 5 ml of 6 *N* acetic acid and sufficient water to make about 800 ml. Heat on a steam bath, protected from light, with frequent agitation until dissolved. Cool to about 25°, add water to volume, and mix. Dilute this solution with water, quantitatively and stepwise, to bring it within the operating sensitivity of the fluorometer used.

In the same manner, prepare a standard solution to contain, in each ml, a quantity of USP Riboflavin Reference Standard, accurately weighed, equivalent to that of the solution prepared as directed in the preceding paragraph, and measure the intensity of its fluorescence in a fluorometer at about 530 nm, using an excitation wavelength of about 440 nm. Immediately after the reading, add to the solution about 10 mg of sodium hydrosulfite, stirring with a glass rod until dissolved, and at once measure the fluorescence again. The difference between the two readings represents the intensity of the fluorescence due to the standard.

Similarly, measure the intensity of the fluorescence of the final solution of the riboflavin being assayed, before and after the addition of sodium hydrosulfite. Calculate the quantity of $C_{17}H_{20}N_4O_6$ in the final solution of riboflavin by the formula $C(I_U/I_S)$, in which C is the concentration, in µg per ml, of USP Riboflavin Reference Standard in the final solution of the standard and I_U and I_S are the corrected fluorescence values observed for the solutions of the riboflavin and the standard, respectively.

Loss on Drying, page 518 Dry at 105° for 2 h.

Lumiflavin Prepare alcohol-free chloroform as follows: Shake 20 ml of chloroform gently but thoroughly with 20 ml of water for 3 min, draw off the chloroform layer, and wash twice more with 20-ml portions of water. Finally, filter the chloroform through a dry filter paper, shake it well for 5 min with 5 g of powdered anhydrous sodium sulfate, allow the mixture to stand for 2 h, and decant or filter the clear chloroform.

Shake 25 mg of the sample with 10 ml of the alcohol-free chloroform for 5 min, and filter. The absorbance of the filtrate, determined in a 1-cm cell at 440 nm with a suitable spectrophotometer, using alcohol-free chloroform as the blank, does not exceed 0.025.

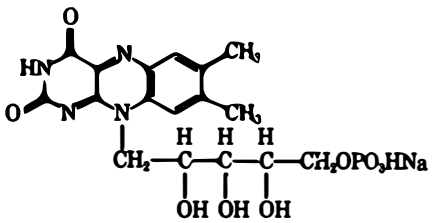
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Riboflavin 5'-Phosphate Sodium

Riboflavin 5'-Phosphate Ester Monosodium Salt



$C_{17}H_{20}N_4NaO_9P.2H_2O$ Mol wt 514.36

DESCRIPTION

A fine, orange yellow, crystalline powder having a slight odor. One g dissolves in about 30 ml of water. When dry, it is not affected by diffused light, but when in solution, light induces deterioration rapidly. It is hygroscopic.

REQUIREMENTS

Identification

A solution of 1.5 mg in 100 ml of water responds to the *Identification Test* under *Riboflavin*, page 262.

Assay Not less than the equivalent of 70.0% and not more than the equivalent of 75.0% of riboflavin ($C_{17}H_{20}N_4O_6$).

Free Phosphate Not more than 1%, calculated as PO_4.

Free Riboflavin Not more than 6%.

Loss on Drying Not more than 7%.

pH of a 1 in 100 Solution Between 5.0 and 6.5.

Residue on Ignition Not more than 25%.

Riboflavin Diphosphate Not more than 6%, calculated as riboflavin.

Specific Rotation $[\alpha]_D^{25°}$: Between +37.0° and +42.0°, calculated on the dried basis.

TESTS

Assay (NOTE: Use low-actinic glassware, and conduct this assay so that all solutions are protected from direct sunlight at all stages.)

Assay Preparation Transfer about 50 mg of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, add 20 ml of pyridine and 75 ml of water, and dissolve the sample by frequent shaking. Transfer the solution to a 1000-ml volumetric flask, dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a second 1000-ml volumetric flask, add sufficient 0.1 *N* sulfuric acid (about 4 ml) so that the final pH of the solution is between 5.9 and 6.1, dilute to volume with water, and mix.

Standard Preparation Transfer about 35 mg of USP Riboflavin Reference Standard, accurately weighed, into a 250-ml Erlenmeyer flask, add 20 ml of pyridine and 75 ml of water, and dissolve the riboflavin by frequent shaking. Transfer the solution to a 1000-ml volumetric flask, dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a second 1000-ml volumetric flask, add sufficient 0.1 *N* sulfuric acid (about 4 ml) so that the final pH of the solution is between 5.9 and 6.1, dilute to volume with water, and mix.

Procedure With a suitable fluorometer, determine the intensity of the fluorescence of each solution at about 530 nm, using an excitation wavelength of about 440 nm. Record the fluorescence of the *Assay Preparation* as I_U, and that of the *Standard Preparation* as I_S. Calculate the quantity, in mg of $C_{17}H_{20}N_4O_6$ in the sample taken, by the formula $100C \times I_U/I_S$, in which C is the exact concentration, in μg per ml, of the *Standard Preparation*, corrected for loss on drying.

Free Phosphate

Standard Preparation Transfer 220.0 mg of monobasic potassium phosphate, KH_2PO_4, into a 1000-ml volumetric flask, dissolve in and dilute to volume with water, and mix. Transfer 20.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

Test Preparation Transfer 300.0 mg of the sample into a 100-ml volumetric flask, dissolve in and dilute to volume with water, and mix.

Acid Molybdate Solution Dilute 25 ml of ammonium molybdate solution (7 g of $(NH_4)_6Mo_7O_{24}4H_2O$ in sufficient water to make 100 ml) to 200 ml with water, and then add slowly 25 ml of 7.5 *N* sulfuric acid.

Ferrous Sulfate Solution Just before use, prepare a 10% aqueous ferrous sulfate solution containing 2 ml of 7.5 *N* sulfuric acid per 100 ml of final solution.

Procedure Transfer 10.0 ml each of the *Standard Preparation* and of the *Test Preparation* into separate 50-ml Erlenmeyer flasks, add 10.0 ml of *Acid Molybdate Solution* and 5.0 ml of *Ferrous Sulfate Solution* to each flask, and mix. Determine the absorbance of each solution in a 1-cm cell at 700 nm with a suitable spectrophotometer, using as the blank a mixture of 10.0 ml of water, 10.0 ml of *Acid Molybdate Solution*, and 5.0 ml of *Ferrous Sulfate Solution*. The absorbance of the solution from the *Test Preparation* is not greater than that of the *Standard Preparation*.

Free Riboflavin and Riboflavin Diphosphate (NOTE: Use low-actinic glassware, and conduct this test so that all solutions are protected from direct sunlight at all stages.)

Standard Preparation Transfer 35.0 mg of USP Riboflavin Reference Standard into a 250-ml Erlenmeyer flask, add 20 ml of pyridine and 75 ml of water, and dissolve the riboflavin by frequent shaking. Transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and

mix. Transfer 20.0 ml of this solution into a second 1000-ml volumetric flask, adjust the pH to 6.0 by the addition of 8 ml of 0.1 *N* sulfuric acid, dilute to volume with water, and mix. Finally, transfer 25.0 ml of the last solution into a 100-ml volumetric flask, dilute to volume with dioxane-water mixture (1:3), and mix. This solution contains 0.175 µg of riboflavin per ml.

pH 7 Buffer Solution Dissolve 15.6 g of monobasic sodium phosphate ($NaH_2PO_4 2H_2O$) in about 100 ml of water, add 59.3 ml of 1 *N* sodium hydroxide, and dilute to 2000 ml with water. Check the pH with a pH meter, and adjust to 7.0 if necessary.

Test Preparation Dissolve 100.0 mg of the sample in 10.0 ml of *pH 7 Buffer Solution*. Prepare a strip of Whatman chromatography paper, Type 3-mm, medium flow rate, or other equivalent paper suitable for electrophoresis, and saturate the paper with *pH 7 Buffer Solution*. Using a micropipet, apply 0.01 ml of the sample solution along a narrow line on the cathode side of the paper strip contained in a suitable paper electrophoresis chamber. Apply a potential of approximately 250 V, allow electrophoresis to continue for 6 h, and then remove the paper from the chamber. Detect any free riboflavin and/or riboflavin diphosphate by observing the strip in daylight or under ultraviolet light. Free riboflavin, if present, will appear as a band nearest to the starting line, and riboflavin diphosphate will appear farthest from the starting line. (*Caution:* The riboflavin will be destroyed if exposed to the ultraviolet light for more than a few seconds.) Cut off the respective bands, place them in separate 250-ml Erlenmeyer flasks containing 35.0 ml of dioxane-water mixture (1:3), and allow to stand until the spots are completely eluted from the strips.

Procedure With a suitable fluorometer, determine the intensity of fluorescence of each sample solution and of the *Standard Preparation* at about 530 nm, using an excitation wavelength of about 440 nm. The fluorescence of the sample solution containing the eluted riboflavin band and riboflavin diphosphate band, respectively, is not greater than that produced by the *Standard Preparation*.

Loss on Drying, page 518 Dry at 100° in a vacuum over phosphorus pentoxide for 5 h.

pH of a 1 in 100 Solution Determine by the *Potentiometric Method*, page 531.

Residue on Ignition Ignite a 1-g sample as directed in the general method, page 533.

Specific Rotation, page 530 Transfer about 750 mg, accurately weighed, into a 50-ml volumetric flask, dissolve in and dilute to volume with 20% hydrochloric acid, and mix. Determine the rotation in a 1-dm tube within 15 min.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Rice Bran Wax

DESCRIPTION

A refined wax obtained from rice bran. It is hard, slightly crystalline, and ranges in color from tan to light brown. It is soluble in chloroform and in benzene, but is insoluble in water.

REQUIREMENTS

Identification

Identify rice bran wax by comparing its infrared absorption spectrum with the respective typical spectrum as shown on page 721. The sample is melted and prepared for analysis on a cesium bromide plate.

Arsenic (as As) Not more than 3 ppm.
Free Fatty Acids Not more than 10%.
Heavy Metals (as Pb) Not more than 0.004%.
Iodine Value Not more than 20.
Lead Not more than 3 ppm.
Melting Range Between 75° and 80°.
Saponification Value Between 75 and 120.

TESTS

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Free Fatty Acids Determine as directed in the general method procedure, page 504.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodine Value Determine by the *Wijs Method*, page 505.

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Melting Range Determine as directed for *Class II* substances in the general procedure, page 520.

Saponification Value Determine as directed in the general procedure, page 509.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base; coating agent.

Rosemary Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh flowering tops of *Rosemarinus officinalis* L. (Fam. *Labiatae*). It is a colorless or pale yellow liquid having the characteristic odor of rosemary and a warm camphoraceous taste.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 605, using the same test conditions as specified therein.

Assay for Esters Not less than 1.5% of esters, calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Assay for Total Borneol Not less than 8.0% of borneol ($C_{10}H_{18}O$).
Angular Rotation Between $-5°$ and $+10°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.464 and 1.476 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.894 and 0.912.

TESTS

Assay for Esters Weigh accurately about 10 ml, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.
Assay for Total Borneol Proceed as directed under *Total Alcohols*, page 499, using 5 ml of the dried, acetylized oil, accurately weighed, for the saponification. Calculate the percentage of total borneol by the formula

$$7.712A(1 - 0.0021E)/(B - 0.021A),$$

in which A is the difference between the number of ml of 0.5 *N* hydrochloric acid required for the sample and the number of ml of 0.5 *N* hydrochloric acid required for the residual blank titration, B is the weight of the acetylized oil taken, and E is the percentage of esters calculated as bornyl acetate ($C_{12}H_{20}O_2$).
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 90% alcohol. Upon further dilution, the solution may become turbid.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers. Avoid exposure to excessive heat.
Functional Use in Foods Flavoring agent.

Rose Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh flowers of *Rosa gallica* L., *Rosa damascena* Miller, *Rosa alba* L., *Rosa centifolia* L., and varieties of these species (Fam. *Rosaceae*). It is a colorless or yellow liquid having the characteristic odor and taste of rose. At 25° it is a viscous liquid. Upon gradual cooling it changes to a translucent, crystalline mass, which may be liquefied by warming.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 605, using the same test conditions as specified therein.

Angular Rotation Between $-1°$ and $-4°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.457 and 1.463 at 30°.
Solubility Passes test.
Specific Gravity Between 0.848 and 0.863 at 30°/15°.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility One ml is miscible with 1 ml of chloroform without turbidity. Add 20 ml of 90% alcohol to this mixture. The resulting liquid is neutral or acid to moistened litmus paper and, upon standing at 20°, deposits crystals within 5 min.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Rue Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh blossoming plants *Ruta graveolens* L., *Ruta montana* L., or *Ruta bracteosa* L. (Fam. *Rutaceae*). It is a yellow to yellow amber liquid having a characteristic fatty odor. It is soluble in most fixed oils and in mineral oil, but it is relatively insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 605, using the same test conditions as specified therein.

Assay Not less than 90.0% of ketones, calculated as methyl nonyl ketone ($C_{11}H_{22}O$).
Angular Rotation Between $-1°$ and $+3°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.430 and 1.440 at 20°.
Solidification Point Between 7.5° and 10.5°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.826 and 0.838.

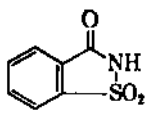
TESTS

Assay Weigh accurately about 1 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, using 85.10 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solidification Point Determine as directed in the general method, page 538.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 4 ml of 70% alcohol, occasionally with opalescence or precipitation of solids.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or aluminum containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Saccharin

o-Benzosulfimide; Gluside; 1,2-Benzisothiazolin-3-one 1,1-Dioxide



$C_7H_5NO_3S$ Mol wt 183.18

DESCRIPTION

White crystals or a white, crystalline powder. It is odorless or has a faint, aromatic odor. It is intensely sweet. Its solutions are acid to litmus. One g is soluble in 290 ml of water at 25°, in 25 ml of boiling water, and in 30 ml of alcohol. It is slightly soluble in chloroform and in ether, and is readily dissolved by dilute solutions of ammonia, solutions of alkali hydroxides, or solutions of alkali carbonates with the evolution of carbon dioxide.

REQUIREMENTS

Identification

A. Dissolve about 100 mg in 5 ml of sodium hydroxide solution (1 in 20), evaporate to dryness, and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with diluted hydrochloric acid TS, and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet color.
B. Mix 20 mg with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid results.

Assay Not less than 98.0% and not more than the equivalent of 101.0% of $C_7H_5NO_3S$ after drying.
Arsenic (as As) Not more than 3 ppm.
Benzoic and Salicylic Acids Passes test.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 1%.
Melting Range Between 226° and 230°.
Readily Carbonizable Substances Passes test.
Residue on Ignition Not more than 0.2%.

Selenium Not more than 0.003%.
Toluenesulfonamides Not more than 0.0025%.

TESTS

Assay Dissolve about 500 mg, previously dried at 105° for 2 h and accurately weighed, in 75 ml of hot water, cool quickly, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Each ml of 0.1 *N* sodium hydroxide is equivalent to 18.32 mg of $C_7H_5NO_3S$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Benzoic and Salicylic Acids To 10 ml of a hot, saturated solution add ferric chloride TS, dropwise. No precipitate or violet color appears in the liquid.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (Solution A).
Loss on Drying, page 518 Dry at 105° for 2 h.
Melting Range, page 519 Determine as directed in *Procedure for Class Ia.*
Readily Carbonizable Substances, page 532 Dissolve 200 mg in 5 ml of sulfuric acid TS, and keep at a temperature of 48° to 50° for 10 min. The color is no darker than *Matching Fluid A*.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.
Toluenesulfonamides Determine as directed under *Sodium Saccharin*, page 298, but use 8.0 ml of sodium carbonate TS to dissolve the sample for the *Test Preparation.*

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nonnutritive sweetener.

Sage Oil, Dalmatian Type

DESCRIPTION

The oil obtained by steam distillation from the partially dried leaves of the plant *Salvia officinalis* L. It is a yellowish or greenish yellow liquid having a warm, camphoraceous and thujonelike odor and flavor. It is soluble in most fixed oils and in mineral oil. Frequently the solutions in mineral oil are opalescent. It is slightly soluble in propylene glycol, but it is practically insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 605, using the same test conditions as specified therein.

Assay Not less than 50.0% of ketones, calculated as thujone ($C_{10}H_{16}O$).
Angular Rotation Between +2° and +29°.
Ester Value after Acetylation Between 25 and 60.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.457 and 1.469 at 20°.
Saponification Value Between 5 and 20.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.903 and 0.925.

TESTS

Assay Weigh accurately about 1 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, using 76.12 as the equivalence factor (*e*) in the calculation.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Ester Value after Acetylation Proceed as directed under *Total Alcohols*, page 499, using about 2.5 g of the acetylized oil. Calculate the *Ester Value after Acetylation* by the formula $A \times 28.05/B$, in which A is the number of ml of 0.5 *N* alcoholic potassium hydroxide consumed in the saponification of the acetylized oil, and B is the weight, in g, of the acetylized oil taken as the sample.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 80% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, or galvanized iron containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Sage Oil, Spanish Type

DESCRIPTION

The volatile oil obtained by distillation from the plants of *Salvia lavandulaefolia* Vahl. or *Salvia hispanorium* Lag. (Fam. *Labiatae*). It is a colorless to slightly yellow oil having a camphora-

ceous odor with a cineole top note. It is soluble in most fixed oils and in glycerin. It is soluble, usually with opalescence, in mineral oil and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 606, using the same test conditions as specified therein.

Angular Rotation Between −3° and +24°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.468 and 1.473 at 20°.
Saponification Value Between 14 and 57.
Saponification Value after Acetylation Between 56 and 98.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.909 and 0.932.

TESTS

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Determine as directed in the general method, page 501, using about 5 g, accurately weighed.

Saponification Value after Acetylation Acetylate a 10-ml sample as directed under *Total Alcohols*, page 499. Weigh accurately about 2.5 g of the dried, acetylated oil, and proceed as directed under *Saponification Value*, page 501, using the weight, in g, of the acetylated oil for *W* in the calculation formula.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 80% alcohol. The solution may become opalescent upon dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass or tin-lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Sandalwood Oil, East Indian Type

DESCRIPTION

The volatile oil obtained by steam distillation from the dried, ground roots and wood of *Santalum album* L. (Fam. *Santalaceae*). It is a pale yellow to yellow, somewhat viscous oily liquid having a strong, persistent characteristic odor. It is soluble in most fixed oils, in propylene glycol, and in mineral oil, sometimes with haziness. It is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 606, using the same test conditions as specified therein.

Assay Not less than 90% of alcohol, calculated as santalol ($C_{15}H_{24}O$).
Angular Rotation Between −15° and −20°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.500 and 1.510 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.965 and 0.980.

TESTS

Assay Proceed as directed under *Total Alcohols*, page 499. Weigh accurately about 1.2 g of the acetylated alcohol for the saponification, and use 110.2 as the equivalence factor (*e*) in the calculation.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 5 ml of 70% alcohol and remains in solution on dilution to 10 ml.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, or suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Savory Oil (Summer Variety)

Summer Savory Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the whole dried plant *Satureia hortensis* L. (Fam. *Labiatae*). It is a light yellow to dark brown liquid having a spicy aromatic note suggestive of thyme or origanum. It is soluble in most fixed oils and in mineral oil, but it is practically insoluble in glycerin and in propylene glycol.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 606, using the same test conditions as specified therein.

Assay Not less than 20.0% and not more than 57.0% of phenols as carvacrol ($C_{10}H_{14}O$).
Angular Rotation Between $-5°$ and $+4°$.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.486 and 1.505 at 20°.
Saponification Value Not more than 6.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.875 and 0.954.

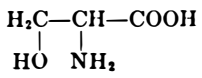
TESTS

Assay Proceed as directed under *Phenols*, page 502.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Saponification Value Determine as directed in the general method, page 501, using 5 g, accurately weighed.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml usually dissolves in 2 ml of 80% alcohol. Some oils may be slightly hazy in 10 ml of 90% alcohol.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

DL-Serine

DL-2-Amino-3-hydroxypropanoic Acid



$C_3H_7NO_3$ Mol wt 105.09

DESCRIPTION

White crystals or a crystalline powder. It is soluble in water, but insoluble in alcohol and in ether. It melts with decomposition at about 246° using a closed capillary tube and a bath preheated to 225°. It is optically inactive.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A bluish purple or purple color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_3H_7NO_3$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.

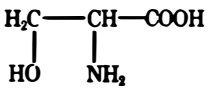
TESTS

Assay Transfer about 300 mg, accurately weighed, into a 100-ml beaker, add 25 ml of water, and heat on a steam bath until dissolved. Add 5 ml of formaldehyde TS, previously neutralized to phenolphthalein TS by the addition of 0.1 *N* sodium hydroxide. Cool the mixture, add a few drops phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 10.51 mg of $C_3H_7NO_3$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

L-Serine

L-2-Amino-3-hydroxypropanoic Acid



$C_3H_7NO_3$ Mol wt 105.10

DESCRIPTION

A white crystalline powder without odor and having a sweet taste. It is soluble in water, but is insoluble in alcohol and in ether. It melts with decomposition at about 228°.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A reddish purple or purple color is produced.

B. Dissolve about 500 mg in 10 ml of water, add 200 mg of periodic acid, and heat. The odor of formaldehyde is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_3H_7NO_3$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20}$: Between +13.5° and +16°, on the dried basis.

TESTS

Assay Transfer about 300 mg, accurately weighed, into a 100-ml beaker, add 25 ml of water, and heat on a steam bath until dissolved. Add 5 ml of formaldehyde TS, previously neutralized to phenolphthalein TS by the addition of 0.1 *N* sodium hydroxide, and cool. Add a few drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 10.51 mg of $C_3H_7NO_3$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution containing 10 g of a previously dried sample in sufficient 2 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Shellac, Bleached

White Shellac; Regular Bleached Shellac

DESCRIPTION

Shellac is obtained from lac, the resinous secretion of the insect *Laccifer* (*Tachardia*) *lacca* Kerr (Fam. *Coccidae*). Bleached shellac is obtained by dissolving the lac in aqueous sodium carbonate, followed by bleaching with sodium hypochlorite, precipitation of the bleached lac with dilute sulfuric acid solution, and drying. It occurs as an off-white, amorphous, granular resin. It is freely (though very slowly) soluble in alcohol, insoluble in water, and slightly soluble in acetone and in ether. Bleached shellac is usually dissolved in a suitable solvent for application to food products.

REQUIREMENTS

Identification

To 50 mg of the sample add a few drops of a solution of 1 g of ammonium molybdate in 3 ml of sulfuric acid. A green color is produced, changing to lilac when the solution is neutralized with ammonia TS.

Acid Value Between 73 and 89.
Arsenic (as As) Not more than 1.5 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 6%.
Rosin Passes test.
Wax Not more than 5.5%.

TESTS

Acid Value Dissolve about 2 g of finely ground sample, accurately weighed, in 50 ml of alcohol previously neutralized to phenolphthalein with sodium hydroxide. Add additional phenolphthalein TS, if necessary, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint. Calculate the acid value by the formula $56.1V \times N/W$, in which *V* is the exact volume, in ml, and *N* is the exact normality of the sodium hydroxide solution, and *W* is the weight of sample taken, in g, calculated on the dried basis.

Arsenic Dissolve 10 g of the sample in 50 ml of nitric acid in a 300-ml Kjeldahl flask, with the aid of heat. Cool, add 15 ml of sulfuric acid, and evaporate until copious fumes of sulfur trioxide are evolved. Cool, and cautiously add 50 ml of water. Evaporate to about 25 ml, cool, and filter into a 50-ml

volumetric flask. Rinse the filter with a few ml of water, dilute to volume with water, and mix. A 10-ml portion of this solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 41° ± 2° to constant weight.

Rosin Dissolve 2 g of the sample in 10 ml of dehydrated alcohol, and add slowly, with shaking, 50 ml of solvent hexane. Transfer to a separator, wash with two 50-ml portions of water, and discard the washings. Filter the solvent layer, evaporate it to dryness, and to the residue add 2 ml of a mixture of 1 volume of liquefied phenol and 2 volumes of carbon tetrachloride. Stir, and transfer a portion of the mixture to a cavity of a color-reaction plate. Fill an adjacent cavity with a mixture of 1 volume of bromine and 4 volumes of carbon tetrachloride, and cover both cavities with an inverted watch glass. No purple or deep indigo blue color is produced in or above the liquid containing the sample residue.

Wax Transfer about 10 g of finely ground sample, accurately weighed, and 2.5 g of sodium carbonate to a 200-ml tall-form beaker. Add 150 ml of hot water, immerse the beaker in a boiling water bath, and stir until the sample is dissolved. Cover the beaker with a watch glass, heat for 3 h without agitation, and cool in a cold water bath. When the wax has floated to the surface, filter the mixture through medium-speed quantitative ashless filter paper, transferring the wax to the paper, and wash the filter with water. Pour 5 to 10 ml of alcohol onto the filter to accelerate drying. Wrap the paper loosely in a larger piece of filter paper, bind with a piece of fine wire, and dry with the aid of gentle heat. Extract with chloroform in a suitable continuous extraction apparatus for 2 h, using a previously dried and accurately weighed flask to receive the extracted wax and solvent. Evaporate the solvent, dry the wax at 105° to constant weight, and calculate the percentage of wax.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; surface-finishing agent; glaze.

Shellac, Bleached, Wax-Free

Refined Bleached Shellac

DESCRIPTION

Shellac is obtained from lac, the resinous secretion of the insect *Laccifer* (*Tachardia*) *lacca* Kerr (Fam. *Coccidae*). Wax-free bleached shellac is obtained by the same process as that described for *Bleached Shellac*, page 270, except that, in addition, wax is removed by filtration. It occurs as an amorphous, light yellow, granular resin. Its solubility is the same as that of *Bleached Shellac*. Wax-free bleached shellac is usually dissolved in a suitable solvent for application to food products.

REQUIREMENTS

Identification

Wax-free bleached shellac responds to the *Identification Test* for *Bleached Shellac*, page 270.

Acid Value Between 75 and 91.

Arsenic (as As) Not more than 1.5 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 6%.

Rosin Passes test.

Wax Not more than 0.2%.

TESTS

Perform as directed under *Bleached Shellac*, page 270.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; surface-finishing agent; glaze.

Silicon Dioxide

Synthetic Amorphous Silica

SiO_2 Mol wt 60.08

DESCRIPTION

Silicon dioxide for food use is an amorphous substance that shows a noncrystalline pattern when examined by X-ray diffraction. It is produced synthetically by either a vapor-phase hydrolysis process, yielding *fumed* (or colloidal) *silica*, or by a wet process, yielding *precipitated silica*, *silica gel*, or *hydrous silica*. Fumed silica is produced in essentially an anhydrous state, whereas the wet-process products are obtained as hydrates or contain surface-adsorbed water.

Fumed silica occurs as a white, fluffy, nongritty powder of extremely fine particle size and is hygroscopic. The wet-process silicas occur as white, fluffy powders or as white, microcellular beads or granules and are hygroscopic or absorb moisture from the air in varying amounts. All of these forms of silicon dioxide are insoluble in water and in organic solvents, but are soluble in hydrofluoric acid and in hot, concentrated solutions of alkalies.

REQUIREMENTS

Identification

A. Place about 5 mg of the sample in a platinum crucible, mix with 200 mg of anhydrous potassium carbonate, and ignite at a red heat for about 10 min over a burner. Cool, dissolve

the melt in 2 ml of freshly distilled water, warming if necessary, and slowly add 2 ml of ammonium molybdate TS. A deep yellow color is produced.

B. Place 1 drop of the solution from *Identification Test A* on a filter paper, and evaporate the solvent. Add 1 drop of a saturated solution of *o*-tolidine in glacial acetic acid, and place the paper over stronger ammonia TS. A greenish blue spot is produced.

Assay *Fumed silica:* not less than 99.0% of SiO_2 after ignition; *precipitated silica, silica gel, and hydrous silica:* not less than 94.0% of SiO_2 after ignition.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 10 ppm.

Loss on Drying *Fumed silica:* not more than 2.5%; *precipitated silica* and *silica gel:* not more than 7%; *hydrous silica:* not more than 70%.

Loss on Ignition *Fumed silica:* not more than 2% after drying; *silica gel, hydrous silica gel,* and *precipitated silica:* not more than 8.5% after drying.

Soluble Ionizable Salts (as Na_2SO_4) *Precipitated silica, silica gel,* and *hydrous silica:* not more than 5%.

Insoluble Substances Not more than 1%.

TESTS

Assay Transfer about 1 g of the sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum crucible, ignite as directed in the test for *Loss on Ignition*, cool in a desiccator, and weigh to obtain the ignited sample weight (*W*). Moisten the residue with 3 or 4 drops of alcohol, add 2 drops of sulfuric acid, and then add enough hydrofluoric acid to cover the wetted sample. Evaporate to dryness on a hot plate, using medium heat (95° to 105°), then add 5 ml of hydrofluoric acid, swirl the dish carefully to wash down the sides, and again evaporate to dryness. Ignite the dried residue to a red heat over a Meker burner, cool in a desiccator, and weigh to obtain the residual weight (*w*). The difference between the ignited sample weight and the residual weight ($W - w$) represents the weight of SiO_2 in the ignited sample.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead Transfer 3.3 g of the sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through a Whatman No. 3 filter paper, or equivalent, into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic A 30-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A 20-ml portion of the *Sample Solution* meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 30-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Loss on Ignition Transfer into a suitable tared crucible about 1 g of an accurately weighed sample that has been previously dried at 105° for 2 h. Place the crucible in a cold muffle furnace, and bring the temperature to 900°–1000° during a 1-h period. Ignite at this temperature for 1 h, cool in a desiccator, and weigh.

Soluble Ionizable Salts Weigh accurately 5 g of the sample, previously dried at 105° for 2 h, and stir it with 150 ml of water for at least 5 min in a high-speed mixer. Filter with the aid of suction, and wash the mixer and filter with 100 ml of water in divided portions, adding the washings to the filtrate. Dilute the filtrate to 250 ml with water, and determine its conductance with a suitable conductance bridge assembly. The conductance is not greater than that produced by a control containing 250 mg of anhydrous sodium sulfate in each 250 ml.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Anticaking agent; defoaming agent; carrier; conditioning agent; chillproofing agent in malt beverages.

Sodium Acetate

$C_2H_3NaO_2.3H_2O$ Mol wt 136.08

DESCRIPTION

Colorless, transparent crystals or a granular crystalline powder. It is odorless or has a faint, acetous odor. It effloresces in warm, dry air. One g dissolves in about 0.8 ml of water and in about 19 ml of alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and *Acetate*, page 515.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $C_2H_3NaO_2$ after drying.

Alkalinity (as Na_2CO_3) Not more than 0.05%.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Between 36% and 41%.

Potassium Compounds Passes test.

TESTS

Assay Weigh accurately about 400 mg of the sample obtained in the test for *Loss on Drying*, dissolve it in 40 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.

Alkalinity Dissolve 2 g in about 20 ml of water, and add 3 drops of phenolphthalein TS. If a pink color is produced, not more than 0.1 ml of 0.1 *N* sulfuric acid is required to discharge it.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using glacial acetic acid to adjust the pH of *Solution B*, and using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 80° overnight and follow by drying at 120° for 4 h.

Potassium Compounds Mix a few drops of sodium bitartrate TS with 5 ml of a clear, saturated solution of the sample. No turbidity is produced within 5 min.

Packaging and Storage Store in tight containers.
Functional Use in Foods Buffer.

Sodium Acetate, Anhydrous

$C_2H_3NaO_2$ Mol wt 82.03

DESCRIPTION

A white, odorless, granular powder. It is hygroscopic. One g dissolves in about 2 ml of water.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Acetate*, page 515.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $C_2H_3NaO_2$ after drying.
Alkalinity (as NaOH) Not more than 0.2%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 2%.
Potassium Compounds Passes test.

TESTS

Assay Weigh accurately about 400 mg of the sample obtained in the test for *Loss on Drying*, dissolve it in 40 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid. Perform a blank determination (see page 2), and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 8.203 mg of $C_2H_3NaO_2$.

Alkalinity Dissolve 2 g in 20 ml of water, and add phenolphthalein TS. If a pink color is produced, not more than 1.0 ml of 0.1 *N* sulfuric acid is required to discharge it.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using glacial acetic acid to adjust the pH of *Solution B*, and using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 80° overnight and follow by drying at 120° for 4 h.

Potassium Compounds Mix a few drops of sodium bitartrate TS with 5 ml of a clear, saturated solution of the sample. No turbidity is produced within 5 min.

Packaging and Storage Store in tight containers.
Functional Use in Foods Buffer.

Sodium Acid Pyrophosphate

Disodium Pyrophosphate; Disodium Dihydrogen Pyrophosphate

$Na_2H_2P_2O_7$ Mol wt 221.94

DESCRIPTION

White, crystalline powder. It is soluble in water. The pH of a 1 in 100 solution is about 4. It may contain a suitable aluminum and/or calcium salt to control the rate of reaction in leavening systems.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.
B. Dissolve 100 mg of the sample in 100 ml of diluted nitric acid TS. Add 0.5 ml of this solution to 30 ml of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 ml of the solution to 30 ml of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 95.0% of $Na_2H_2P_2O_7$.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 0.002%.
Insoluble Substances Not more than 1%.
Lead Not more than 5 ppm.

TESTS

Assay Dissolve about 500 mg, accurately weighed, in 100 ml of water in a 400-ml beaker. Adjust the pH of the solution to 3.8 with hydrochloric acid, using a pH meter, then add 50 ml of a 1 in 8 solution of zinc sulfate (125 g of $ZnSO_4.7H_2O$ dissolved in water, diluted to 1000 ml, filtered, and adjusted to pH 3.8), and allow to stand for 2 min. Titrate the liberated acid with 0.1 *N* sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the endpoint, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 *N* sodium hydroxide is equivalent to 11.10 mg of $Na_2H_2P_2O_7$. (NOTE: The 0.1 *N* sodium hydroxide used in this titration must be standardized against sodium pyrophosphate, $Na_4P_2O_7$, that has been recrystallized three times from water and dried at 400° to constant weight.)

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; leavening agent; sequestrant.

Sodium Alginate

Algin

$(C_6H_7O_6Na)_n$ Equiv wt, *Calculated*, 198.11
Equiv wt, *Actual* (Avg), 222.00

DESCRIPTION

The sodium salt of alginic acid (see *Alginic Acid*, page 13), occurs as a white to yellowish, fibrous or granular powder. It is nearly odorless and tasteless. It dissolves in water to form a viscous, colloidal solution. It is insoluble in alcohol and in hydroalcoholic solutions in which the alcohol content is greater than about 30% by weight. It is insoluble in chloroform, in ether, and in acids having a pH lower than about 3.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 100 solution add 1 ml of calcium chloride TS. A voluminous, gelatinous precipitate is formed.

B. To 10 ml of a 1 in 100 solution add 1 ml of diluted sulfuric acid TS. A heavy gelatinous precipitate is formed.

C. Sodium alginate meets the requirements of *Identification Test C* under *Alginic Acid*, page 13.

D. Extract the *Ash* from sodium alginate with diluted hydrochloric acid TS, and filter. The filtrate gives positive tests for *Sodium*, page 517.

Assay It yields not less than 18% and not more than 21% of carbon dioxide (CO_2), corresponding to between 90.8% and 106% of sodium alginate (equiv wt 222.00).

Arsenic (as As) Not more than 3 ppm.

Ash Between 18% and 27% after drying.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 15%.

TESTS

Assay Proceed as directed in the *Alginates Assay*, page 463. Each ml of 0.25 *N* sodium hydroxide consumed in the assay is equivalent to 27.75 mg of sodium alginate (equiv wt 222.00).

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash Determine as directed under *Ash* in the monograph on *Alginic Acid*, page 14.

Heavy Metals Determine as directed in the test for *Heavy Metals* under *Alginic Acid*, page 14.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 4 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Stabilizer; thickener; emulsifier.

Sodium Aluminosilicate

Sodium Silicoaluminate

DESCRIPTION

A series of hydrated sodium aluminum silicates having an $Na_2O/Al_2O_3/SiO_2$ mol ratio of approximately 1/1/13.2, respectively. It occurs as a fine, white, amorphous powder, or as beads. It is odorless and tasteless. It is insoluble in water and in alcohol and other organic solvents, but at 80° to 100° is partially soluble in strong acids and solutions of alkali hydroxides. The

pH of a 20% slurry, prepared with carbon dioxide–free water, is between 6.5 and 10.5.

REQUIREMENTS

Identification

A. Mix 500 mg of the sample with 2.5 g of anhydrous potassium carbonate, and heat the mixture in a platinum or nickel crucible until it melts completely. Cool, add 5 ml of water, and allow to stand for 3 min. Heat the bottom of the crucible gently, detach the melt, and transfer it to a beaker with the aid of about 50 ml of water. Add gradually hydrochloric acid until no effervescence is observed, then add 10 ml more of the acid, and evaporate to dryness on a steam bath. Cool, add 20 ml of water, boil, and filter through ash-free filter paper. An insoluble residue of silica remains. (NOTE: Retain the filtrate for *Identification Test B*.) Transfer the gelatinous residue to a platinum dish, and cautiously add 5 ml of hydrofluoric acid. The precipitate dissolves. (If it does not dissolve, repeat the treatment with hydrofluoric acid.) Heat and hold in the vapors a glass stirring rod with a drop of water on the tip. The drop becomes turbid.

B. Portions of the filtrate obtained in *Identification Test A* give positive tests for *Aluminum*, page 515, and for *Sodium*, page 517.

Assay

Silicon Dioxide Not less than 66.0% and not more than 71.0% of SiO_2 after drying.

Aluminum Oxide Not less than 9.0% and not more than 13.0% of Al_2O_3 after drying.

Sodium Oxide Not less than 4.0% and not more than 7.0% of Na_2O after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 8%.

Loss on Ignition Between 8% and 11%.

TESTS

Assay

Silicon Dioxide Transfer about 500 mg, previously dried at 105° for 2 h and accurately weighed, into a 250-ml beaker, wash the sides of the beaker with a few ml of water, and then add 30 ml of 72% perchloric acid and 15 ml of hydrochloric acid. Heat on a hot plate in a hood until dense white fumes are evolved, cool, add 15 ml of hydrochloric acid, and heat again to dense white fumes. Cool, add 70 ml of water, and filter through Whatman No. 40 or equivalent filter paper. Wash the filter paper and precipitate with hot water until free from perchloric acid, collecting the filtrate in a 250-ml Erlenmeyer flask. Retain the filtrate for the determination of *Aluminum Oxide*.

Transfer the filter paper and precipitate into a tared platinum crucible, char, and ignite at 900° to constant weight. Moisten the residue with a few drops of water, then add 15 ml of hydrofluoric acid and 8 drops of sulfuric acid, and heat on a hot plate until white fumes of sulfur trioxide are evolved. Cool, add 5 ml of water, 10 ml of hydrofluoric acid, and 3 drops of sulfuric acid, and evaporate to dryness on the hot plate. Heat cautiously over an open flame until sulfur trioxide fumes have ceased, and ignite at 900° to constant weight. The weight loss after the addition of hydrofluoric acid represents the weight of SiO_2 in the sample taken. Retain the residue for the determination of *Aluminum Oxide*.

Aluminum Oxide Add 2 g of potassium pyrosulfate to the residue obtained in the *Silicon Dioxide* determination, heat over a Meker burner until a clear melt is obtained, and cool. Add a few ml of water and a few drops of sulfuric acid, and heat until the residue is dissolved, adding more water if necessary. Transfer this solution into the Erlenmeyer flask containing the filtrate obtained in the *Silicon Dioxide* determination, and add ammonia TS until the aluminum hydroxide precipitate formed goes back slowly into solution after the last drop of the TS is added. Add 50.0 ml of 0.05 *M* disodium EDTA, and boil gently for 5 min. Cool, and add in the order given and with continuous stirring 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone TS. Titrate with 0.05 *M* zinc sulfate until the color changes from green violet to rose pink, and perform a blank determination (see page 2). Each ml of 0.05 *M* disodium EDTA is equivalent to 2.549 mg of Al_2O_3.

Sodium Oxide Transfer about 500 mg of the sample, previously dried at 105° for 2 h and accurately weighed, into a tared platinum dish, and moisten with 8 to 10 drops of water. Add 25 ml of 70% perchloric acid and 10 ml of hydrofluoric acid, and heat on a hot plate in a hood until dense white fumes of perchloric acid appear. Add 10 ml of hydrofluoric acid, heat again to dense white fumes, and dissolve the residue in sufficient water to make 250.0 ml.

Set a suitable flame photometer to a wavelength of 589 nm. Adjust the instrument to zero transmittance against water, then adjust it to 100.0% transmittance with a standard solution containing 200 µg of sodium, in the form of the chloride, per ml. Read the percent transmittance of three other standard solutions containing 50, 100, and 150 µg each of sodium per ml, and plot the standard curve as percent transmittance versus concentration of sodium.

Place a portion of the sample solution in the photometer, read the percent transmittance in the same manner, and by reference to the standard curve determine the concentration (C) of sodium, in µg per ml, in the sample solution. Calculate the quantity, in mg, of Na_2O in the sample taken by the formula

$$(250C \times 1.348/1000) - F,$$

in which F, as determined below, is the quantity of sodium oxide equivalent to any sodium sulfate present in the sample.

Correction for Sodium Sulfate Content Weigh accurately 12.5 g of the sample, previously dried at 105° for 2 h, and stir it with 240 ml of water for at least 5 min with a high-speed mixer. Transfer the mixture into a 250-ml graduate, and wash the mixer container with water, adding the washings to the graduate to make 250 ml. Stopper the graduate, invert it several times to mix the sample, and determine the conduc-

tivity of the slurry using a suitable conductance bridge assembly. By means of a standard curve, obtained from solutions containing 50, 100, 200, and 500 mg of sodium sulfate in each 100 ml, determine the concentration (C'), in mg per 100 ml, of sodium sulfate in the sample slurry, and calculate the correction factor (F) by the formula

$$0.437(2.5C' \times w/W),$$

in which w is the weight of the sample taken for the *Sodium Oxide* determination, and W is the weight of the sample taken for the preparation of the slurry.

Sample Solution for the Determination of Arsenic and Heavy Metals Transfer 10.0 g of the sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat slowly to boiling. Boil gently for 15 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 4, or equivalent, filter paper into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A 20-ml portion of the *Sample Solution* meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 2 h.

Loss on Ignition Transfer about 5 g, previously dried at 105° for 2 h and accurately weighed, into a suitable tared crucible, and ignite at 900° to constant weight.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Anticaking agent.

Sodium Aluminum Phosphate, Acidic

SALP

$NaAl_3H_{14}(PO_4)_8{\cdot}4H_2O$ Mol wt 949.88
or
$Na_3Al_2H_{15}(PO_4)_8$ Mol wt 897.82

DESCRIPTION

A white, odorless powder. It is insoluble in water, but is soluble in hydrochloric acid.

REQUIREMENTS

Identification

A 1 in 10 solution in dilute hydrochloric acid (1 in 2) gives positive tests for *Aluminum*, page 515, and for *Phosphate*, page 517, and it responds to the flame test for *Sodium*, page 517.

Assay Not less than 95.0% of $NaAl_3H_{14}(PO_4)_8{\cdot}4H_2O$, or not less than 95.0% of $Na_3Al_2H_{15}(PO_4)_8$.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.0025%.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Ignition $NaAl_3H_{14}(PO_4)_8{\cdot}4H_2O$: between 19.5% and 21%; $Na_3Al_2H_{15}(PO_4)_8$: between 15% and 16%.

TESTS

Assay Transfer about 2.5 g, accurately weighed, into a 250-ml volumetric flask, add 15 ml of hydrochloric acid and one glass bead, and boil gently for about 5 min. Cool, dilute to volume with water, and mix. Transfer 10.0 ml of this solution to a 250-ml beaker, add phenolphthalein TS, and neutralize with ammonia TS. Add dilute hydrochloric acid (1 in 2) until the precipitate just dissolves, then dilute to 100 ml with water, and heat to 70°–80°. Add 10 ml of 8-hydroxyquinoline TS and sufficient ammonium acetate TS until a yellow precipitate forms, then add 30 ml in excess. Digest at 70° for 30 min, filter through a previously dried and weighed Gooch crucible, and wash thoroughly with hot water. Dry at 105° for 2 h, cool, and weigh. Each mg of the precipitate so obtained corresponds to 0.689 mg of $NaAl_3H_{14}(PO_4)_8{\cdot}4H_2O$, or to 0.977 mg of $Na_3Al_2H_{15}(PO_4)_8$.

Arsenic A solution of 1 g in 10 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the *Arsenic Test*, page 464.

Fluoride Weigh accurately 2.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Heavy Metals Dissolve 500 mg in 2.5 ml of diluted hydrochloric acid TS, and add water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Ignition Ignite at 750° to 800° for 2 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Leavening agent.

Sodium Aluminum Phosphate, Basic

Kasal

DESCRIPTION

A white, odorless powder consisting of an autogenous mixture of an alkaline sodium aluminum phosphate [approximately $Na_8Al_2(OH)_2(PO_4)_4$] with about 30% dibasic sodium phosphate. It is soluble in hydrochloric acid; the sodium phosphate moiety is soluble in water, whereas the sodium aluminum phosphate moiety is only sparingly soluble in water.

REQUIREMENTS

Identification

A 1 in 10 solution in dilute hydrochloric acid (1 in 2) gives positive tests for *Aluminum*, page 515, and for *Phosphate*, page 517, and it responds to the flame test for *Sodium*, page 517.

Assay Not less than 9.5% and not more than 12.5% of Al_2O_3, calculated on the ignited basis.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.0025%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Ignition Not more than 9%.

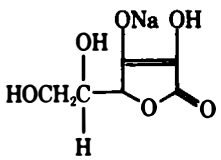
TESTS

Assay Transfer about 2.5 g, accurately weighed, into a 250-ml volumetric flask, add 15 ml of hydrochloric acid and one glass bead, and boil gently for about 5 min. Cool, dilute to volume with water, and mix. Transfer 10.0 ml of this solution to a 250-ml beaker, add phenolphthalein TS, and neutralize with ammonia TS. Add dilute hydrochloric acid (1 in 2) until the precipitate just dissolves, then dilute to 100 ml with water and heat to 70°–80°. Add 10 ml of 8-hydroxyquinoline TS and sufficient ammonium acetate TS until a yellow precipitate forms, then add 30 ml in excess. Digest at 70° for 30 min, filter through a previously dried and weighed Gooch crucible, and wash thoroughly with hot water. Dry at 105° for 2 h, cool, and weigh. Each mg of the precipitate so obtained corresponds to 0.111 mg of Al_2O_3.
Arsenic A solution of 1 g in 10 ml of dilute hydrochloric acid (1 in 2) meets the requirements of the *Arsenic Test*, page 464.
Fluoride Weigh accurately 2.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.
Heavy Metals Dissolve 500 mg in 2.5 ml of diluted hydrochloric acid TS, and add water to make 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Ignition Ignite at 750° to 800° for 2 h.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Emulsifier.

Sodium Ascorbate

Vitamin C Sodium; Sodium L-Ascorbate



$C_6H_7NaO_6$ Mol wt 198.11

DESCRIPTION

A white to yellowish crystalline solid. One g is soluble in 2 ml of water. The pH of a 1 in 10 solution is about 7.5.

REQUIREMENTS

Identification

A. A 1 in 50 solution slowly reduces alkaline cupric tartrate TS at 25°, but more readily upon heating.
B. To 2 ml of a 1 in 50 solution of the sample acidified with 0.5 ml of 0.1 *N* hydrochloric acid add 4 drops of methylene blue TS, and warm to 40°. The deep blue color is practically discharged within 3 min.
C. Dissolve 15 mg of the sample in 15 ml of a 1 in 20 solution of trichloroacetic acid, add about 200 mg of activated charcoal, shake vigorously for 1 min, and filter through a small fluted filter, returning the filtrate, if necessary, until clear. To 5 ml of the filtrate add 1 drop of pyrrole, agitate gently until dissolved, and then heat in a water bath at 50°. A blue color develops.
D. It gives positive tests for *Sodium*, page 517.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $C_6H_7NaO_6$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.25%.
Specific Rotation $[\alpha]_D^{25°}$: Between + 103° and + 108°.

TESTS

Assay Dissolve about 400 mg, previously dried over phosphorus pentoxide for 24 h and accurately weighed, in a mixture of 100 ml of water, recently boiled and cooled, and 25 ml of diluted sulfuric acid TS, and titrate with 0.1 *N* iodine, adding starch TS near the endpoint. Each ml of 0.1 *N* iodine is equivalent to 9.905 mg of $C_6H_7NaO_6$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

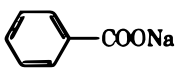
Loss on Drying Dry in vacuum over phosphorus pentoxide at 60° for 4 h.

Specific Rotation, page 530 Determine in a solution containing 1 g in each 10 ml.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Antioxidant; nutrient; dietary supplement.

Sodium Benzoate



$C_7H_5NaO_2$ Mol wt 144.10

DESCRIPTION

White, odorless or nearly odorless granules, crystalline powder, or flakes. One g dissolves in 2 ml of water, in 75 ml of alcohol, and in 50 ml of 90% alcohol.

REQUIREMENTS

Identification

It gives positive tests for *Sodium*, page 517, and for *Benzoate*, page 516.

Assay Not less than 99.0% of $C_7H_5NaO_2$, calculated on the dried basis.

Alkalinity (as NaOH) Not more than 0.04%.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Water Not more than 1.5%.

TESTS

Assay Transfer about 600 mg, accurately weighed, to a 250-ml beaker, add 100 ml of glacial acetic acid, and stir until the sample is completely dissolved. Add crystal violet TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid. Each ml of 0.1 *N* perchloric acid is equivalent to 14.41 mg of $C_7H_5NaO_2$.

Alkalinity Dissolve 2 g in 20 ml of hot water, and add 2 drops of phenolphthalein TS. If a pink color is produced, not more than 0.2 ml of 0.1 *N* sulfuric acid is required to discharge it.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 4 g in 40 ml of water, add dropwise, with vigorous stirring, 10 ml of diluted hydrochloric acid TS, and filter. A 25-ml portion of the filtrate meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Preservative; antimicrobial agent.

Sodium Bicarbonate

Baking Soda

$NaHCO_3$ Mol wt 84.01

DESCRIPTION

A white crystalline powder. It is stable in dry air, but slowly decomposes in moist air. Its solutions, when freshly prepared with cold water without shaking, are alkaline to litmus. The alkalinity increases as the solutions stand, are agitated, or are heated. One g dissolves in 10 ml of water. It is insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Sodium*, page 517, and for *Bicarbonate*, page 516.

Assay Not less than 99.0% of $NaHCO_3$ after drying.

Ammonia Passes test.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 5 ppm.

Insoluble Substances Passes test.

Loss on Drying Not more than 0.25%.

TESTS

Assay Weigh accurately about 3 g, previously dried over silica gel for 4 h, dissolve it in 25 ml of water, add methyl orange TS, and titrate with 1 *N* sulfuric acid. Each ml of 1 *N* sulfuric acid is equivalent to 84.01 mg of $NaHCO_3$.

Ammonia Heat 1 g in a test tube. No odor of ammonia is detected.

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 4 g in 10 ml of diluted hydrochloric

acid TS, boil gently for 1 min, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances One g dissolves completely in 20 ml of water to give a clear solution.

Loss on Drying, page 518 Dry over silica gel for 4 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Alkali; leavening agent.

Sodium Bisulfate

Sodium Acid Sulfate; Nitre Cake

$NaHSO_4$ Mol wt 120.06

DESCRIPTION

White, odorless crystals or granules. It is soluble in water, and its solutions are strongly acid. It is decomposed by alcohol into sodium sulfate and free sulfuric acid.

REQUIREMENTS

Identification

It gives positive tests for *Sodium*, page 517, and for *Sulfate*, page 517.

Assay Not less than 35.0% and not more than 39.0% of available H_2SO_4, equivalent to not less than 85.4% and not more than 95.2% of $NaHSO_4$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.8%.

Selenium Not more than 0.003%.

Water-Insoluble Substances Not more than 0.05%.

TESTS

Assay Dissolve about 5 g, accurately weighed, in about 125 ml of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 49.04 mg of H_2SO_4, or to 120.06 mg of $NaHSO_4$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 670 mg in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 25 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry in a desiccator over phosphorus pentoxide for 24 h.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Water-Insoluble Substances Dissolve 50 g in 300 ml of hot water in a 600-ml beaker, allow the insoluble matter to settle, and filter by decanting through a tared Gooch crucible, washing the insoluble matter into the crucible with additional hot water. Dry at 100° to 110° for 1 h, cool in a desiccator, and weigh.

Packaging and Storage Store in tight containers.

Functional Use in Foods Acid.

Sodium Bisulfite

Sodium Acid Sulfite; Sodium Hydrogen Sulfite

DESCRIPTION

Sodium bisulfite consists of sodium bisulfite ($NaHSO_3$) and sodium metabisulfite ($Na_2S_2O_5$) in varying proportions, and for all practical purposes possesses properties of the true bisulfite. It occurs as white or yellowish white crystals or granular powder having an odor of sulfur dioxide. It is unstable in air. One g dissolves in 4 ml of water. It is slightly soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Sodium*, page 517, and for *Sulfite*, page 517.

Assay Not less than 58.5% and not more than 67.4% of SO_2.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Iron Not more than 0.005%.

Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 200 mg, add it to exactly 50 ml of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 3.203 mg of SO_2.

Arsenic Dissolve 1 g of the sample in 10 ml of water in a 150-ml beaker, cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur dioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool, repeat the washing and fuming

procedure, and cool again. This solution meets the requirements of the *Arsenic Test*, page 464, omitting the addition of 20 ml of dilute sulfuric acid (1 in 5).

Heavy Metals Dissolve 2 g in 10 ml of water, add 5 ml of hydrochloric acid, evaporate to dryness on a steam bath, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron To 500 mg of the sample add 2 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 2 ml of hydrochloric acid and 20 ml of water, add a few drops of bromine TS, and boil the solution to remove the bromine. Cool, dilute with water to 25 ml, then add 50 mg of ammonium persulfate and 5 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced in a control containing 2.5 ml of *Iron Standard Solution* (25 µg Fe).

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-filled, tight containers, and avoid exposure to excessive heat.

Functional Use in Foods Preservative.

Sodium Carbonate

$Na_2CO_3 \cdot xH_2O$ Mol wt (anhydrous) 105.99

DESCRIPTION

Sodium carbonate is anhydrous or may contain 1 or 10 molecules of water of hydration. It occurs as colorless crystals or as a white, granular or crystalline powder. It is freely soluble in water, and its solutions are alkaline to litmus. The anhydrous salt is hygroscopic, and the two hydrates are efflorescent. The decahydrate melts at about 32°.

REQUIREMENTS

Identification

It gives positive tests for *Sodium*, page 517, and for *Carbonate*, page 516.

Assay Not less than 99.5% of Na_2CO_3 after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Na_2CO_3 (anhydrous): not more than 1%; $Na_2CO_3 \cdot H_2O$: between 12% and 15%; $Na_2CO_3 \cdot 10H_2O$: between 55% and 65%.

TESTS

Assay Weigh accurately about 2 g of the dried salt, obtained as directed under *Loss on Drying*, dissolve in 50 ml of water, add methyl orange TS, and titrate with 1 *N* sulfuric acid. Each ml of 1 *N* sulfuric acid is equivalent to 53.00 mg of Na_2CO_3.

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Mix 2 g with 5 ml of water and 10 ml of diluted hydrochloric acid TS, boil for 1 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry about 3 g of the anhydrous salt or the monohydrate, accurately weighed, at 250° to 300° to constant weight. For the decahydrate, weigh accurately about 8 g, heat it first at 70°, then gradually raise the temperature, and finally dry at 250° to 300° to constant weight.

Packaging and Storage Store the anhydrous salt and the decahydrate in tight containers; the monohydrate may be stored in well-closed containers.

Functional Use in Foods Alkali.

Sodium Carboxymethylcellulose

CMC; Cellulose Gum

DESCRIPTION

It occurs as a white- to cream-colored powder or as granules. The powder is hygroscopic. A 1 in 100 aqueous suspension has a pH between 6.5 and 8.5. It is readily dispersed in water to form colloidal solutions. It is insoluble in most solvents.

REQUIREMENTS

Identification

Add about 1 g of powdered sample to 50 ml of warm water, while stirring, to produce a uniform dispersion. Continue the stirring until a colloidal solution is produced, and then cool to room temperature.

A. To about 10 ml of the solution add 10 ml of cupric sulfate TS. A fluffy, bluish white precipitate is formed.

B. The filtrate from *Identification Test A* gives positive tests for *Sodium*, page 517.

Assay Not less than 99.5% of sodium carboxymethylcellulose, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Degree of Substitution Not more than 0.95 carboxymethyl groups ($—CH_2COOH$) per anhydroglucose unit after drying.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 10%.

Sodium Not more than 9.5% after drying.

Viscosity of a 2%, Weight in Weight, Solution Not less than 25 centipoises.

TESTS

Assay Calculate the percentage of sodium carboxymethylcellulose by subtracting from 100 the percentages of *Sodium Chloride* and *Sodium Glycolate* determined as follows:

Sodium Chloride Weigh accurately about 5 g of the sample into a 250-ml beaker, add 50 ml of water and 5 ml of 30% hydrogen peroxide, and heat on a steam bath for 20 min, stirring occasionally to ensure complete dissolution. Cool, add 100 ml of water and 10 ml of nitric acid, and titrate with 0.05 *N* silver nitrate to a potentiometric endpoint, using silver and mercurous sulfate–potassium sulfate electrodes and stirring constantly. Calculate the percentage of sodium chloride in the sample by the formula

$$584.4 \times V \times N/(100 - b)W,$$

in which V and N represent the volume, in ml, and the normality, respectively, of the silver nitrate, b is the percentage of *Loss on Drying*, determined separately, W is the weight of the sample, in g, and 584.4 is an equivalence factor for sodium chloride.

Sodium Glycolate Transfer about 500 mg, accurately weighed, of the sample into a 100-ml beaker, moisten thoroughly with 5 ml of acetic acid, followed by 5 ml of water, and stir with a glass rod until solution is complete (usually about 15 min). Slowly add 50 ml of acetone, with stirring, then add 1 g of sodium chloride, and stir for several min to ensure complete precipitation of the carboxymethylcellulose. Filter through a soft, open-textured paper, previously wetted with a small amount of acetone, and collect the filtrate in a 100-ml volumetric flask. Use an additional 30 ml of acetone to facilitate transfer of the solids and to wash the filter cake, then dilute to volume with acetone, and mix.

Prepare a series of standard solutions as follows: Transfer 100 mg of glycolic acid, previously dried in a desiccator at room temperature overnight and accurately weighed, into a 100-ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Use this solution within 30 days. Transfer 1.0 ml, 2.0 ml, 3.0 ml, and 4.0 ml of the solution into separate 100-ml volumetric flasks, add sufficient water to each flask to make 5 ml, then add 5 ml of glacial acetic acid, and dilute to volume with acetone.

Transfer 2.0 ml of the sample solution and 2.0 ml of each standard solution into separate 25-ml volumetric flasks, and prepare a blank flask containing 2.0 ml of a solution containing 5% each of glacial acetic acid and water in acetone. Place the uncovered flasks in a boiling water bath for exactly 20 min to remove the acetone, remove from the bath, and cool. Add to each flask 5.0 ml of 2,7-dihydroxynaphthalene TS, mix thoroughly, add an additional 15 ml, and again mix thoroughly. Cover the mouth of each flask with a small piece of aluminum foil. Place the flasks upright in a boiling water bath for 20 min, then remove from the bath, cool, dilute to volume with sulfuric acid, and mix.

Determine the absorbance of each solution at 540 nm, with a suitable spectrophotometer, against the blank, and prepare a standard curve using the absorbances obtained from the standard solutions. From the standard curve and the absorbance of the sample, determine the weight (w), in mg, of glycolic acid in the sample, and calculate the percentage of sodium glycolate in the sample by the formula

$$12.9 \times w/(100 - b)W,$$

in which 12.9 is a factor converting glycolic acid to sodium glycolate, b is the percentage of *Loss on Drying*, determined separately, and W is the weight of the sample, in g.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Degree of Substitution Weigh accurately about 200 mg of the sample, previously dried at 105° for 3 h, and transfer it into a 250-ml glass-stoppered Erlenmeyer flask. Add 75 ml of glacial acetic acid, connect the flask with a water-cooled condenser, and reflux gently on a hot plate for 2 h. Cool, transfer the solution to a 250-ml beaker with the aid of 50 ml of glacial acetic acid, and titrate with 0.1 *N* perchloric acid in dioxane while stirring with a magnetic stirrer. Determine the endpoint potentiometrically with a pH meter equipped with a standard glass electrode and a calomel electrode modified as follows: Discard the aqueous potassium chloride solution contained in the electrode, rinse and fill with the supernatant liquid obtained by shaking thoroughly 2 g each of potassium chloride and silver chloride (or silver oxide) with 100 ml of methanol, then add a few crystals of potassium chloride and silver chloride (or silver oxide) to the electrode.

Record the ml of 0.1 *N* perchloric acid versus mV (0- to 700-mV range), and continue the titration to a few ml beyond the endpoint. Plot the titration curve, and read the volume (A), in ml, of 0.1 *N* perchloric acid at the inflection point.

Calculate the degree of substitution (*DS*) by the formula

$$\frac{16.2A/G}{1.000 - (8.0A/G)},$$

in which A is the volume, in ml, of 0.1 *N* perchloric acid required, G is the weight, in mg, of the sample taken, 16.2 is one-tenth the molecular weight of one anhydroglucose unit, and 8.0 is one-tenth the molecular weight of one sodium carboxymethyl group.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, adding 1 ml of hydroxylamine hydrochloride solution (1 in 5) to the solution of the residue. Any color does not exceed that produced in a control (*Solution A*) containing 20 µg of lead ion (Pb) and 1 ml of the hydroxylamine hydrochloride solution.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry to constant weight at 105°.

Sodium From the weight of the sample and the number of ml of 0.1 *N* perchloric acid consumed in the determination of *Degree of Substitution*, calculate the percentage of sodium.

Each ml of 0.1 *N* perchloric acid is equivalent to 2.299 mg of Na.

Viscosity Determine as directed under *Viscosity of Sodium Carboxymethylcellulose*, page 550.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Thickener; stabilizer.

Sodium Chloride

Salt

NaCl Mol wt 58.44

DESCRIPTION

Salt is a generic term applied to commercially produced sodium chloride. It is available in various crystalline forms, referred to as evaporated salt, rock salt, solar salt, or simply salt. It may contain up to 2% (total) of suitable food-grade anticaking, free-flowing, or conditioning agents, either singly or in combination. It may contain not more than 13 ppm of sodium ferrocyanide, or not more than 25 ppm of green ferric ammonium citrate as crystal-modifying and anticaking agents. If labeled as iodized, it contains not less than 0.006% and not more than 0.010% of potassium iodide.

Sodium chloride is a transparent to opaque, white crystalline solid of variable particle size. (Rock salt may be white to off-white in color.) It remains dry in air at a relative humidity below 75%, but becomes deliquescent at higher humidities. One g is soluble in 2.8 ml of water at 25°, in 2.7 ml of boiling water, and in about 10 ml of glycerin. Sodium chloride containing water-insoluble anticaking, free-flowing, and conditioning agents may produce cloudy solutions, or may dissolve incompletely. A 1 in 20 solution usually has a pH between 5.5 and 8.5 (the pH may be higher if alkaline conditioning agents have been added).

REQUIREMENTS

Identification

It gives positive tests for *Sodium*, page 517, and for *Chloride*, page 516.

Assay

Evaporated salt with up to 2% of suitable free-flowing or conditioning agents and anticaking agents such as sodium ferrocyanide: Not less than 97.5% of NaCl after drying at 625° for 2 h.

Evaporated salt with only anticaking agents such as sodium ferrocyanide: Not less than 99.0% after drying at 625° for 2 h.

Rock or solar salt: Not less than 97.5% of NaCl after drying at 625° for 2 h, the remainder consisting chiefly of minor amounts of naturally occurring components such as alkaline and/or alkaline earth sulfates and chlorides.

Arsenic (as As) Not more than 1 ppm.

Calcium and Magnesium Not more than 2%.

Heavy Metals (as Pb) Not more than 4 ppm.

Iodine Not less than 0.006% and not more than 0.010% of potassium iodide. (NOTE: This specification applies only to iodized salt.)

Iron Not more than 0.0016% of Fe. (NOTE: This specification applies only to products to which green ferric ammonium citrate has been added.)

Loss on Drying Not more than 0.5%.

Sodium Ferrocyanide Not more than 0.0013% of anhydrous $Na_4Fe(CN)_6$. (NOTE: This specification applies only to products to which sodium ferrocyanide has been added.)

TESTS

NOTE: In the following procedures, it may be necessary to filter the sample solutions to avoid interference by insoluble or suspended anticaking, free-flowing, or conditioning agents.

Assay Weigh accurately about 250 mg of the sample, previously dried at 625° for 2 h, and dissolve it in 50 ml of water in a glass-stoppered flask. Add, while agitating, 3 ml of nitric acid, 5 ml of nitrobenzene, 50.0 ml of 0.1 *N* silver nitrate, and 2 ml of ferric ammonium sulfate TS. Shake well, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each ml of 0.1 *N* silver nitrate is equivalent to 5.844 mg of NaCl.

Arsenic A solution of 3 g of the sample in 25 ml of water meets the requirements of the *Arsenic Test*, page 464.

Calcium and Magnesium

Standard EDTA Solution Dissolve 4.0 g of disodium EDTA, $C_{10}H_{14}N_2Na_2O_8.2H_2O$, in sufficient water to make 1000 ml.

Magnesium Sulfate Solution Dissolve 2.6 g of magnesium sulfate, $MgSO_4.7H_2O$, in sufficient water to make 1000 ml.

Buffer Solution (*a*) Initial Preparation: Transfer 67.5 g of ammonium chloride into a 1000-ml volumetric flask, and dissolve in 570 ml of concentrated ammonium hydroxide. Use 2 ml of this solution as directed below under *Titer Determination*. (*b*) Final preparation: Pipet 50.0 ml of *Magnesium Sulfate Solution* into the flask, add exactly the volume *T*, in ml, of *Standard EDTA Solution*, determined as directed below under *Titer Determination*, then dilute to volume with water, and mix.

Titer Determination Pipet 50.0 ml of *Magnesium Sulfate Solution* into a 400-ml beaker, and add 200 ml of water, 2 ml of *Buffer Solution* (initial preparation), 1.0 ml of potassium cyanide solution (1 in 20), and 5 drops of eriochrome black TS or other suitable indicator. Titrate with the *Standard EDTA Solution*, while stirring with a magnetic stirrer, to a true blue endpoint. Record the volume *T*, in ml, of *Standard EDTA Solution* equivalent to 50.0 ml of the *Magnesium Sulfate Solution*.

Standardization of EDTA Solution Transfer about 1 g, accurately weighed, of primary standard calcium carbonate, $CaCO_3$, into a 1000-ml volumetric flask, dissolve in 800 ml of water containing 5 ml of concentrated hydrochloric acid,

dilute to volume with water, and mix. Pipet 25.0 ml of this solution into a 400-ml beaker, and add 200 ml of water, 2 ml of *Buffer Solution* (final preparation), 1.0 ml of potassium cyanide solution (1 in 20), and 20 drops of eriochrome black TS or other suitable indicator. Titrate with the *Standard EDTA Solution*, stirring with a magnetic stirrer, to a true blue endpoint. Calculate the factor F, giving the number of mg of Ca equivalent to 1.0 ml of *Standard EDTA Solution*, by the formula $10.011w/v$, in which w is the exact weight, in g, of the primary standard calcium carbonate taken, and v is the volume, in ml, of the *Standard EDTA Solution* required in the titration.

Sample Preparation for Rock and Solar Salt Transfer 50.0 g of the sample into a 500-ml volumetric flask, dissolve in 400 ml of water containing 2 ml of concentrated hydrochloric acid, dilute to volume with water, and mix. Filter a 50-ml aliquot, then pipet 10.0 ml of the filtrate into a 400-ml beaker, and add 190 ml of water.

Sample Preparation for Evaporated Salt Transfer 10.0 g of the sample into a 400-ml beaker, and dissolve in 100 ml of water. If free-flowing agents are present, filter and rinse quantitatively. Dilute the solution or filtrate to 200 ml with water.

Procedure To the *Sample Preparation* add 5 ml of *Buffer Solution* (final preparation), 1 ml of potassium cyanide solution (1 in 20), and 5 drops of eriochrome black TS or other suitable indicator. Begin stirring with a magnetic stirrer, and titrate with *Standard EDTA Solution* to a true blue endpoint, recording the volume, in ml, required as V. Calculate the ppm of total calcium and magnesium (both expressed as Ca) in the sample by the formula

$$V \times F \times 1000/W,$$

in which W is the weight, in g, of salt sample in the final solution titrated.

Heavy Metals A solution of 5 g of the sample in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iodine Transfer about 20 g of the sample, accurately weighed, into a 600-ml beaker, and dissolve in about 300 ml of water. Add a few drops of methyl orange TS, neutralize the solution with phosphoric acid (85%), and then add 1 ml excess of the acid. Add 25 ml of bromine TS and a few glass beads, boil until the solution is clear, then boil for an additional 5 min. Add about 50 mg of salicylic acid crystals, 1 ml of phosphoric acid, and 10 ml of potassium iodide solution (1 in 20), and titrate to a pale yellow color with 0.01 N sodium thiosulfate. Add 1 ml of starch TS, and continue the titration to the disappearance of the blue color. Each ml of 0.01 N sodium thiosulfate is equivalent to 0.2767 mg of KI.

Iron Dissolve 625.0 mg of the sample in 10 ml of diluted hydrochloric acid TS, and dilute to 50 ml with water. Add about 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 µg Fe) in an equal volume of solution containing 2 ml of hydrochloric acid and the quantities of ammonium persulfate and ammonium thiocyanate used in the test.

Loss on Drying, page 518 Dry at 110° for 2 h.

Sodium Ferrocyanide Dissolve 9.62 g of the sample in 80 ml of water in a 150-ml glass-stoppered cylinder or flask. Prepare a standard solution containing 125 µg of $Na_4Fe(CN)_6$ in each ml by dissolving 99.5 mg of $Na_4Fe(CN)_6.10H_2O$ in 500.0 ml of water, then transfer 1.0 ml of this solution into a similar 150-ml container for the control. To each container add 2 ml of ferrous sulfate TS and 1 ml of diluted sulfuric acid TS, dilute to 100 ml with water, and mix. Transfer 50-ml portions of the respective solutions into matched color-comparison tubes. The sample solution shows no more blue color than the control.

Packaging and Storage Store in well-closed containers.

Labeling Label the product to indicate whether or not it is iodized.

Functional Use in Foods Nutrient; preservative; flavoring agent and intensifier; curing agent; dough conditioner.

Sodium Citrate

$CH_2(COONa)C(OH)(COONa)CH_2COONa$

$C_6H_5Na_3O_7.2H_2O$ Mol wt 294.10

DESCRIPTION

Sodium citrate is anhydrous or contains two molecules of water of crystallization. It occurs as colorless crystals or as a white, crystalline powder. One g of the dihydrate dissolves in 1.5 ml of water at 25° and in 0.6 ml of boiling water. It is insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Citrate*, page 516.

Assay Not less than 99.0% of $C_6H_5Na_3O_7$, calculated on the anhydrous basis.

Alkalinity Passes test.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Water *Anhydrous sodium citrate:* not more than 1%; *sodium citrate dihydrate:* between 10% and 13%.

TESTS

Assay Transfer about 350 mg, accurately weighed, to a 250-ml beaker. Add 100 ml of glacial acetic acid, stir until completely dissolved, and titrate with 0.1 N perchloric acid, using crystal violet TS as the indicator. Each ml of 0.1 N perchloric acid is equivalent to 8.602 mg of $C_6H_5Na_3O_7$.

Alkalinity A solution of 1 g in 20 ml of water is alkaline to litmus paper, but after the addition of 0.2 ml of 0.1 N sulfuric

acid no pink color is produced by 1 drop of phenolphthalein TS.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

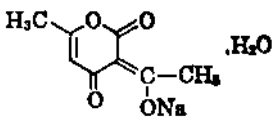
Water Determine by drying at 180° for 18 h (see page 518), or by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; sequestrant; nutrient for cultured buttermilk.

Sodium Dehydroacetate

Sodium 3-(1-Hydroxyethylidene)-6-methyl-1,2-pyran-2,4(3*H*)-dione



$C_8H_7NaO_4 \cdot H_2O$ Mol wt 208.15

DESCRIPTION

A white or nearly white, odorless powder having a slight characteristic taste. One g dissolves in about 3 ml of water, in 2 ml of propylene glycol, and in 7 ml of glycerin.

REQUIREMENTS

Identification

Dissolve about 1.5 g in 10 ml of water, add 5 ml of diluted hydrochloric acid TS, collect the crystals with suction, wash with 10 ml of water, and dry between 75° and 80° for 4 h. The crystals melt between 109° and 111° (see page 519).

Assay Not less than 98.0% of $C_8H_7NaO_4$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Water Between 8.5% and 10%.

TESTS

Assay Transfer about 500 mg, accurately weighed, to a 125-ml Erlenmeyer flask, dissolve it in 25 ml of glacial acetic acid containing 1 drop of a 1 in 100 solution of *p*-naphtholbenzein in glacial acetic acid that has been previously neutralized to a blue color, and titrate with 0.1 *N* perchloric acid to the original blue color. Each ml of 0.1 *N* perchloric acid is equivalent to 19.01 mg of $C_8H_7NaO_4$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Preservative.

Sodium Diacetate

Sodium Hydrogen Diacetate

$CH_3COONa \cdot CH_3COOH \cdot xH_2O$

$C_4H_7NaO_4 \cdot xH_2O$ Mol wt (anhydrous) 142.09

DESCRIPTION

Sodium diacetate is a molecular compound of sodium acetate and acetic acid. It is a white, hygroscopic, crystalline solid having an odor of acetic acid. One g is soluble in about 1 ml of water. The pH of a 1 in 10 solution is between 4.5 and 5.0.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Acetate*, page 515, and for *Sodium*, page 517.

Assay Not less than 39.0% and not more than 41.0% of free acetic acid (CH_3COOH), and not less than 58.0% and not more than 60.0% of sodium acetate (CH_3COONa).

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Readily Oxidizable Substances (as formic acid) Not more than 0.2%.

Water Not more than 2%.

TESTS

Assay

Free Acetic Acid Weigh accurately about 4 g, dissolve it in 50 ml of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 60.05 mg of CH_3COOH.

Sodium Acetate Content Weigh accurately about 500 mg, dissolve it in 50 ml of glacial acetic acid, and titrate with 0.1 *N* perchloric acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 8.203 mg of CH_3COONa.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

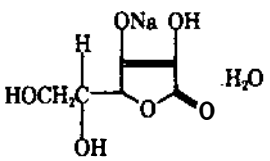
Readily Oxidizable Substances Dissolve 1.0 g in about 50 ml of water, add 10 ml of diluted sulfuric acid TS, and heat the solution to between 80° and 90°. Titrate the hot solution with 0.1 *N* potassium permanganate to a faint pink color that persists for at least 15 s. Each ml of 0.1 *N* potassium permanganate is equivalent to 2.301 mg of CH_2O_2.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Sequestrant; preservative; mold and rope inhibitor.

Sodium Erythorbate



$C_6H_7NaO_6.H_2O$ Mol wt 216.12

DESCRIPTION

White, odorless, crystalline powder or granules. In the dry state it is reasonably stable in air, but in solution it deteriorates in the presence of air, trace metals, heat, and light. One g dissolves in about 7 ml of water. The pH of a 1 in 20 solution is between 5.5 and 8.0.

REQUIREMENTS

Identification

A. A 1 in 50 solution slowly reduces alkaline cupric tartrate TS at 25°, but more readily upon heating.

B. To 2 ml of a 1 in 50 solution acidified with 0.5 ml of 0.1 *N* hydrochloric acid add a few drops of sodium nitroferricyanide TS, followed by 1 ml of 0.1 *N* sodium hydroxide. A transient blue color is produced immediately.

C. It gives positive tests for *Sodium*, page 517.

Assay Not less than 98.0% of $C_6H_7NaO_6.H_2O$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.003%.

Lead Not more than 10 ppm.

Oxalate Passes test.

Specific Rotation $[\alpha]_D^{25°}$: Between +95.5° and +98.0°.

TESTS

Assay Dissolve about 400 mg, accurately weighed, in a mixture of 100 ml of water, recently boiled and cooled, and 25 ml of diluted sulfuric acid TS, and immediately titrate with 0.1 *N* iodine, adding starch TS near the endpoint. Each ml of 0.1 *N* iodine is equivalent to 10.81 mg of $C_6H_7NaO_6.H_2O$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 670-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Oxalate To a solution of 1 g in 10 ml of water add 2 drops of glacial acetic acid and 5 ml of a 1 in 10 solution of calcium acetate. The solution remains clear.

Specific Rotation, page 530 Determine in a solution containing 1 g in each 10 ml.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Preservative; antioxidant.

Sodium Ferric Pyrophosphate

Sodium Iron Pyrophosphate

$Na_8Fe_4(P_2O_7)_6.xH_2O$ Mol wt (anhydrous) 1277.02

DESCRIPTION

A white to tan, odorless powder. It is insoluble in water, but is soluble in hydrochloric acid.

REQUIREMENTS

Identification

Dissolve 500 mg in 5 ml of dilute hydrochloric acid (1 in 2), and add an excess of sodium hydroxide TS. A reddish brown precipitate forms. Age the solution for several min, and then filter, discarding the first few ml. To 5 ml of the clear filtrate add 1 drop of bromophenol blue TS, and titrate with 1 *N* hydrochloric acid to a green color. Add 10 ml of a 1 in 8 solution of zinc sulfate, and readjust the pH to 3.8 (green color). A white precipitate forms (distinction from orthophosphates).

Assay Not less than 14.5% and not more than 16.0% of Fe.

Fluoride Not more than 0.005%.

Lead Not more than 10 ppm.

Loss on Ignition Not more than 8%.

Mercury Not more than 3 ppm.

TESTS

Assay Proceed as directed in the *Assay* under *Ferric Phosphate*, page 118.

Fluoride Weigh accurately 1.0 g, and proceed as directed in the *Fluoride Limit Test*, page 510.

Lead Proceed as directed in the test for *Lead* under *Ferric Phosphate*, page 118.

Loss on Ignition Ignite at 800° for 1 h.

Mercury Determine as directed in the test for *Mercury* under *Ferric Phosphate*, page 119.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Sodium Ferrocyanide

Yellow Prussiate of Soda

$Na_4Fe(CN)_6.10H_2O$ Mol wt 484.06

DESCRIPTION

Yellow crystals or crystalline powder. It is soluble in water, but is practically insoluble in most organic solvents.

REQUIREMENTS

Identification

To 10 ml of a 1% solution of the sample add 1 ml of ferric chloride TS. A dark blue precipitate forms.

Assay Not less than 99.0% of $Na_4Fe(CN)_6.10H_2O$.

Chloride Not more than 0.2%.

Cyanide Passes test.

Ferricyanide Passes test.

Free Moisture Not more than 1%.

Insoluble Matter Not more than 0.03%.

Sulfate Not more than 0.07%.

TESTS

Assay Transfer about 3 g, accurately weighed, into a 400-ml beaker, dissolve in 225 ml of water, and add cautiously about 25 ml of sulfuric acid TS. Add, with stirring, 1 drop of orthophenanthroline TS, and titrate with 0.1 *N* ceric sulfate until the color changes sharply from orange to pure yellow. Each ml of 0.1 *N* ceric sulfate is equivalent to 96.81 mg of $Na_4Fe(CN)_6.10H_2O$.

Chloride, page 471 Dissolve 100 mg of the sample in 100 ml of water. Any turbidity produced by a 10-ml portion of this solution does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Cyanide Dissolve 10 mg of copper sulfate in a mixture of 8 ml of water and 2 ml of ammonia TS. Wet a strip of filter paper with this solution, and place the wet paper in a stream of hydrogen sulfide. When 1 drop of a 1% solution of the sample is placed on the brown reagent paper, a white circle is not produced.

Ferricyanide Dissolve about 10 mg of the sample in 10 ml of water, and place 1 drop of this solution on a spot plate. Add 1 drop of a 1% solution of lead nitrate followed by a few drops of a solution prepared by saturating cold 2 *N* acetic acid with benzidine. No blue precipitate or blue coloration appears.

Free Moisture Heat 20 g of the sample at 105° for 6 h, cool in a desiccator, and weigh. Grind the dried sample rapidly, then heat 3 g of the powder to constant weight at 105°, and calculate the total water content (*W*). Calculate the percentage of free moisture in the sample by the formula $W - 0.3721A$, in which *A* is the percentage of $Na_4Fe(CN)_6.10H_2O$ found in the *Assay*.

Insoluble Matter Dissolve 50 g of the sample in 300 ml of hot water, and filter off the insoluble matter on a tared Gooch crucible. Wash the residue thoroughly with hot water, dry the crucible in an oven at 105°, cool in a desiccator, and weigh.

Sulfate, page 471 Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 350 µg of sulfate (SO_4).

Packaging and Storage Store in tight containers.

Functional Use in Foods Anticaking agent for sodium chloride.

Sodium Gluconate

$CH_2OH(CHOH)_4COONa$

$C_6H_{11}NaO_7$ Mol wt 218.14

DESCRIPTION

A white to tan, granular to fine, crystalline powder. It is very soluble in water, and is sparingly soluble in alcohol. It is insoluble in ether.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.

B. To 5 ml of a warm solution (1 in 10) add 0.7 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, heat on a steam bath for 30 min, and cool. Induce crystallization by scratching the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

Assay Not less than 98.0% of $C_6H_{11}NaO_7$.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.
Reducing Substances Not more than 0.5%.

TESTS

Assay Transfer about 150 mg, accurately weighed, into a clean, dry 200-ml Erlenmeyer flask, add 75 ml of glacial acetic acid, and dissolve by heating on a hot plate. Cool, add quinaldine red TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid, using a 10-ml microburet, to a colorless endpoint. Each ml of 0.1 *N* perchloric acid is equivalent to 21.81 mg of $C_6H_{11}NaO_7$.
Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A solution of 1 g in 25 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Reducing Substances Determine as directed in the test for *Reducing Substances* under *Copper Gluconate*, page 90.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement; sequestrant.

Sodium Hydroxide

Caustic Soda

NaOH Mol wt 40.00

DESCRIPTION

White, or nearly white, pellets, flakes, sticks, fused masses, or other forms. Upon exposure to air it readily absorbs carbon dioxide and moisture. One g dissolves in 1 ml of water. It is freely soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 25 solution gives positive tests for *Sodium*, page 517.

Assay Not less than 95.0% of total alkali, calculated as NaOH.
Arsenic (as As) Not more than 3 ppm.
Carbonate (as Na_2CO_3) Not more than 3%.
Heavy Metals (as Pb) Not more than 0.003%.
Insoluble Substances and Organic Matter Passes test.
Lead Not more than 10 ppm.
Mercury Not more than 0.1 ppm.

TESTS

Assay Dissolve about 1.5 g, accurately weighed, in 40 ml of recently boiled and cooled water, cool to 15°, add phenolphthalein TS, and titrate with 1 *N* sulfuric acid. At the discharge of the pink color, record the volume of acid required, then add methyl orange TS, and continue the titration until a persistent pink color is produced. Record the total volume of acid required for the titration. Each ml of 1 *N* sulfuric acid is equivalent to 40.00 mg of total alkali, calculated as NaOH.
Arsenic Dissolve 1 g in about 10 ml of water, cautiously neutralize to litmus paper with sulfuric acid, and cool. This solution meets the requirements of the *Arsenic Test*, page 464.
Carbonate Each ml of 1 N sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* is equivalent to 106.0 mg of Na_2CO_3.
Heavy Metals Dissolve 670 mg in a mixture of 5 ml of water and 7 ml of diluted hydrochloric acid TS. Heat to boiling, cool, dilute to 25 ml with water, and filter. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Insoluble Substances and Organic Matter A 1 in 20 solution is complete, clear, and colorless to slightly colored.
Lead Dissolve 1 g in a mixture of 5 ml of water and 11 ml of diluted hydrochloric acid TS, and cool. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Mercury Determine as directed under *Mercury Limit Test*, page 520, preparing the *Standard Preparation* and the *Sample Preparation* as follows:

Standard Preparation Prepare the stock solution and dilutions to obtain a solution containing 1 µg of Hg per ml, as directed on page 520. Transfer 1.0 ml of the final solution (1 µg of Hg) to a 50-ml beaker, and add 20 ml of water, 1 ml of dilute sulfuric acid solution (1 in 5), and 1 ml of potassium permanganate solution (1 in 25). Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Transfer 10.0 g of the sample into a 100-ml beaker, dissolve in 15 ml of water, add 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with dilute hydrochloric acid solution (1 in 2). Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.
Functional Use in Foods Alkali.

Sodium Hydroxide Solution

DESCRIPTION

Sodium hydroxide solutions are usually available in nominal concentrations of 50% and 73% of NaOH, weight in weight,

having freezing points of about 15° and 63°, respectively. These solutions are clear or slightly turbid, colorless or slightly colored, strongly caustic, and hygroscopic, and when exposed to the air they absorb carbon dioxide, forming sodium carbonate.

REQUIREMENTS

Identification

Solutions of sodium hydroxide give positive tests for *Sodium*, page 517.

Assay Not less than 97.0% and not more than 103.0%, by weight, of the labeled amount of NaOH, calculated as total alkalinity.

Arsenic (as As) Not more than 3 ppm, calculated on the basis of NaOH determined in the *Assay*.

Carbonate (as Na_2CO_3) Not more than 3%, calculated on the basis of NaOH determined in the *Assay*.

Heavy Metals (as Pb) Not more than 0.003%, calculated on the basis of NaOH determined in the *Assay*.

Lead Not more than 10 ppm, calculated on the basis of NaOH determined in the *Assay*.

Mercury Not more than 1 ppm, calculated on the basis of NaOH determined in the *Assay*.

TESTS

Assay Based on the stated or labeled percentage of NaOH, weigh accurately a volume of the solution equivalent to about 1.5 g of sodium hydroxide, and dilute it to 40 ml with recently boiled and cooled water. Continue as directed in the *Assay* under *Sodium Hydroxide*, page 287, beginning with " . . . cool to 15°. . . . "

Arsenic Dilute the equivalent of 1 g of NaOH, calculated on the basis of the *Assay*, to 10 ml with water, cautiously neutralize to litmus paper with sulfuric acid, and cool. This solution meets the requirements of the *Arsenic Test*, page 464.

Carbonate Each ml of 1 *N* sulfuric acid required between the phenolphthalein and methyl orange endpoints in the *Assay* is equivalent to 106.0 mg of Na_2CO_3.

Heavy Metals Dilute the equivalent of 670 mg of NaOH, calculated on the basis of the *Assay*, with a mixture of 5 ml of water and 7 ml of diluted hydrochloric acid TS, and heat to boiling. Cool, dilute to 25 ml with water, and filter. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Dilute the equivalent of 1 g of NaOH, calculated on the basis of the *Assay*, with a mixture of 5 ml of water and 11 ml of diluted hydrochloric acid TS. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Mercury Determine as directed under *Mercury Limit Test*, page 520, using the following as the *Sample Preparation*: Transfer an accurately weighed amount of the sample, equivalent to 2.0 g of NaOH, into a 50-ml beaker, add 10 ml of water and 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with dilute hydrochloric acid solution (1 in 2). Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker with a watch glass, boil for a few seconds, and cool.

Packaging and Storage Store in tight containers.

Functional Use in Foods Alkali.

Sodium Hypophosphite

$NaH_2PO_2.H_2O$ Mol wt 106.01

DESCRIPTION

Sodium hypophosphite occurs as a white crystalline powder, as white granules, or as colorless, pearly crystalline plates. It is very deliquescent. One ml of water dissolves about 1 g at 25° and about 6 g at 100°. It is slightly soluble in alcohol.

Caution: Care should be observed in mixing sodium hypophosphite with nitrates, chlorates, or other oxidizing agents, as an explosion may occur if triturated or heated.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Hypophosphites*, page 516.

Assay Not less than 97.0% and not more than 103.0% of $NaH_2PO_2.H_2O$.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 10 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Substances Not more than 0.1%.

TESTS

Assay Dissolve about 100 mg of the sample, accurately weighed, in 20 ml of water, add 40.0 ml of 0.1 *N* ceric sulfate, mix well, and add 2 ml of silver sulfate solution (5 g of Ag_2SO_4 dissolved in 95 ml of concentrated sulfuric acid). Cover, heat nearly to boiling, and continue heating for 30 min. Cool to room temperature, and titrate with 0.1 *N* ferrous sulfate to a pale yellow color. Add 2 drops of orthophenanthroline TS, and continue the titration to a salmon-colored endpoint, recording the volume required as *S*, in ml. Perform a residual blank titration (see page 2), and record the volume required as *B*. Each ml of the volume $B - S$ is equivalent to 2.650 mg of $NaH_2PO_2.H_2O$.

Arsenic A solution of 1 g in 10 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Proceed as directed in the *Fluoride Limit Test*, page 510.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g of the sample in 100 ml of hot water, and filter through a tared filtering crucible. Wash the residue with hot water, dry at 105° for 2 h, cool, and weigh.

Packaging and Storage Store in tight containers.

Functional Use in Foods Preservative; antioxidant.

Sodium Lauryl Sulfate

DESCRIPTION

Sodium lauryl sulfate is a mixture of sodium alkylsulfates consisting chiefly of sodium lauryl sulfate [$CH_3(CH_2)_{10}CH_2OSO_3Na$]. It occurs as small, white or light yellow crystals having a slight, characteristic odor. One g dissolves in 10 ml of water, forming an opalescent solution.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Sodium*, page 517, and, after acidification with hydrochloric acid and boiling gently for 20 min, responds to the tests for *Sulfate*, page 517.

Assay Not less than 59.0% of total alcohols.

Alkalinity (as NaOH) Passes test (about 0.25%).

Arsenic (as As) Not more than 3 ppm.

Combined Sodium Chloride and Sodium Sulfate Not more than 8%.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 5 ppm.

Unsulfated Alcohols Not more than 4%.

TESTS

Assay Transfer about 5 g, accurately weighed, to an 800-ml Kjeldahl flask, and add 150 ml of water, 50 ml of hydrochloric acid, and a few boiling chips. Attach a reflux condenser to the flask, heat carefully to avoid excessive frothing, and then boil for about 4 h. Cool the flask, rinse the condenser with ether, collecting the ether in the flask, and transfer the contents to a 500-ml separator, rinsing the flask twice with ether and adding the washings to the separator. Extract the solution with two 75-ml portions of ether, evaporate the combined ether extracts in a tared beaker on a steam bath, dry the residue at 105° for 30 min, cool, and weigh. The residue represents the total alcohols.

Alkalinity Dissolve 1 g in 100 ml of water, add phenol red TS, and titrate with 0.1 *N* hydrochloric acid. Not more than 0.5 ml is required for neutralization.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Combined Sodium Chloride and Sodium Sulfate

Sodium Chloride Dissolve about 5 g, accurately weighed, in about 50 ml of water. Neutralize the solution with dilute nitric acid (1 in 20), using litmus paper as the indicator, add 2 ml of potassium chromate TS, and titrate with 0.1 *N* silver nitrate. Each ml of 0.1 *N* silver nitrate is equivalent to 5.844 mg of sodium chloride.

Sodium Sulfate Transfer about 1 g, accurately weighed, to a 400-ml beaker, add 10 ml of water, heat the mixture, and stir until completely dissolved. To the hot solution add 100 ml of alcohol, cover, and digest at a temperature just below the boiling point for 2 h. Filter while hot through a Gooch crucible, and wash the precipitate with 100 ml of hot alcohol. Dissolve the precipitate in the crucible by washing with about 150 ml of water, collecting the washings in a beaker. Acidify with 10 ml of hydrochloric acid, heat to boiling, add 25 ml of barium chloride TS, and allow to stand overnight. Collect the precipitate of barium sulfate on a tared Gooch crucible, wash until free from chloride, dry, ignite, and weigh. The weight of barium sulfate so obtained, multiplied by 0.6086, represents the weight of Na_2SO_4.

Heavy Metals A solution of 1 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Unsulfated Alcohols Dissolve about 10 g, accurately weighed, in 100 ml of water, and add 100 ml of alcohol. Transfer the solution to a separator, and extract with three 50-ml portions of solvent hexane. If an emulsion forms, sodium chloride may be added to promote separation of the two layers. Wash the combined solvent hexane extracts with three 50-ml portions of water, and dry with anhydrous sodium sulfate. Filter the solvent hexane extract into a tared beaker, evaporate on a steam bath until the odor of solvent hexane no longer is perceptible, dry the residue at 105° for 30 min, cool, and weigh. The residue represents the unsulfated alcohols.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Surface-active agent.

Sodium Metabisulfite

Sodium Pyrosulfite

$Na_2S_2O_5$ Mol wt 190.10

DESCRIPTION

Colorless crystals or a white to yellowish crystalline powder having an odor of sulfur dioxide. It is freely soluble in water and slightly soluble in alcohol. Its solutions are acid to litmus.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Sodium*, page 517, and for *Sulfite*, page 517.

Assay Not less than 90.0% of $Na_2S_2O_5$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Iron Not more than 0.002%.
Lead Not more than 10 ppm.
Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 200 mg, add it to exactly 50 ml of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 4.752 mg of $Na_2S_2O_5$.

Arsenic Dissolve 1 g of the sample in 10 ml of water in a 150-ml beaker, cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur trioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool, repeat the washing and fuming procedure, and cool again. This solution meets the requirements of the *Arsenic Test*, page 464, omitting the addition of 20 ml of dilute sulfuric acid (1 in 5).

Heavy Metals Dissolve 1 g in 10 ml of water, add 5 ml of hydrochloric acid, evaporate to dryness on a steam bath, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron To 500 mg of the sample add 2 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Dissolve the residue in 2 ml of hydrochloric acid and 20 ml of water, add a few drops of bromine TS, and boil the solution to remove the bromine. Cool, dilute with water to 25 ml, then add 50 mg of ammonium persulfate and 5 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced in a control containing 1.0 ml of *Iron Standard Solution* (10 µg Fe).

Lead Dissolve 1 g in 10 ml of water, add 5 ml of hydrochloric acid, evaporate to dryness on a steam bath, and dissolve the residue in about 20 ml of water. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in well-filled, tight containers, and avoid exposure to excessive heat.

Functional Use in Foods Preservative; antioxidant.

Sodium Metaphosphate, Insoluble

Insoluble Sodium Polyphosphate; IMP; Maddrell's Salt

DESCRIPTION

A high-molecular-weight sodium polyphosphate composed of two long metaphosphate chains ($NaPO_3$) that spiral in opposite directions about a common axis. The Na_2O/P_2O_5 ratio is about 1.0. It occurs as a white crystalline powder. It is practically insoluble in water but dissolves in mineral acids and in solutions of potassium and ammonium (but not sodium) chlorides. The pH of a 1 in 3 slurry in water is about 6.5.

REQUIREMENTS

Identification

A. Finely powder about 1 g of the sample, and add it slowly to 100 ml of a 1 in 20 solution of potassium chloride while stirring vigorously. A gelatinous mass is formed.
B. Mix 500 mg of the sample with 10 ml of nitric acid and 50 ml of water, boil for about 30 min, and cool. The resulting solution gives positive tests for *Sodium*, page 517, and for *Phosphates*, page 517.

Assay Not less than 68.7% and not more than 70.0% of P_2O_5.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 10 ppm.

TESTS

Assay Transfer about 800 mg of the sample, accurately weighed, into a 500-ml volumetric flask, add 100 ml of water and 25 ml of nitric acid, and boil for 10 min on a hot plate. Cool, dilute to volume with water, and mix. Pipet 20.0 ml of this solution into a 500-ml Erlenmeyer flask, add 100 ml of water, and heat just to boiling. Add with stirring 50 ml of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared Gooch crucible (or fritted-glass crucible of medium porosity), and wash with five 25-ml portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 µg of P_2O_5.

Arsenic A solution of 1 g in 15 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 1-g sample, dissolved in 5 ml of a 1 to 1 hydrochloric acid solution, as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals Warm 2 g with 25 ml of diluted hydrochloric acid TS until no more dissolves, dilute with water to 25 ml, and filter if necessary. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Packaging and Storage Store in tight containers.
Functional Use in Foods Emulsifier; sequestrant; texturizer.

Sodium Methylate

Sodium Methoxide

CH_3ONa Mol wt 54.03

DESCRIPTION

A white, amorphous, hygroscopic, free-flowing powder. It is soluble in fats, in esters, and in alcohols. It decomposes without melting above 127°.

Caution: Sodium methylate and its solutions are caustic and flammable. Avoid contact with the eyes, skin, and clothing, and do not inhale vapors from sodium methylate solutions.

REQUIREMENTS

Identification

It reacts with oxygen and carbon dioxide and is decomposed by water. The resulting solution gives positive tests for *Sodium*, page 517.

Assay Not less than 97.0% of CH_3NaO.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.0025%.
Lead Not more than 10 ppm.
Mercury Not more than 1 ppm.
Sodium Carbonate Not more than 0.4%.
Sodium Hydroxide Not more than 1.7%.

TESTS

NOTE: The tests in the following section must be conducted with a minimum exposure of the sample to air. Preferably the tests should be conducted in a nitrogen hood.

Assay, Sodium Carbonate, and Sodium Hydroxide

Sample Preparation Select two tared weighing bottles, each approximately 30 mm in diameter and 80 mm high, nearly fill each with the sample, which should weigh between 12 and 15 g, securely fit the covers, and weigh.

Determination of Alkalinity as CH_3ONa Remove the top from one of the sample bottles, and quickly drop the bottle into a 500-ml Erlenmeyer flask containing 200 ml of ice-cold, carbon dioxide–free water, sliding the sample bottle gently down the side of the flask to prevent splashing. Immediately stopper the flask with a rubber stopper, and swirl until the sample dissolves. Wash the sample solution into a 250-ml volumetric flask, and nearly dilute to volume with carbon dioxide–free water. Allow the solution to reach room temperature, then dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a 500-ml glass-stoppered Erlenmeyer flask, add 150 ml of carbon dioxide–free water and 5 ml of barium chloride TS, stopper the flask, mix, and allow to stand for 5 min. Add 3 drops of phenolphthalein TS, and titrate with 1 *N* hydrochloric acid to the disappearance of the pink color. Retain the titrated solution for the *Determination of Sodium Carbonate*. Calculate the percentage of alkalinity as CH_3ONa (*% A*) by the formula

$$(V_1 \times N \times 5.403)/(W \times 0.2),$$

in which V_1 is the volume, in ml, and N the exact normality of the hydrochloric acid used, and W is the weight of the sample, in g.

Determination of Sodium Carbonate Add 2 drops of methyl orange TS to the solution retained above, and continue the titration with 1 *N* hydrochloric acid to a permanent pink color. Calculate the percentage of Na_2CO_3 by the formula

$$(V_2 \times N \times 5.30)/(W \times 0.2),$$

in which V_2 is the volume, in ml, of 1 *N* hydrochloric acid consumed in the second titration, and N and W are as defined above.

Determination of Sodium Hydroxide The *Karl Fischer Titrimetric Method*, page 552, may be adapted for this determination at the discretion of the analyst, or the following procedure may be used:

Solution A Add 400 ml of colorless pyridine, containing no more than 0.05% of water, to a 500-ml Florence flask filled with a 2-hole rubber stopper and a 7-mm glass tube extending nearly to the bottom of the flask. Place the flask in a cooling bath of running water, and pass dry sulfur dioxide from an upright cylinder until 80 ± 0.5 g has been added. Disconnect the hose from the delivery tube before closing the gas valve. Transfer the solution into a dry glass-stoppered bottle, add 400 ml of absolute methanol, and mix. Store in a dark place.

Solution B Add 75 g of iodine to 900 ml of absolute methanol contained in a dry glass-stoppered bottle, and shake until the iodine dissolves. Transfer to a dry automatic buret protected by drying tubes.

Standardization of Solution B Add 15 ml of *Solution A*, measured with a dry graduate, to a dry 125-ml iodine flask, and titrate with *Solution B* to a brownish yellow color. Stopper the flask immediately to prevent moisture contamination and endpoint fading. Disregard the volume of *Solution B* added. To the flask add 50.0 ml of methanol-water standard solution, containing 1.0 mg of H_2O per ml, and immediately titrate with *Solution B* to the same brownish yellow endpoint. Calculate the equivalence factor, *F*, in mg of water per ml of *Solution B*. Restandardize on each day of use.

Procedure Select two 120-ml wide-mouth glass jars with plastic screw caps, wash with hydrochloric acid, rinse with water followed by isopropanol, and dry with a current of air. Bore a hole through an extra screw cap to accommodate the tip from the automatic buret. Place a magnetic stirring bar in each jar, and flush the jars with dry nitrogen to remove carbon dioxide. To each jar add 30 ml of *Solution A* and 15

ml of absolute methanol, and screw on the caps. Replace the screw cap of one of the jars with the extra cap, insert the buret tip, and begin stirring. Titrate with *Solution B* to a distinct brownish yellow color that persists for at least 5 min. Replace the original cap, and titrate the other solution in the same manner. Remove the caps from both jars, and to one of the jars add about 2 g of the sample, accurately weighed, from the remaining weighing bottle prepared as directed under *Sample Preparation*. Replace the cap on the second jar, and titrate the sample solution with *Solution B* to the brownish yellow color. Titrate the blank to the same color. Calculate the percentage H_2O in the sample by the formula

$$(F \times V \times 100)/(W \times 1000),$$

in which F is the equivalence factor, in mg per ml, of *Solution B*, V is the net volume of *Solution B* required in the titration of the sample, in ml, and W is the weight of the sample taken, in g.

Calculations Calculate the percentage of NaOH by the formula

$$2.222 \times [\%\ H_2O - (\%\ Na_2CO_3 \times 0.170)].$$

Finally, calculate the percentage of CH_3ONa by the formula

$$\%\ A - (\%\ NaOH \times 1.350).$$

Arsenic Cautiously dissolve 1 g of the sample in 10 ml of water, neutralize to litmus paper with diluted sulfuric acid TS, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Cautiously dissolve 800 mg of the sample in 10 ml of water, add 10 ml of diluted hydrochloric acid TS, and heat to boiling. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Cautiously dissolve 1 g of the sample in 10 ml of water, add 10 ml of diluted hydrochloric acid, and heat to boiling. Cool, and dilute to 25 ml with water. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Mercury Determine as directed under *Mercury Limit Test*, page 520, using the following as the *Sample Preparation*: Cautiously dissolve 2 g of the sample in 10 ml of water in a small beaker, add 2 drops of phenolphthalein TS, and slowly neutralize, with constant stirring, with dilute sulfuric acid solution (1 in 5). Add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), and mix.

Packaging and Storage Store in air-tight containers, and take all necessary precautions to prevent combustion during handling.

Functional Use in Foods Catalyst for the transesterification of fats.

Sodium Nitrate

$NaNO_3$ Mol wt 84.99

DESCRIPTION

Colorless, odorless crystals or crystalline granules. It is moderately deliquescent in moist air. It is freely soluble in water, and is sparingly soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 5 solution is neutral to litmus and gives positive tests for *Sodium*, page 517, and for *Nitrate*, page 517.

Assay Not less than 99.0% of $NaNO_3$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Total Chlorine Passes test (approximately 0.2%).

TESTS

Assay Weigh accurately about 350 mg, previously dried at 105° for 4 h, dissolve in 10 ml of hydrochloric acid in a small beaker or porcelain dish, and evaporate to dryness on a steam bath. Dissolve the residue in 10 ml of hydrochloric acid, and again evaporate to dryness, continuing the heating until the residue, when dissolved in water, is neutral to litmus. Transfer the residue with the aid of 25 ml of water to a glass-stoppered flask, add exactly 50 ml of 0.1 *N* silver nitrate, then add 3 ml of nitric acid and 3 ml of nitrobenzene, and shake vigorously. Add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Each ml of 0.1 *N* silver nitrate is equivalent to 8.50 mg of $NaNO_3$.

Arsenic Dissolve 1 g in 1 ml of water, add 2 ml of sulfuric acid, and evaporate to strong fumes of sulfur trioxide. Cool, wash down the sides of the container with water, and heat again to strong fuming. Repeat the washing and fuming three more times, then cool and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 3 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 1 g of the sample in the control (*Solution A*).

Total Chlorine Dissolve 1 g in 100 ml of water, add enough 6% sulfurous acid to give the solution a distinct odor of sulfur dioxide, boil gently until the odor of the sulfur dioxide is no longer apparent, and adjust the volume to 100 ml by the addition of water. Add 1.0 ml of 0.1 *N* silver nitrate followed by 3 ml of nitric acid and 3 ml of nitrobenzene, and shake vigorously. Add ferric ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. No more than 0.6 ml of the 0.1 *N* silver nitrate is consumed.

Packaging and Storage Store in tight containers.
Functional Use in Foods Antimicrobial agent; preservative.

Sodium Nitrite

$NaNO_2$ Mol wt 69.00

DESCRIPTION

It occurs as a white to slightly yellow, granular powder, or as white or nearly white, opaque, fused masses or sticks. It has a mild, saline taste, and is deliquescent in air. Its solutions are alkaline to litmus. One g of sodium nitrite dissolves in 1.5 ml of water, but it is sparingly soluble in alcohol.

REQUIREMENTS

Identification

Its solutions give positive tests for *Sodium*, page 517, and for *Nitrite*, page 517.

Assay Not less than 97.0% of $NaNO_2$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.25%.

TESTS

Assay Dissolve about 3 g, previously dried over silica gel for 4 h and accurately weighed, in water to make 100 ml. Pipet 10 ml of this solution into a mixture of 100.0 ml of 0.1 *N* potassium permanganate, 50 ml of water, and 5 ml of sulfuric acid, keeping the tip of the pipet well below the surface of the liquid. Warm the solution to 40°, allow it to stand for 5 min, and add 25.0 ml of 0.1 *N* oxalic acid. Heat the mixture to about 80°, and titrate with 0.1 *N* potassium permanganate. Each ml of 0.1 *N* potassium permanganate is equivalent to 3.450 mg of $NaNO_2$.

Arsenic Dissolve 1 g in 10 ml of diluted sulfuric acid TS, boil gently for 1 min, cool, and dilute to 35 ml with water. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Dissolve 1 g in a mixture of 10 ml of water and 2 ml of hydrochloric acid, and evaporate to dryness on a steam bath. Add another 2-ml portion of hydrochloric acid, again evaporate to dryness, and dissolve the residue in 25 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A solution of 1 g in 10 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry over silica gel for 4 h.

Packaging and Storage Store in tight containers.
Functional Use in Foods Color fixative in meat and meat products; antimicrobial agent; preservative.

Sodium Phosphate, Dibasic

Disodium Monohydrogen Phosphate; Disodium Phosphate

Na_2HPO_4 Mol wt 141.96

DESCRIPTION

Dibasic sodium phosphate is anhydrous or contains two molecules of water of hydration. It occurs as a white, crystalline powder or as granules. The anhydrous form is hygroscopic. Both forms are freely soluble in water and insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Phosphate*, page 517, and for *Sodium*, page 517.

Assay Not less than 98.0% of Na_2HPO_4 after drying.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 10 ppm.
Insoluble Substances Not more than 0.2%.
Loss on Drying Na_2HPO_4 (anhydrous): not more than 5%; $Na_2HPO_4.2H_2O$ (dihydrate): between 18% and 22%.

TESTS

Assay Transfer about 6.5 g of the sample, previously dried at 105° for 4 h and accurately weighed, into a 250-ml beaker, add 50.0 ml of 1 *N* hydrochloric acid and 50 ml of water, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and titrate the excess acid with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*) of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 to pH 8.8). When *A* is equal to or less than *B*, each ml of the volume *A* of 1 *N* hydrochloric acid is equivalent to 142.0 mg of Na_2HPO_4. When *A* is greater than *B*, each ml of the volume $2B - A$ of 1 *N* sodium hydroxide is equivalent to 142.0 mg of Na_2HPO_4.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 510, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water,

and filter through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Loss on Drying, page 518 Dry at 120° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Emulsifier; texturizer; buffer; nutrient; dietary supplement.

Sodium Phosphate, Monobasic

Monosodium Phosphate; Sodium Biphosphate; Monosodium Dihydrogen Phosphate

NaH_2PO_4 Mol wt 119.98

DESCRIPTION

Monobasic sodium phosphate is anhydrous or contains one or two molecules of water of hydration. It is odorless and is slightly hygroscopic. The anhydrous form is a white, crystalline powder or granules. The hydrated forms occur as white or transparent crystals or granules. All forms are freely soluble in water, but are insoluble in alcohol. The pH of a 1 in 100 solution is between 4.1 and 4.7.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Phosphate*, page 517, and for *Sodium*, page 517.

Assay Not less than 98.0% and not more than the equivalent of 103.0% of NaH_2PO_4 after drying.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Substances Not more than 0.2%.

Loss on Drying NaH_2PO_4 (anhydrous): not more than 2%; $NaH_2PO_4.H_2O$ (monohydrate): between 10% and 15%; $NaH_2PO_4.2H_2O$ (dihydrate): between 20% and 25%.

TESTS

Assay Transfer about 5 g of the sample, previously dried at 105° for 4 h and accurately weighed, into a 250-ml beaker, add 100 ml of water and 50.0 ml of 1 *N* hydrochloric acid, and stir until the sample is completely dissolved. Place the electrodes of a suitable pH meter in the solution, and slowly titrate the excess acid, stirring constantly, with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4. Record the buret reading, and calculate the volume (*A*), if any, of 1 *N* hydrochloric acid consumed by the sample. Continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached, record the buret reading, and calculate the volume (*B*) of 1 *N* sodium hydroxide required in the titration between the two inflection points (pH 4 and pH 8.8). Each ml of the volume *B* − *A* of 1 *N* sodium hydroxide is equivalent to 120.0 mg of NaH_2PO_4.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.2 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Loss on Drying, page 518 Dry first at 60° for 1 h, then at 105° for 4 h.

Packaging and Storage Store in tight containers.

Functional Use in Foods Buffer; emulsifier; nutrient; dietary supplement.

Sodium Phosphate, Tribasic

Trisodium Phosphate

Na_3PO_4 Mol wt (anhydrous) 163.94

DESCRIPTION

Tribasic sodium phosphate is anhydrous or contains 1 or 12 molecules of water of hydration. The formula for the dodecahydrate is approximately $4(Na_3PO_4.12H_2O)NaOH$. It occurs as white, odorless crystals or granules or as a crystalline powder. It is freely soluble in water, but is insoluble in alcohol. The pH of a 1 in 100 solution is between 11.5 and 12.0.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Phosphate*, page 517.

Assay Na_3PO_4 (anhydrous) and $Na_3PO_4.H_2O$ (monohydrate): not less than 97.0% of Na_3PO_4, calculated on the ignited basis; $4(Na_3PO_4.12H_2O)NaOH$ (dodecahydrate): not less than 92.0% of Na_3PO_4, calculated on the ignited basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Substances Not more than 0.2%.

Loss on Ignition Na_3PO_4 (anhydrous): not more than 2%; $Na_3PO_4.H_2O$ (monohydrate): between 8% and 11%;

$4(Na_3PO_4.12H_2O)NaOH$ (dodecahydrate): between 45% and 57%.

TESTS

Assay Dissolve an accurately weighed quantity of the sample, equivalent to between 5.5 and 6 g of anhydrous Na_3PO_4, in 40 ml of water in a 400-ml beaker, and add 100.0 ml of 1 *N* hydrochloric acid. Pass a stream of carbon dioxide–free air, in fine bubbles, through the solution for 30 min to expel carbon dioxide, covering the beaker loosely to prevent any loss by spraying. Wash the cover and sides of the beaker with a few ml of water, and place the electrodes of a standard pH meter in the solution. Titrate the solution with 1 *N* sodium hydroxide to the inflection point occurring at about pH 4, then calculate the volume (*A*) of 1 *N* hydrochloric acid consumed. Protect the solution from absorbing carbon dioxide from the air, and continue the titration with 1 *N* sodium hydroxide until the inflection point occurring at about pH 8.8 is reached. Calculate the volume (*B*) of 1 *N* sodium hydroxide consumed in the titration. When *A* is equal to or greater than 2*B*, each ml of the volume *B* of 1 *N* sodium hydroxide is equivalent to 163.9 mg of Na_3PO_4. When *A* is less than 2*B*, each ml of the volume $A - B$ of 1 *N* sodium hydroxide is equivalent to 163.9 mg of Na_3PO_4.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.2 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible (not glass). Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Loss on Ignition Ignite at about 800° for 30 min after drying at 110° for 5 h.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Buffer; emulsifier; nutrient; dietary supplement.

Sodium Polyphosphates, Glassy

Sodium Hexametaphosphate; Sodium Tetrapolyphosphate; Graham's Salt

DESCRIPTION

A class consisting of several amorphous, water-soluble polyphosphates composed of linear chains of metaphosphate units, $(NaPO_3)_x$ where $x \geq 2$, terminated by Na_2PO_4— groups. These substances occur as colorless or white transparent platelets, granules, or powders. They are usually identified by their Na_2O/P_2O_5 ratio or their P_2O_5 content. The Na_2O/P_2O_5 ratios vary from about 1.3 for sodium tetrapolyphosphate, where x = approximately 4; through about 1.1 for Graham's salt, commonly called sodium hexametaphosphate, where x = 13 to 18; to about 1.0 for the higher molecular weight sodium polyphosphates, where x = 20 to 100 or more. The pH of their solutions varies from about 7.7 to 6.0, in the same order. The glassy sodium polyphosphates are very soluble in water.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.

B. Dissolve about 100 mg in 5 ml of hot diluted nitric acid TS, warm on a steam bath for 10 min, and cool. Neutralize to litmus paper with sodium hydroxide TS, and add silver nitrate TS. A yellow precipitate is formed that is soluble in diluted nitric acid TS.

Assay Between 60.0% and 71.0% of P_2O_5.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Substances Not more than 0.1%.

TESTS

Assay Transfer about 800 mg of the sample, accurately weighed, into a 500-ml volumetric flask, add 100 ml of water and 25 ml of nitric acid, and boil for 10 min on a hot plate. Cool, dilute to volume with water, and mix. Pipet 20.0 ml of this solution into a 500-ml Erlenmeyer flask, add 100 ml of water, and heat just to boiling. Add with stirring 50 ml of quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared Gooch crucible (or fritted-glass crucible of medium porosity), and wash with five 25-ml portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 µg of P_2O_5.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution B* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals Dissolve 20 g of the sample in 80 ml of water in a 250-ml beaker, cautiously add 20 ml of sulfuric acid, and boil for 1 h. Cool the solution, dilute it to 100 ml with water, mix, and filter through a fritted-disk funnel. Dilute a 10-ml aliquot to 25 ml with water, and adjust the pH to between 3.0 and 4.0 with ammonium hydroxide. Dilute to 40 ml with water, mix, and add 10 ml of freshly prepared hydrogen sulfide TS. Allow to stand for 5 min, and view downward over a white surface. The color of the sample solution is no darker than that of a standard prepared with 20 µg of lead ion (2.0 ml of *Standard Lead Solution*, page 512, treated in the same manner as the sample.

Insoluble Substances Dissolve 10 g in 100 ml of hot water,

and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Packaging and Storage Store in tight containers.
Functional Use in Foods Emulsifier; sequestrant; texturizer.

Sodium Potassium Tartrate

Rochelle Salt

$$KOOCCH(OH)CH(OH)COONa4H_2O$$

$C_4H_4KNaO_6 4H_2O$ Mol wt 282.22

DESCRIPTION

Sodium potassium tartrate is a salt of L(+)-tartaric acid. It occurs as colorless crystals or as a white, crystalline powder, and has a cooling, saline taste. As it effloresces slightly in warm, dry air, the crystals are often coated with a white powder. One g dissolves in 1 ml of water. It is practically insoluble in alcohol.

REQUIREMENTS

Identification

A. Upon ignition, it emits the odor of burning sugar and leaves a residue that is alkaline to litmus and that effervesces with acids.
B. To 10 ml of a 1 in 20 solution add 10 ml of acetic acid. A white crystalline precipitate is formed within 15 min.
C. A 1 in 10 solution gives positive tests for *Tartrate*, page 517.

Assay Not less than 99.0% and not more than the equivalent of 102.0% of $C_4H_4KNaO_6.4H_2O$.
Alkalinity Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Water Between 21% and 26%.

TESTS

Assay Weigh accurately about 2 g, and proceed as directed under *Alkali Salts of Organic Acids Assay*, page 463. Each ml of 0.5 *N* sulfuric acid is equivalent to 70.56 mg of $C_4H_4KNaO_6.4H_2O$.
Alkalinity A 1 in 20 solution is alkaline to litmus, but after the addition of 0.2 ml of 0.1 *N* sulfuric acid to 10 ml of this solution, no pink color is produced by the addition of 1 drop of phenolphthalein TS.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Water Determine by the *Karl Fischer Titrimetric Method*, page 552, using a 200-mg sample and 35 ml of methanol in the *Procedure*.

Packaging and Storage Store in tight containers.
Functional Use in Foods Buffer; sequestrant.

Sodium Propionate

$$CH_3CH_2COONa$$

$C_3H_5NaO_2$ Mol wt 96.06

DESCRIPTION

Colorless, transparent crystals or a granular crystalline powder. It is odorless or has a faint acetic-butyric odor. It is deliquescent in moist air. One g is soluble in about 1 ml of water at 25°, in about 0.65 ml of boiling water, and in about 24 ml of alcohol. The pH of a 1 in 10 solution is between 8.0 and 10.5.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.
B. Upon ignition, it yields an alkaline residue that effervesces with acids.
C. Warm a small sample with sulfuric acid. Propionic acid, recognized by its odor, is evolved.

Assay Not less than 99.0% of $C_3H_5NaO_2$ after drying.
Alkalinity (as Na_2CO_3) Passes test (about 0.15%).
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iron Not more than 0.003%.
Water Not more than 1%.

TESTS

Assay Weigh accurately about 250 mg, previously dried at 105° for 1 h, and dissolve it in 40 ml of glacial acetic acid, warming if necessary to effect solution. Cool to room temperature, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 9.606 mg of $C_3H_5NaO_2$.
Alkalinity Dissolve 4 g in 20 ml of water, and add 3 drops of phenolphthalein TS. If a pink color is produced, not more than 0.6 ml of 0.1 *N* sulfuric acid is required to discharge it.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron Dissolve 300 mg in 40 ml of water, and add 2 ml of hydrochloric acid, about 40 mg of ammonium persulfate, and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 0.9 ml of *Iron Standard Solution* (9 µg Fe) in an equal volume of solution containing the quantities of reagents used in the test.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Preservative; mold and rope inhibitor.

Sodium Pyrophosphate

Tetrasodium Diphosphate; Tetrasodium Pyrophosphate

$Na_4P_2O_7$ Mol wt 265.90

DESCRIPTION

Sodium pyrophosphate is anhydrous or contains 10 molecules of water of hydration. It occurs as a white, crystalline or granular powder. The decahydrate effloresces slightly in dry air. It is soluble in water, but is insoluble in alcohol. The pH of a 1 in 100 solution is about 10.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.

B. Dissolve 100 mg of the sample in 100 ml of diluted nitric acid TS. Add 0.5 ml of this solution to 30 ml of quimociac TS. A yellow precipitate does not form. Heat the remaining portion of the sample solution for 10 min at 95°, and then add 0.5 ml of the solution to 30 ml of quimociac TS. A yellow precipitate forms immediately.

Assay Not less than 95.0% of $Na_4P_2O_7$, calculated on the ignited basis.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Substances Not more than 0.2%.

Loss on Ignition $Na_4P_2O_7$ (anhydrous): not more than 0.5%; $Na_4P_2O_7.10H_2O$ (decahydrate): between 38% and 42%.

TESTS

Assay Dissolve an accurately weighed quantity of the sample, equivalent to 500 mg of anhydrous $Na_4P_2O_7$, in 100 ml of water in a 400-ml beaker. Adjust the pH of the solution to 3.8 with hydrochloric acid, using a pH meter, then add 50 ml of a 1 in 8 solution of zinc sulfate (125 g of $ZnSO_4.7H_2O$ dissolved in water, diluted to 1000 ml, filtered, and adjusted to pH 3.8), and allow to stand for 2 min. Titrate the liberated acid with 0.1 *N* sodium hydroxide until a pH of 3.8 is again reached. After each addition of sodium hydroxide near the endpoint, time should be allowed for any precipitated zinc hydroxide to redissolve. Each ml of 0.1 *N* sodium hydroxide is equivalent to 13.30 mg of $Na_4P_2O_7$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

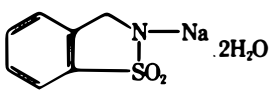
Loss on Ignition Dry at 110° for 4 h, and then ignite at about 800° for 30 min.

Packaging and Storage Store in tight containers.

Functional Use in Foods Emulsifier; buffer; nutrient; dietary supplement.

Sodium Saccharin

1,2-Benzisothiazolin-3-one 1,1-Dioxide Sodium Salt; Sodium *o*-Benzosulfimide; Soluble Saccharin



$C_7H_4NNaO_3S.2H_2O$ Mol wt 241.19

DESCRIPTION

White crystals or a white, crystalline powder. It is odorless or has a faint, aromatic odor. It has an intensely sweet taste, even in dilute solutions. In powdered form, it effloresces to the extent that it usually contains only about one third the amount of water indicated in its molecular formula. One g is soluble in 1.5 ml of water and in about 50 ml of alcohol.

REQUIREMENTS

Identification

A. Dissolve about 100 mg in 5 ml of sodium hydroxide solution (1 in 20), evaporate to dryness, and gently fuse the residue over a small flame until it no longer evolves ammonia. After the residue has cooled, dissolve it in 20 ml of water, neutralize the solution with diluted hydrochloric

acid TS, and filter. The addition of a drop of ferric chloride TS to the filtrate produces a violet color.

B. Mix 20 mg with 40 mg of resorcinol, add 10 drops of sulfuric acid, and heat the mixture in a liquid bath at 200° for 3 min. After cooling, add 10 ml of water and an excess of sodium hydroxide TS. A fluorescent green liquid results.

C. The residue obtained by igniting a 2-g sample gives positive tests for *Sodium*, page 517.

D. Add 1 ml of hydrochloric acid to 10 ml of a 1 in 10 solution of the sample, wash the crystalline precipitate well with cold water, and dry at 105° for 2 h. The saccharin thus obtained melts between 226° and 230° (see page 519).

Assay Not less than 98.0% and not more than the equivalent of 101.0% of $C_7H_4NNaO_3S$, calculated on the anhydrous basis.

Alkalinity Passes test.

Arsenic (as As) Not more than 3 ppm.

Benzoate and Salicylate Passes test.

Heavy Metals (as Pb) Not more than 10 ppm.

Readily Carbonizable Substances Passes test.

Selenium Not more than 0.003%.

Toluenesulfonamides Not more than 25 ppm.

Water Not more than 15%.

TESTS

Assay Transfer about 500 mg of the sample, accurately weighed, into a separator with the aid of 10 ml of water, add 2 ml of diluted hydrochloric acid TS, and extract the precipitated saccharin first with 30 ml, then with five 20-ml portions, of a solvent composed of 9 volumes of chloroform and 1 volume of alcohol. Filter each extract through a small filter paper moistened with the solvent mixture, and evaporate the combined filtrates on a steam bath to dryness with the aid of a current of air. Dissolve the residue in 40 ml of alcohol, add 40 ml of water, mix, add 3 drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Perform a blank determination on a mixture of 40 ml of alcohol and 40 ml of water (see page 2). Each ml of 0.1 *N* sodium hydroxide is equivalent to 20.52 mg of $C_7H_4NNaO_3S$.

Alkalinity A 1 in 10 solution is neutral or alkaline to litmus, but produces no red color with phenolphthalein TS.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Benzoate and Salicylate To 10 ml of a 1 in 20 solution, previously acidified with 5 drops of acetic acid, add 3 drops of ferric chloride TS. No precipitate or violet color appears.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Readily Carbonizable Substances, page 532 Dissolve 200 mg in 5 ml of sulfuric acid TS, and keep at a temperature of 48° to 50° for 10 min. The color is no darker than *Matching Fluid A*.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Toluenesulfonamides

Methylene Chloride Use a suitable grade (such as that obtainable from Burdick & Jackson Laboratories, Inc.), equivalent to the product obtained by distillation in all-glass apparatus.

Internal Standard Stock Solution Transfer 100.0 mg of *n*-tricosane (95%, obtainable from Chemical Samples Co.) into a 10-ml volumetric flask, dissolve in *n*-heptane, dilute to volume with the same solvent, and mix.

Stock Standard Preparation Transfer 20.0 mg each of reagent-grade *o*-toluenesulfonamide and *p*-toluenesulfonamide into a 10-ml volumetric flask, dissolve in methylene chloride, dilute to volume with the same solvent, and mix.

Diluted Standard Preparations Pipet into five 10-ml volumetric flasks 0.1, 0.25, 1.0, 2.5, and 5.0 ml, respectively, of the *Stock Standard Preparation*. Pipet 0.25 ml of the *Internal Standard Stock Solution* into each flask, dilute each to volume with methylene chloride, and mix. These solutions contain, respectively, 20, 50, 200, 500, and 1000 µg per ml of each toluenesulfonamide, plus 250 µg of *n*-tricosane.

Test Preparation Dissolve 2.00 g of the sample in 8.0 ml of 5% sodium bicarbonate solution, and mix the solution thoroughly with 10.0 g of chromatographic siliceous earth (Celite 545, Johns-Manville, or equivalent). Transfer the mix into a 25- × 250-mm chromatographic tube having a fritted-glass disk and a Teflon stopcock at the bottom and a reservoir at the top. Pack the contents of the tube by tapping the column on a padded surface, and then by tamping firmly from the top. Place 100 ml of methylene chloride in the reservoir, and adjust the stopcock so that 50 ml of eluate is collected in 20 to 30 min. To the eluate add 25 µl of *Internal Standard Stock Solution*, mix, and then concentrate the solution to a volume of 1.0 ml in a suitable concentrator tube fitted with a modified Snyder column, using a Kontes tube heater maintained at 90°.

Procedure Inject 2.5 µl of the *Test Preparation* into a suitable gas chromatograph equipped with a flame-ionization detector. The column is of glass, 10 ft (approximately 3 m) in length and 2 mm in inside diameter, and it is packed with 3% phenyl methyl silicone (OV-17, Applied Science Laboratories, Inc., or equivalent) on 100- to 120-mesh silanized calcined diatomaceous silica (Gas-Chrom Q, Applied Science, or equivalent). [*Caution:* The glass column should extend into the injector for on-column injection and into the detector base to avoid contact with metal.] The carrier is helium, flowing at a rate of 30 ml per min. The injection port, column, and detector are maintained at 225°, 180°, and 250°, respectively. The instrument attenuation setting should be such that 2.5 µl of the *Diluted Standard Preparation* containing 200 µg per ml of each toluenesulfonamide gives a response of 40% to 80% of full-scale deflection. Record the chromatogram, note the peaks for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and the *n*-tricosane internal standard, and calculate the areas for each peak by suitable means. The retention times for *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane are about 5, 6, and 15 min, respectively.

In a similar manner, obtain the chromatograms for 2.5-µl portions of each of the five *Diluted Standard Preparations*,

and for each solution determine the areas of the *o*-toluenesulfonamide, *p*-toluenesulfonamide, and *n*-tricosane peaks. From the values thus obtained, prepare standard curves by plotting concentration of each toluenesulfonamide, in μg per ml, versus the ratio of the respective toluenesulfonamide peak area to that of *n*-tricosane. From the standard curve determine the concentration, in μg per ml, of each toluenesulfonamide in the *Test Preparation*. Divide each value by 2 to convert the result to ppm of the toluenesulfonamide in the 2-g sample taken for analysis. [NOTE: If the toluenesulfonamide content of the sample is greater than about 500 ppm, the impurity may crystallize out of the methylene chloride concentrate (see *Test Preparation*). Although this level of impurity exceeds that permitted by the specification, the analysis may be completed by diluting the concentrate with methylene chloride containing 250 μg of *n*-tricosane per ml, and by applying appropriate dilution factors in the calculation. Care must be taken to redissolve completely any crystalline toluenesulfonamide to give a homogeneous solution.]

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nonnutritive sweetener.

Sodium Sesquicarbonate

$Na_2CO_3.NaHCO_3.2H_2O$ Mol wt 226.03

DESCRIPTION

White crystals, flakes, or a crystalline powder. It is soluble in water, and its solutions are alkaline to litmus.

REQUIREMENTS

Identification

A 1 in 10 solution gives positive tests for *Sodium*, page 517, and for *Carbonate*, page 516.

Assay *Sodium bicarbonate:* not less than 35.0% and not more than 38.6% of $NaHCO_3$; *sodium carbonate:* not less than 46.4% and not more than 50.0% of Na_2CO_3.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iron Not more than 0.002%.
Sodium Chloride Not more than 0.5%.
Water Between 13.8% and 16.7%.

TESTS

Assay for Sodium Bicarbonate Dissolve about 3 g of the sample, accurately weighed, in 150 ml of carbon dioxide–free water in a 600-ml beaker containing 50.0 ml of 0.5 *N* sodium hydroxide. While stirring add 200 ml of 0.48 *M* barium chloride that has been adjusted to a pH of 8.0 with a pH meter. Using a pH meter that has been standardized to pH 9.0, titrate the solution with 0.5 *N* hydrochloric acid until a pH of 8.8 remains for 1 min, and record the volume of 0.5 *N* hydrochloric acid required as *S*, in ml. Perform a blank determination using 2.1 g of primary standard sodium carbonate, and record the volume of 0.5 *N* hydrochloric acid required as *B*, in ml. Each ml of the volume $B - S$ of 0.5 *N* hydrochloric acid is equivalent to 42.00 mg of $NaHCO_3$.

Assay for Sodium Carbonate Determine the total alkalinity (as Na_2O) of the sample as follows: Dissolve about 4.2 g, accurately weighed, in 100 ml of water in a 250-ml beaker, add methyl orange TS, and titrate with 1 *N* sulfuric acid, stirring vigorously near the endpoint to expel carbon dioxide. Each ml of 1 *N* sulfuric acid is equivalent to 30.99 mg of Na_2O. Calculate the percentage of sodium oxide (% Na_2O) in the sample taken.

Calculate the percentage of sodium carbonate in the sample by the formula

$$[\%\ Na_2O - (\%\ NaHCO_3 \times 0.3689)] \times 1.7099,$$

in which % $NaHCO_3$ is the percentage of sodium bicarbonate determined in the *Assay for Sodium Bicarbonate*, 0.3689 is a factor converting $NaHCO_3$ to Na_2O, and 1.7099 is a factor converting Na_2O to Na_2CO_3.

Arsenic A solution of 1 g in 5 ml of diluted hydrochloric acid TS meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Mix 2 g with 5 ml of water and 10 ml of diluted hydrochloric acid TS, boil for 1 min, cool, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Iron Dissolve 500 mg in 10 ml of diluted hydrochloric acid TS, and dilute to 50 ml with water. Add about 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 μg Fe) in an equal volume of solution containing 2 ml of hydrochloric acid and the quantities of ammonium persulfate and ammonium thiocyanate used in the test.

Sodium Chloride Dissolve about 10 g, accurately weighed, in 50 ml of water in a 250-ml beaker, add sufficient nitric acid to make the solution slightly acid, then add 1 ml of ferric ammonium sulfate TS and 1.00 ml of 0.05 *N* ammonium thiocyanate, and titrate with 0.05 *N* silver nitrate, stirring constantly, until the red color is completely discharged. Finally, back titrate with 0.05 *N* ammonium thiocyanate until a faint reddish color is obtained. Subtract the total volume of 0.05 *N* ammonium thiocyanate added from the volume of 0.05 *N* silver nitrate required. Each ml of 0.05 *N* silver nitrate is equivalent to 2.922 mg of NaCl. Calculate the percentage of sodium chloride in the sample taken.

Water Calculate the percentage of water by subtracting from 100 the sum of the percentages of *Sodium Bicarbonate*, *Sodium Carbonate*, and *Sodium Chloride* found in the sample.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Alkali; neutralizer in dairy products.

Sodium Stearoyl Lactylate

DESCRIPTION

A mixture of sodium salts of stearoyl lactylic acids and minor proportions of other sodium salts of related acids, manufactured by the reaction of stearic acid and lactic acid, neutralized to the sodium salts. It is a slightly hygroscopic, cream-colored powder having a mild, caramellike odor. It is soluble in hot oil or fat, and is dispersible in warm water. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

A. Heat 1 g with a mixture of 25 ml of water and 5 ml of hydrochloric acid. Fatty acids are liberated, floating as an oily layer on the surface of the liquid. The water layer gives positive tests for *Sodium*, page 517.

B. Mix 25 g of the sample with 50 g of a 15% alcoholic potassium hydroxide solution in an Erlenmeyer flask, and reflux for 1 h or until saponification is complete. Cool, add 150 ml of water, and mix. After complete solution of the soap, add 60 ml of diluted sulfuric acid TS, and heat the mixture, with frequent stirring, until the fatty acids separate cleanly as a transparent layer. Wash the fatty acids with boiling water until free from sulfate, collect them in a small beaker, and warm on a steam bath until the water has separated and the fatty acids are clear. Allow the acids to cool, pour off the water layer, then melt the acids, filter into a dry beaker, and dry at 105° for 20 min. The solidification point of the fatty acids so obtained is not below 54° (see page 519).

Acid Value Between 60 and 80.
Arsenic (as As) Not more than 3 ppm.
Ester Value Between 150 and 190.
Heavy Metals (as Pb) Not more than 10 ppm.
Sodium Content Between 3.5% and 5.0%.
Total Lactic Acid Between 31.0% and 34.0%.

TESTS

Acid Value Transfer about 1 g, accurately weighed, to a 125-ml Erlenmeyer flask, add 25 ml of alcohol, previously neutralized to phenolphthalein TS, and heat on a hot plate until the sample is dissolved. Cool, add 5 drops of phenolphthalein TS, and titrate rapidly with 0.1 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula $56.1V \times N/W$, in which V is the volume, in ml, and N is the normality, respectively, of the sodium hydroxide solution, and W is the weight, in g, of the sample taken. Retain the neutralized solution for the determination of *Ester Value*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ester Value To the neutralized solution retained in the test for *Acid Value* add 10.0 ml of alcoholic potassium hydroxide solution prepared by dissolving 11.2 g of potassium hydroxide in 250 ml of alcohol and diluting with 25 ml of water. Add 5 drops of phenolphthalein TS, connect a suitable condenser, and reflux for 2 h. Cool, add 5 additional drops of phenolphthalein TS, and titrate the excess alkali with 0.1 *N* sulfuric acid. Perform a blank determination using 10.0 ml of the alcoholic potassium hydroxide solution. Calculate the ester value by the formula $56.1(B - S)N/W$, in which $B - S$ represents the difference between the volumes of 0.1 *N* sulfuric acid required for the blank and sample, respectively, N is the normality of the sulfuric acid, and W is the weight, in g, of the sample taken.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Sodium Content [NOTE: Ordinary glassware should not be used in this test because of possible contamination by sodium; use suitable plastic (e.g., polyethylene) vessels where necessary.]

Stock Lanthanum Solution Transfer 5.86 g of lanthanum oxide, La_2O_3, to a 100-ml volumetric flask, wet with a few ml of water, slowly add 25 ml of hydrochloric acid, and swirl until the material is completely dissolved. Dilute to volume with water, and mix.

Stock Sodium Solution Use a solution containing 1 mg of Na in each ml (1000 ppm Na). The solution may be obtained commercially or prepared as follows: Transfer 1.271 g of sodium chloride, previously dried at 105° for 2 h and accurately weighed, to a 500-ml volumetric flask, dilute to volume with water, and mix.

Standard Preparations Transfer 10.0 ml of the *Stock Lanthanum Solution* to each of three 100-ml volumetric flasks. Using a microliter syringe, transfer 0.20 ml of the *Stock Sodium Solution* to the first flask, 0.40 ml to the second flask, and 0.50 ml to the third flask. Dilute each flask to volume with water, and mix. The flasks contain 2.0, 4.0, and 5.0 µg of Na per ml, respectively. Prepare these solutions fresh daily.

Sample Preparation Transfer about 250 mg of the sample, accurately weighed, to a 30-ml beaker, dissolve with heating in 10 ml of alcohol, and quantitatively transfer the solution into a 25-ml volumetric flask. Wash the beaker with two 5-ml portions of alcohol, adding the washings to the flask, dilute to volume with alcohol, and mix. Transfer 2.5 ml of the *Stock Lanthanum Solution* to a second 25-ml volumetric flask. Using a microliter syringe, transfer 0.25 ml of the alcoholic solution of the sample to the second flask, dilute to volume with water, and mix.

Procedure Concomitantly determine the absorbance of each *Standard Preparation* and of the *Sample Preparation* at 589 nm, with a suitable atomic absorption spectrophotometer, following the operating parameters as recommended by the manufacturer of the instrument. Plot the absorbances of the *Standard Preparations* versus concentration of Na, in µg per ml, and from the curve so obtained determine the

concentration, *C*, in μg per ml, of Na in the *Sample Preparation*. Calculate the quantity, in mg, of Na in the sample taken by the formula 2.5*C*.

Total Lactic Acid

Standard Curve Dissolve 1.067 g of lithium lactate in sufficient water to make 1000.0 ml. Transfer 10.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 1.0, 2.0, 4.0, 6.0, and 8.0 ml of the diluted standard solution into separate 100-ml volumetric flasks, dilute each flask to volume with water, and mix. These standards represent 1, 2, 4, 6, and 8 μg of lactic acid per ml, respectively. Transfer 1.0 ml of each solution into separate test tubes, and continue as directed in the *Procedure*, beginning with "Add 1 drop of cupric sulfate TS. . . . " After color development and reading the absorbance values, construct a *Standard Curve* by plotting absorbance versus μg of lactic acid.

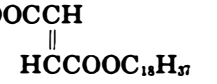
Test Preparation Transfer about 200 mg of the sample, accurately weighed, into a 125-ml Erlenmeyer flask, add 10 ml of 0.5 *N* alcoholic potassium hydroxide and 10 ml of water, attach an air condenser, and reflux gently for 45 min. Wash the sides of the flask and the condenser with about 40 ml of water, and heat on a steam bath until no odor of alcohol remains. Add 6 ml of dilute sulfuric acid (1 in 2), heat until the fatty acids are melted, then cool to about 60°, and add 25 ml of petroleum ether. Swirl the mixture gently, and transfer quantitatively to a separator. Collect the water layer in a 100-ml volumetric flask, and wash the petroleum ether layer with two 20-ml portions of water, adding the washings to the volumetric flask. Dilute to volume with water, and mix. Transfer 1.0 ml of this solution into a second 100-ml volumetric flask, dilute to volume with water, and mix.

Procedure Transfer 1.0 ml of the *Test Preparation* into a test tube, and transfer 1.0 ml of water to a second test tube to serve as the blank. Treat each tube as follows: Add 1 drop of cupric sulfate TS, swirl gently, and then add rapidly from a buret 9.0 ml of sulfuric acid. Loosely stopper the tube, and heat in a water bath at 90° for exactly 5 min. Cool immediately to below 20° in an ice bath for 5 min, add 3 drops of *p*-phenylphenol TS, shake immediately, and heat in a water bath at 30° for 30 min, shaking the tube twice during this time to disperse the reagent. Heat the tube in a water bath at 90° for exactly 90 s, and then cool immediately to room temperature in an ice water bath. Determine the absorbance of the solution in a 1-cm cell, at 570 nm, with a suitable spectrophotometer, using the blank to set the instrument. Obtain the weight, in μg, of lactic acid in the portion of the *Test Preparation* taken for the *Procedure* by means of the *Standard Curve*.

Packaging and Storage Store in tight containers in a cool, dry place.

Functional Use in Foods Emulsifier; dough conditioner; stabilizer; whipping agent.

Sodium Stearyl Fumarate



$C_{22}H_{39}NaO_4$ Mol wt 390.54

DESCRIPTION

A fine, white powder. It is slightly soluble in methanol, but is practically insoluble in water.

REQUIREMENTS

Identification

When the sample is analyzed by ascending thin-layer chromatography as directed in the test for *Sodium Stearyl Maleate and Stearyl Alcohol*, the R_f value of the large yellow spot obtained from the *Sample Solution* corresponds to that obtained from *Standard Solution A*, and the R_f value of the main spot obtained after spraying the plate with sulfuric acid and heating at 150° corresponds to that obtained from *Standard Solution B*.

Assay Not less than 99.0% and not more than the equivalent of 101.5% of $C_{22}H_{39}NaO_4$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Saponification Value Between 142.2 and 146, calculated on the anhydrous basis.

Sodium Stearyl Maleate Not more than 0.25%.

Stearyl Alcohol Not more than 0.5%.

Water Not more than 5%.

TESTS

Assay Transfer about 250 mg, accurately weighed, into a 50-ml Erlenmeyer flask, mix it with 1 ml of chloroform, and add 20 ml of glacial acetic acid to dissolve the sample. Add quinaldine red TS, and titrate with 0.1 *N* perchloric acid in glacial acetic acid. Each ml of 0.1 *N* perchloric acid is equivalent to 39.05 mg of $C_{22}H_{39}NaO_4$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Saponification Value Transfer about 450 mg of sodium stearyl fumarate, accurately weighed, into a 300-ml Erlenmeyer flask, and add 50.0 ml of ethanolic potassium hydroxide

solution, rinsing down the inside of the flask during the addition. (Prepare the ethanolic potassium hydroxide solutions as follows: Dissolve about 5.5 g of potassium hydroxide in absolute ethanol, heating if necessary to effect solution, and dilute to 1000 ml with absolute ethanol. Prepare fresh daily, and filter if necessary to remove carbonate.) Reflux the mixture gently on a steam bath for at least 2 h, swirling gently occasionally but avoiding splashing the mixture up into the condenser. Rinse the condenser with 10 ml of 70% alcohol, followed by three 10-ml portions of water, collecting the rinsings in the flask. Cool, rinse the sides of the flask with two 10-ml portions of 70% alcohol, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid to the disappearance of any pink color. Perform a blank determination using the same amount of ethanolic potassium hydroxide solution (see page 0). Calculate the saponification value by the formula

$$56.1(B - S) \times N/W,$$

in which $B - S$ represents the difference between the volumes of 0.1 *N* hydrochloric acid required for the blank and the sample, respectively, *N* is the exact normality of the hydrochloric acid, and *W* is the weight, in g, of the sample taken.

Sodium Stearyl Maleate and Stearyl Alcohol

Apparatus Assemble a suitable apparatus for ascending thin-layer chromatography (see page 474). Prepare a slurry of 24 g of chromatographic silica gel G in 75 ml of water, apply a uniformly thin layer to 23-cm square glass plates, or other convenient size, and dry in the air at room temperature for 2 h.

Sample Solution Weigh accurately 200 mg of the sample into a glass-stoppered 10-ml volumetric flask, dilute to volume with a solution of 10% acetic acid in chloroform, and mix. The mixture may be heated carefully, if necessary, to dissolve the sample, and then cooled before diluting to volume with the solvent mixture.

Standard Solution A Weigh accurately 10 mg of sodium stearyl maleate into a 100-ml volumetric flask, dilute to volume with 10% acetic acid in chloroform, and shake well.

Standard Solution B Weigh accurately 20 mg of stearyl alcohol into a 100-ml volumetric flask, dilute to volume with 10% acetic acid in chloroform, and shake well.

Standard Solution C Mix 25.0 ml of *Standard Solution A* with 25.0 ml of *Standard Solution B*, and shake well. This mixture represents 0.25% of sodium stearyl maleate and 0.5% of stearyl alcohol, based upon the weight (200 mg) of the sample taken.

Procedure Spot 10 µl each of the *Sample Solution* and of *Standard Solution C* at the bottom of the plate. Allow the spots to dry, then place the plate in a suitable chromatographic chamber containing a mixture of 10 volumes of benzene, 10 volumes of hexane, and 1 volume of acetic acid, previously equilibrated, and develop by ascending chromatography for 30 min to effect one pass. Remove the plate from the tank, dry in the air for 10 min, and then heat in an oven at 90° for 2 min. After cooling to room temperature, replace the plate in the chamber for a second pass of 30 min. After the second pass, remove the plate from the chamber and dry in the air for 15 to 20 min. Spray evenly with a mixture consisting of 0.5% of potassium permanganate and 0.3% of sodium carbonate in water. Maleate and fumarate appear as yellow spots against a pink background. Spray with sulfuric acid and heat in an oven at 150° for the detection of stearyl alcohol.

Visually compare any spots from the sample against the R_f of the spots from the standards. The spots from the sample do not appear to be stronger than the respective spots from the standards.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Dough conditioner.

Sodium Sulfate

Na_2SO_4 Mol wt 142.04

DESCRIPTION

Sodium sulfate is anhydrous or contains 10 molecules of water of crystallization. It occurs as colorless crystals or as a fine, white, crystalline powder. The decahydrate is efflorescent. Sodium sulfate is freely soluble in water and practically insoluble in alcohol. A 1 in 20 solution is neutral or slightly alkaline to litmus paper.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Sulfate*, page 517.

Assay Not less than 99.0% of Na_2SO_4 after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying *Anhydrous form:* not more than 1%; *decahydrate:* between 51% and 57%.

Selenium Not more than 0.003%.

TESTS

Assay Dissolve about 500 mg, previously dried at 105° for 3 h and accurately weighed, in 200 ml of water, add 1 ml of hydrochloric acid, and heat to boiling. Gradually add, in small portions at a time and while stirring constantly, an excess of hot barium chloride TS (about 10 ml), and heat the mixture on a steam bath for 1 h. Collect the precipitate on a filter, wash until free from chloride, dry, ignite, and weigh. The weight of the barium sulfate so obtained, multiplied by 0.6086, indicates its equivalent of Na_2SO_4.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 3 g in 25 ml of water meets the

requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) and 1 g of the sample in the control (*Solution A*).

Loss on Drying, page 518 Dry at 105° for 3 h.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in well-closed container.

Functional Use in Foods Agent in caramel production.

Sodium Sulfite

Exsiccated Sodium Sulfite

Na_2SO_3 Mol wt 126.04

DESCRIPTION

A white or tan to slightly pink, odorless or nearly odorless powder having a cooling, saline, sulfurous taste. It undergoes oxidation in air. Its solutions are alkaline to litmus and to phenolphthalein. One g dissolves in about 4 ml of water. It is sparingly soluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Sulfite*, page 517.

Assay Not less than 95.0% of Na_2SO_3.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Selenium Not more than 0.003%.

TESTS

Assay Weigh accurately about 250 mg, add it to exactly 50 ml of 0.1 *N* iodine contained in a glass-stoppered flask, and stopper the flask. Allow to stand for 5 min, add 1 ml. of hydrochloric acid, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 6.302 mg of Na_2SO_3.

Arsenic Dissolve 1 g of the sample in 10 ml of water in a 150-ml beaker, cautiously add 10 ml of nitric acid and 5 ml of sulfuric acid, and evaporate on a steam bath to a volume of about 5 ml. Place the beaker on a hot plate, and heat just to dense fumes of sulfur trioxide. Cool, cautiously wash down the side of the beaker with about 10 ml of water, and again heat to dense fumes. Cool, repeat the washing and fuming procedure, and cool again. This solution meets the requirements of the *Arsenic Test*, page 464, omitting the addition of 20 ml of dilute sulfuric acid (1 in 5).

Heavy Metals Dissolve 2 g in 10 ml of water, add 4 ml of hydrochloric acid, and evaporate to dryness on a steam bath. To the residue add 5 ml of hot water and 1 ml of hydrochloric acid, and again evaporate to dryness. Dissolve the residue in water, and dilute to 25 ml. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample and 100 mg of magnesium oxide.

Packaging and Storage Store in tight containers.

Functional Use in Foods Preservative; antioxidant.

Sodium Tartrate

Disodium Tartrate; Disodium *d*-Tartrate

$NaOOCCH(OH)CH(OH)COONa.2H_2O$

$C_4H_4Na_2O_6.2H_2O$ Mol wt 230.08

DESCRIPTION

Sodium tartrate is the disodium salt of L(+)-tartaric acid. It occurs as transparent, colorless, odorless crystals. One g dissolves in 3 ml of water. It is insoluble in alcohol. The pH of a 1 in 20 solution is between 7 and 9. Upon ignition, it emits the odor of burning sugar and leaves a residue that is alkaline to litmus and that effervesces with acids.

REQUIREMENTS

Identification

It gives positive tests for *Sodium*, page 517, and for *Tartrate*, page 517.

Assay Not less than 99.0% of $C_4H_4Na_2O_6$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Between 14% and 17%.

Oxalate Passes test (limit about 0.1%).

TESTS

Assay Weigh accurately about 250 mg, previously dried at 150° for 3 h, and transfer it to a 250-ml beaker. Add 150 ml of glacial acetic acid, heat to near boiling, stir until the sample is dissolved (preferably with a magnetic stirrer), and cool to room temperature. Titrate with 0.1 *N* perchloric acid in glacial acetic acid, determining the endpoint potentiometrically. Each ml of 0.1 *N* perchloric acid is equivalent to 9.703 mg of $C_4H_4Na_2O_6$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry at 150° for 3 h.
Oxalate Dissolve 1 g in 10 ml of water, and add 5 drops of diluted acetic acid TS and 2 ml of calcium chloride TS. No turbidity develops within 1 h.

Packaging and Storage Store in tight containers.
Functional Use in Foods Sequestrant.

Sodium Thiosulfate

Sodium Hyposulfite

$Na_2S_2O_3 5H_2O$ Mol wt 248.17

DESCRIPTION

Large, colorless crystals or a coarse, crystalline powder. It is deliquescent in moist air and effloresces in dry air at a temperature above 33°. Its solutions are neutral or faintly alkaline to litmus. One g dissolves in 0.5 ml of water. It is insoluble in alcohol.

REQUIREMENTS

Identification

A. To a 1 in 10 solution add a few drops of iodine TS. The color is discharged.
B. A 1 in 20 solution gives positive tests for *Sodium*, page 517, and for *Thiosulfate*, page 517.

Assay Not less than 99.0% of $Na_2S_2O_3$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Selenium Not more than 0.003%.
Water Between 32% and 37%.

TESTS

Assay Weigh accurately about 500 mg of the dried sample obtained in the test for *Water*, dissolve it in 30 ml of water, and titrate with 0.1 *N* iodine, using starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 15.81 mg of $Na_2S_2O_3$.
Arsenic Dissolve 1.0 g in about 5 ml of water, add 9 ml of nitric acid, and cautiously evaporate to dryness on a steam bath. Take up the residue in a few ml of water, filter, wash thoroughly, and evaporate the combined filtrate and washings to dryness. Cool, and dissolve the residue in water to make 35 ml. This solution meets the requirements of the *Arsenic Test*, page 464.

Sample Solution for the Determination of Heavy Metals and Lead Dissolve 5.0 g in 40 ml of water, slowly add 25 ml of diluted hydrochloric acid TS, and evaporate to dryness on a steam bath. Add 30 ml of water to the residue, boil gently for 2 min, and filter. Heat the filtrate to boiling, add sufficient bromine TS to produce a clear solution, then add a slight excess of bromine. Boil to expel the excess bromine, cool, and dilute to 50.0 ml with water.

Heavy Metals Dilute 10.0 ml of the *Sample Solution* (1-g sample) to 25 ml with water, and proceed as directed under the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A 10.0-ml portion of the *Sample Solution* (1-g sample) meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.
Water Dry about 1 g, accurately weighed, in a vacuum at 40° to 45° for 16 h, cool, and weigh.

Packaging and Storage Store in tight containers.
Functional Use in Foods Sequestrant; antioxidant.

Sodium Trimetaphosphate

$(NaPO_3)_3$ Mol wt 305.92

DESCRIPTION

A cyclic polyphosphate composed of three metaphosphate units. It occurs as white crystals or as a white crystalline powder. It is freely soluble in water. The pH of a 1 in 100 solution is about 6.0.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.
B. Dissolve about 100 mg in 5 ml of hot diluted nitric acid TS, warm on a steam bath for 10 min, and cool. Neutralize to litmus paper with sodium hydroxide TS, and add silver nitrate TS. A yellow precipitate is formed that is soluble in diluted nitric acid TS.

Assay Between 68.0% and 70.0% of P_2O_5.
Arsenic (as As) Not more than 3 ppm.
Fluoride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 10 ppm.
Insoluble Substances Not more than 0.1%.

TESTS

Assay Transfer about 800 mg of the sample, accurately weighed, into a 500-ml volumetric flask, add 100 ml of water and 25 ml of nitric acid, and boil for 10 min on a hot plate. Cool, dilute to volume with water, and mix. Pipet 20.0 ml of this solution into a 500-ml Erlenmeyer flask, add 100 ml of water, and heat just to boiling. Add with stirring 50 ml of

quimociac TS, then cover with a watch glass, and boil for 1 min in a well-ventilated hood. Cool to room temperature, swirling occasionally while cooling, then filter through a tared Gooch crucible (or fritted-glass crucible of medium porosity), and wash with five 25-ml portions of water. Dry at about 225° for 30 min, cool, and weigh. Each mg of precipitate thus obtained is equivalent to 32.074 μg of P_2O_5.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Packaging and Storage Store in tight containers.

Functional Use in Foods Starch-modifying agent.

Sodium Tripolyphosphate

Pentasodium Triphosphate; Triphosphate; Sodium Triphosphate

$Na_5P_3O_{10}$ Mol wt 367.86

DESCRIPTION

Sodium tripolyphosphate is anhydrous or contains six molecules of water of hydration. It occurs as white, slightly hygroscopic granules, or as a powder. It is freely soluble in water. The pH of a 1 in 100 solution is about 9.5.

REQUIREMENTS

Identification

A. A 1 in 20 solution gives positive tests for *Sodium*, page 517.

B. To 1 ml of a 1 in 100 solution add a few drops of silver nitrate TS. A white precipitate is formed that is soluble in diluted nitric acid TS.

Assay *Anhydrous:* Not less than 85.0% of $Na_5P_3O_{10}$; *hexahydrate:* not less than 65.0% of $Na_5P_3O_{10}$.

Arsenic (as As) Not more than 3 ppm.

Fluoride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 10 ppm.

Insoluble Substances Not more than 0.1%.

Lead Not more than 5 ppm.

TESTS

Assay

Potassium Acetate Buffer (pH 5.0) Dissolve 78.5 g of potassium acetate in 1000 ml of water, and adjust the pH of the solution to 5.0 with acetic acid. Add a few mg of mercuric iodide to inhibit mold growth.

0.3 M Potassium Chloride Solution Dissolve 22.35 g of potassium chloride in water, add 5 ml of *Potassium Acetate Buffer*, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

0.6 M Potassium Chloride Solution Dissolve 44.7 g of potassium chloride in water, add 5 ml of *Potassium Acetate Buffer*, dilute with water to 1000 ml, and mix. Add a few mg of mercuric iodide.

1 M Potassium Chloride Solution Dissolve 74.5 g of potassium chloride in water, add 5 ml of *Potassium Acetate Buffer*, dilute to 1000 ml with water, and mix. Add a few mg of mercuric iodide.

Chromatographic Column Use a standard chromatographic column, 20- to 40-cm in length, 20- to 28-mm in inside diameter, with a sealed-in, coarse-porosity fritted disk. If a stopcock is not provided, attach a stopcock having a 3- to 4-mm diameter bore to the outlet of the column with a short length of flexible vinyl tubing.

Procedure Close the column stopcock, fill the space between the fritted disk and the stopcock with water, and connect a vacuum line to the stopcock. Prepare a 1:1 water slurry of Dowex 1 × 8, chloride form, 100–200 or 200–400 mesh, or a comparable grade of styrene-divinylbenzene ion exchange resin, and decant off any very fine particles and any foam. Do this two or three times or until no more finely suspended material or foaming is observed. Fill the column with the slurry, and open the stopcock to allow the vacuum to pack the resin bed until the water level is slightly above the top of the resin, then immediately close the stopcock. Do not allow the liquid level to fall below the resin level at any time. Repeat this procedure until the packed resin column is 15 cm (about 6 in.) above the fritted disk. Place one circle of tightly fitting glass-fiber filter paper on top of the resin bed, then place a perforated polyethylene disk on top of the paper. Alternatively, a loosely packed plug of glass wool may be placed on top of the bed. Close the top of the column with a rubber stopper in which a 7.6 cm length of capillary tubing (1.5-mm id, 7 mm od) has been inserted through the center, so that about 12 mm of the tubing extends through the bottom of the stopper. Connect the top of the capillary tubing to the stem of a 500-ml separator with flexible vinyl tubing, and clamp the separator to a ring stand above the column. Wash the column by adding 100 ml of water to the separator with all stopcocks closed. First open the separator stopcock, then open the column stopcock. The rate of flow should be about 5 ml per min. When the separator is empty, close the stopcock on the column, then close the separator stopcock.

Transfer about 500 mg of the sample, accurately weighed, into a 250-ml volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 10.0 ml of this solution into the separator, open both stopcocks, and allow the solution to drain into the column, rinsing the separator with 20 ml of water. Discard the eluate.

Add 370 ml of *0.3 M Potassium Chloride Solution* to the separator, and allow this solution to pass through the column, discarding the eluate. Add 250 ml of *0.6 M Potassium Chloride Solution* to the column, allow the solution to pass through the column, and receive the eluate in a 400-ml beaker. (To ensure a clean column for the next run, pass 100 ml of *1 M Potassium Chloride Solution* through the column, and then follow with 100 ml of water. Discard all washings.) To the beaker add 15 ml of nitric acid, mix, and boil for 15 to 20 min. Add methyl orange TS, and neutralize the solution with stronger ammonia TS. Add 1 g of ammonium nitrate crystals, stir to dissolve, and cool. Add 15 ml of ammonium molybdate TS, with stirring, and stir vigorously for 3 min, or allow to stand with occasional stirring for 10 to 15 min. Filter the contents of the beaker with suction through a 6- to 7-mm paper-pulp filter pad supported in a 25-mm porcelain disk. The filter pad should be covered with a suspension of infusorial earth. After the contents of the beaker have been transferred to the filter, wash the beaker with five 10-ml portions of a 1 in 100 solution of sodium or potassium nitrate, passing the washings through the filter, then wash the filter with five 5-ml portions of the wash solution. Return the filter pad and the precipitate to the beaker, wash the funnel thoroughly with water into the beaker, and dilute to about 150 ml. Add 0.1 *N* sodium hydroxide from a buret until the yellow precipitate is dissolved, then add 5 to 8 ml in excess. Add phenolphthalein TS, and titrate the excess alkali with 0.1 *N* nitric acid. Finally, titrate with 0.1 *N* sodium hydroxide to the first appearance of the pink color. The difference between the total volume of 0.1 *N* sodium hydroxide added and the volume of nitric acid required represents the volume, *V*, in ml, of 0.1 *N* sodium hydroxide consumed by the phosphomolybdate complex. Calculate the quantity, in mg, of $Na_5P_3O_{10}$ in the sample taken by the formula $0.533 \times 25V$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Fluoride Determine on a 2-g sample as directed in *Method IV* under the *Fluoride Limit Test*, page 512, using *Buffer Solution A* and 0.1 ml of *Fluoride Standard Solution*.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Insoluble Substances Dissolve 10 g in 100 ml of hot water, and filter through a tared filtering crucible. Wash the insoluble residue with hot water, dry at 105° for 2 h, cool, and weigh.

Lead A solution of 1 g in 20 ml of water meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers.

Functional Use in Foods Texturizer.

Sorbic Acid

2,4-Hexadienoic Acid

$$CH_3CH=CHCH=CHCOOH$$

$C_6H_8O_2$ Mol wt 112.13

DESCRIPTION

A white, free-flowing powder with a characteristic odor. It is slightly soluble in water. One g dissolves in about 10 ml of alcohol and in about 20 ml of ether.

REQUIREMENTS

Identification

A. To 2 ml of a 1 in 10 solution of the sample in alcohol add a few drops of bromine TS. The color is discharged.

B. A 1 in 400,000 solution in isopropanol exhibits an absorbance maximum at 254 ± 2 nm.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $C_6H_8O_2$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Melting Range Between 132° and 135°.

Residue on Ignition Not more than 0.2%.

Water Not more than 0.5%.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 50 ml of anhydrous methanol that previously has been neutralized with 0.1 *N* sodium hydroxide, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 11.21 mg of $C_6H_8O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Melting Range, page 519 Determine as directed for *Class Ia*, but heat at a rate of rise of 1° per min until the melting is complete.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers protected from light, preferably at a temperature not exceeding 38°.

Functional Use in Foods Preservative.

Sorbitan Monostearate

DESCRIPTION

A mixture of partial stearic and palmitic acid esters of sorbitol and its mono- and dianhydrides. It is manufactured by reacting edible commercial stearic acid (usually containing associated fatty acids, chiefly palmitic) with sorbitol. It is a light cream to tan-colored, hard, waxy solid with a bland odor and taste. It is soluble at temperatures above its melting point in toluene, dioxane, carbon tetrachloride, ether, ethanol, methanol, and aniline. It is insoluble in cold water, and in mineral spirits and acetone, but is dispersible in warm water and soluble, with haze, above 50° in mineral oil and in ethyl acetate. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Identification

A. The fatty acid residue obtained in the *Assay* has an *Acid Value* between 200 and 212 (*Method I*, page 503) and a *Solidification Point* not below 53°.

B. *Apparatus* Assemble a suitable apparatus for ascending thin-layer chromatography. Prepare a slurry of chromatographic silica gel and water (1 g to 2 ml), apply a uniformly thin layer to glass plates of convenient size, dry in the air for 10 min, and activate by drying at 100° for 1 h. Store the cooled plates in a desiccator over anhydrous calcium sulfate until ready for use.

Sample Solution Transfer 500 mg of the polyols obtained in the *Assay* into a 2-ml volumetric flask, dissolve in and dilute to volume with water, and mix.

Standard Solution Transfer 50 mg each of sorbitol, of USP 1,4-Sorbitan Reference Standard, and of USP Isosorbide Reference Standard into a 1-ml volumetric flask, dissolve in and dilute to volume with water, and mix.

Procedure By means of the template provided with the apparatus, mark the sample spot area 1.5 cm from the bottom of the plate, then mark the solvent front line 15 cm above the sample spot area, using a sharp needle to cut through the silica gel. In the same manner, mark a spot for the standard separated from the sample spot by about 1.5 cm. Using micropipets, spot 2 µl of the *Sample Solution* and 1 µl of the *Standard Solution* at the appropriate marks. Allow the spots to dry, then place the plate in a suitable chromatographic chamber containing a mixture of 100 volumes of acetone and 2 volumes of acetic acid as the developing solvent, and develop by ascending chromatography until the solvent front reaches the 15-cm line (about 45 min). Remove the plate from the chamber, dry thoroughly in air, and spray evenly with sulfuric acid solution (1 in 2) until the surface is uniformly wet. (*Caution:* Do not overspray.) Immediately place the sprayed plate on a hot plate maintained at 200° in a hood. Char until white fumes of sulfur trioxide cease, and cool on an asbestos mat to room temperature. The spots from the sample are located at the same R_f values as those of the polyols from the standard. The approximate R_f values are: sorbitol, 0.07; 1,4-sorbitan, 0.40; and isosorbide, 0.77.

Assay Not less than 29.5 g and not more than 33.5 g of polyols (as sorbitol and its mono- and dianhydrides) per 100 g of sample, and not less than 71 g and not more than 75 g of fatty acids per 100 g of sample.

Acid Value Between 5 and 10.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Hydroxyl Value Between 235 and 260.

Saponification Value Between 147 and 157.

Water Not more than 1.5%.

TESTS

Assay Transfer about 25 g of the sample, accurately weighed, into a 500-ml round-bottom boiling flask, add 250 ml of alcohol and 7.5 g of potassium hydroxide, and mix. Connect a suitable condenser to the flask, reflux the mixture for 1 to 2 h, then transfer to an 800-ml beaker, rinsing the flask with about 100 ml of water and adding it to the beaker. Heat on a steam bath to evaporate the alcohol, adding water occasionally to replace the alcohol, and evaporate until the odor of alcohol can no longer be detected. Adjust the final volume to about 250 ml with hot water. Neutralize the soap solution with dilute sulfuric acid (1 in 2), add 10% in excess, and heat, while stirring, until the fatty acid layer separates. Transfer the fatty acids to a 500-ml separator, wash with three or four 20-ml portions of hot water to remove polyols, and combine the washings with the original aqueous polyol layer from the saponification. Extract the combined aqueous layer with three 20-ml portions of petroleum ether, add the extracts to the fatty acid layer, evaporate to dryness in a tared dish, cool, and weigh.

Neutralize the polyol solution with a 1 in 10 solution of potassium hydroxide to pH 7 using a suitable pH meter. Evaporate this solution to a moist residue, and separate the polyols from the salts by several extractions with hot alcohol. Evaporate the alcohol extracts on a steam bath to dryness in a tared dish, cool, and weigh. Avoid excessive drying and heating.

Acid Value Determine as directed under *Method II* in the general procedure, page 504.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

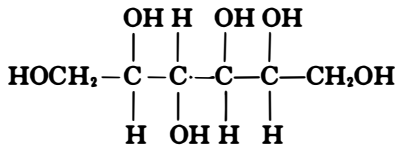
Saponification Value Determine as directed in the general procedure, page 509, using about 4 g, accurately weighed.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Emulsifier; stabilizer; defoaming agent.

Sorbitol

D-Sorbitol; D-Glucitol; D-Sorbite; D-Sorbol; 1,2,3,4,5,6-Hexanehexol



$C_6H_{14}O_6$ Mol wt 182.17

DESCRIPTION

White, hygroscopic powder, flakes, or granules having a sweet taste. Its density is about 1.49. One g dissolves in about 0.45 ml of water. It is slightly soluble in alcohol, in methanol, and in acetic acid. Sorbitol can exist in any of several crystalline forms with melting points ranging from 89° to 101°.

REQUIREMENTS

Identification

Dissolve about 5 g in 6 ml of water, add 7 ml of methanol, 1 ml of benzaldehyde, and 1 ml of hydrochloric acid, and shake in a mechanical shaker until crystals appear. Filter with the aid of suction, dissolve the crystals in 20 ml of boiling water containing 1 g of sodium bicarbonate, filter while hot, cool the filtrate, filter with suction, wash with 5 ml of methanol-water mixture (1 in 2), and dry in air. The sorbitol monobenzylidene derivative so obtained melts between 174° and 179° (see page 519).

Assay Not less than 91.0% of sorbitol ($C_6H_{14}O_6$). Small amounts of mannitol and other polyhydric alcohols may be present.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 1%.
Reducing Sugars Not more than 0.3%.
Residue on Ignition Not more than 0.1%.
Sulfate Not more than 0.01%.
Total Sugars Not more than 1%.

TESTS

Assay Proceed as directed in the *Assay for Sorbitol Solution*, page 309.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Chloride, page 471 Any turbidity produced by a 400-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).
Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Loss on Drying, page 518 Dry at 80° and at a pressure not exceeding 5 mm of Hg for 6 h.
Reducing Sugars Dissolve 7.0 g in 35 ml of water in a 400-ml beaker, and mix. Add 50 ml of alkaline cupric tartrate TS, cover the beaker with glass, heat the mixture at such a rate that it comes to a boil in about 4 min, and boil for exactly 2 min. Collect the precipitated cuprous oxide in a tared Gooch crucible previously washed with hot water, alcohol, and ether and dried at 100° for 30 min. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 ml of alcohol, and finally with 10 ml of ether, and dry at 100° for 30 min. The weight of the cuprous oxide does not exceed 50 mg.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.
Sulfate, page 471 Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 200 µg of sulfate (SO_4).
Total Sugars Transfer 2.1 g into a 250-ml flask fitted with a ground-glass joint, add 40 ml of approximately 0.1 *N* hydrochloric acid, attach a reflux condenser, and reflux for 4 h. Transfer the solution to a 400-ml beaker, rinsing the flask with about 10 ml of water, neutralize with 6 *N* sodium hydroxide, and continue as directed under *Reducing Sugars*, beginning with "Add 50 ml of alkaline cupric tartrate TS. . . . " The weight of the cuprous oxide does not exceed 50 mg.

Packaging and Storage Store in tight containers.
Functional Use in Foods Humectant; texturizing agent; sequestrant.

Sorbitol Solution

DESCRIPTION

A water solution of sorbitol ($C_6H_{14}O_6$) containing a small amount of mannitol and other isomeric polyhydric alcohols. It is a clear, colorless, syrupy liquid having a sweet taste. It is neutral to litmus. It is miscible with water, with glycerin, and with propylene glycol. It is soluble in alcohol, and is practically insoluble in other common organic solvents.

REQUIREMENTS

Identification

To 6 ml add 7 ml of methanol, 1 ml of benzaldehyde, and 1 ml of hydrochloric acid, mix, and shake in a mechanical shaker until crystals appear. Filter with the aid of suction, dissolve the

crystals in 20 ml of boiling water containing 1 g of sodium bicarbonate, filter while hot, cool the filtrate, filter with suction, wash with 5 ml of methanol-water mixture (1 in 2), and dry in air. The sorbitol monobenzylidene derivative so obtained melts between 174° and 179° (see page 519).

Assay Not less than 64.0% of sorbitol ($C_6H_{14}O_6$).
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.005%.
Heavy Metals (as Pb) Not more than 10 ppm.
Reducing Sugars Not more than 0.21%.
Residue on Ignition Not more than 0.1%.
Specific Gravity Not less than 1.285.
Sulfate Not more than 0.01%.
Total Sugars Not more than 0.7%.
Water Between 29% and 31%.

TESTS

Assay

Reagent–Internal Standard Preparation Dissolve a suitable quantity of *n*-butylboronic acid in pyridine to obtain a solution having a concentration of about 10 mg per ml. This is the reagent solution. Add a suitable quantity of methyl nonadecanoate, the internal standard, to the reagent solution to obtain a solution having a concentration of about 2 mg per ml. This is the *Reagent–Internal Standard Preparation.*

Standard Preparation Dissolve an accurately weighed quantity of USP Sorbitol Reference Standard in water to obtain a solution having a concentration of about 1.5 mg per ml.

Assay Preparation Dissolve an accurately weighed quantity of the sample, equivalent to about 150 mg of anhydrous sorbitol, in water in a 100-ml volumetric flask, dilute to volume with water, and mix.

Procedure Pipet 1-ml portions of the *Standard Preparation* and of the *Assay Preparation* into separate 25-ml vials, and heat the vials in a vacuum oven at 50° to dryness. Add 1.0 ml of the *Reagent–Internal Standard Preparation* to each residue, and mix. Inject a 1.0-µl portion of the solution from the *Assay Preparation* into a suitable gas chromatograph in which the detector is the hydrogen flame-ionization type and the column is 4 mm × 1.8 m, packed with 3% cyanopropylphenyl silicone liquid phase on 80- to 100-mesh silanized diatomaceous earth. The carrier is nitrogen flowing at 50 ml per min. The injection port temperature is 245°, the column temperature is 205°, and the detector temperature is 260°. The retention time of the internal standard is about 4 min, and that of sorbitol about 9 min. In a suitable chromatogram, the resolution factor, R, is not less than 5.0 between the peaks for sorbitol and the internal standard, and six replicate injections of the *Standard Preparation* show a relative standard deviation of not more than 1.5%. Similarly, inject a 1.0-µl portion of the solution from the *Standard Preparation.* Calculate the quantity, in mg, of $C_6H_{14}O_6$ in the sample taken by the formula $100C(R_U/R_S)$, in which C is the concentration of USP Sorbitol Reference Standard in the *Standard Preparation*, in mg per ml, and R_U and R_S are the ratios of the peak areas of sorbitol to those of the internal standard of the *Assay Preparation* and *Standard Preparation*, respectively.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 400-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Heavy Metals A mixture of 2 g with 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Reducing Sugars Transfer 10.0 g, accurately weighed, to a 400-ml beaker, with the aid of 35 ml of water, and mix. Add 50 ml of alkaline cupric tartrate TS, cover the beaker with glass, heat the mixture at such a rate that it comes to a boil in approximately 4 min, and boil for exactly 2 min. Collect the precipitated cuprous oxide in a tared Gooch crucible previously washed successively with hot water, alcohol, and ether and dried at 100° for 30 min. Thoroughly wash the collected cuprous oxide on the filter with hot water, then with 10 ml of alcohol, and finally with 10 ml of ether, and dry at 100° for 1 h. The weight of the cuprous oxide does not exceed 50 mg.

Residue on Ignition, page 533 Ignite 2 g as directed under *Method II* (for liquids).

Specific Gravity Determine by any reliable method (see page 3).

Sulfate, page 471 Any turbidity produced by a 2-g sample does not exceed that shown in a control containing 200 µg of sulfate (SO_4).

Total Sugars Transfer 3.0 g into a 250-ml flask fitted with a ground-glass joint, add 40 ml of approximately 0.1 *N* hydrochloric acid, attach a reflux condenser, and reflux for 4 h. Transfer the solution to a 400-ml beaker, rinsing the flask with about 10 ml of water, neutralize with 6 *N* sodium hydroxide, and continue as directed under *Reducing Sugars*, beginning with "Add 50 ml of alkaline cupric tartrate TS. . . . " The weight of the cuprous oxide does not exceed 50 mg.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Humectant; texturizing agent; sequestrant.

Spearmint Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the fresh overground parts of the flowering plant *Mentha spicata* L. (Common Spearmint), or of *Mentha cardiaca* Gerard ex Baker (Scotch Spearmint) (Fam. *Labiatae*). It is a colorless, yellow or greenish yellow liquid having the characteristic odor and taste of spearmint. It may be rectified by distillation.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 612, using the same test conditions as specified therein.

Assay Not less than 55%, by volume, of ketones.
Angular Rotation Between −48° and −59°.
Heavy Metals (as Pb) Passes test.
Reaction Passes test.
Refractive Index Between 1.484 and 1.491 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.917 and 0.934.

TESTS

Assay Proceed as directed under *Aldehydes and Ketones—Neutral Sulfite Method*, page 500.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Reaction A recently prepared solution of the sample in 80% alcohol is neutral or only slightly acid to moistened litmus paper.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 1 ml of 80% alcohol. On further dilution the solution may become turbid.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.
Labeling Label spearmint oil to indicate whether it is natural or rectified.
Functional Use in Foods Flavoring agent.

Spice Oleoresins

DESCRIPTION

Spice oleoresins used in foods are derived from spices and contain the total sapid, odorous, and related characterizing principles normally associated with the respective spices. The oleoresins are produced by one of the following processes: (1) by extraction of the spice with any suitable solvent or solvents, in combination or sequence, followed by removal of the solvent or solvents in conformance with applicable residual solvent regulations (see *General Requirements* below), or (2) by removal of the volatile portion of the spice by distillation, followed by extraction of the nonvolatile portion, which after solvent removal is combined with the total volatile portion.

Spice oleoresins may contain suitable food-grade diluents, preservatives, antioxidants, and other substances consistent with good manufacturing practice, as provided for under *Added Substances*, page 5. When added substances are used, they must be declared on the label in accordance with current U.S. regulations or with the regulations of other countries that recognize the *Food Chemicals Codex*.

The spice oleoresins covered by this monograph are:

Oleoresin Black Pepper Obtained by the solvent extraction of the dried fruit of *Piper nigrium* L. as a dark green, olive green, or olive drab extract usually consisting of an upper oily layer and a lower crystalline layer. It may appear as a homogeneous emulsion if examined shortly after the oleoresin has been homogenized, but the product separates on standing. It may be decolorized by partial removal of chlorophyll.

Oleoresin Capsicum Obtained by the solvent extraction of dried pods of *Capsicum frutescens* L. or *Capsicum annum* L. (var. conoides Irish) as a clear red to dark red, somewhat viscous liquid of characteristic odor, flavor, and bite. It may be decolorized through good manufacturing practice. It is partly soluble in alcohol (with oily separation and/or sediment) and is soluble in most fixed oils. The bite is usually standardized according to the label declaration.

Oleoresin Celery Obtained by the solvent extraction of the dried ripe seed of *Apium graveolens* L. as a dark green, somewhat viscous, nonhomogeneous liquid with the characteristic odor and flavor of celery. It may be decolorized by the partial removal of chlorophyll. It is partly soluble in alcohol (with oily separation), and is soluble in most fixed oils.

Oleoresin Ginger Obtained by the solvent extraction of the dried rhizomes of *Zingiber officinalis* L. Roscoe as a dark brown, viscous to highly viscous liquid with the characteristic odor and flavor of ginger. It is soluble in alcohol (with sediment).

Oleoresin Paprika Obtained by the solvent extraction of the pods of *Capsicum annum* L. as a deep red to deep purplish red, somewhat viscous liquid of characteristic odor and flavor. It frequently occurs as a two-phase mixture. The color is usually standardized according to the label declaration. It is partly soluble in alcohol (with oily separation), and is soluble in most fixed oils.

Oleoresin Turmeric Obtained by the solvent extraction of the dried rhizomes of *Curcuma longa* L. as a yellow orange to red brown viscous liquid with a characteristic odor and flavor. The content of curcumin normally varies, and the product is generally standardized according to the label declaration.

GENERAL REQUIREMENTS

Identification

The volatile oil distilled from an oleoresin is similar in its physical and chemical properties, including the infrared spectrum (see page 584), to that distilled from the spice of the same origin. To obtain the volatile oil from the oleoresin, proceed as directed under *Volatile Oil Content*, page 529.

Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Residual Solvent *Chlorinated hydrocarbons (total):* not more than 0.003%; *acetone:* not more than 30 ppm; *isopropanol:* not more than 0.003%; *methanol:* not more than 0.005%; *hexane:* not more than 0.0025%.

ADDITIONAL REQUIREMENTS

Oleoresin Black Pepper *Piperine:* not less than 36%; *Volatile Oil Content:* between 15 ml and 35 ml per 100 g.
Oleoresin Capsicum *Scoville Heat Units:* between 100,000 and 2,000,000, as specified on the label.
Oleoresin Celery *Volatile Oil Content:* between 7 ml and 20 ml per 100 g.
Oleoresin Ginger *Volatile Oil Content:* between 18 ml and 35 ml per 100 g.
Oleoresin Paprika *Color Value:* between 500 and 4500 units, as specified on the label (according to the method of analysis); *Scoville Heat Units (pungency):* not more than 100,000.
Oleoresin Turmeric *Curcumin (or Color Value equivalent):* between 4% and 45%, as specified on the label.

TESTS (GENERAL REQUIREMENTS)

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Residual Solvent Determine as directed in the general method, page 528.

TESTS (ADDITIONAL REQUIREMENTS)

Color Value Determine as directed in the general method, page 527.
Curcumin Determine as directed in the general method, page 527.
Piperine Determine as directed in the general method, page 527.
Scoville Heat Units Determine as directed in the general method, page 529.
Volatile Oil Content Determine as directed in the general method, page 529.

Packaging and Storage Store in full, tight, preferably glass or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent; color (oleoresins paprika and turmeric only).

Spike Lavender Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the flowers of *Lavandula latifolia*, Vill. (*Lavandula spica*, D.C.) (Fam. *Labiatae*). It is a pale yellow to yellow liquid having a camphoraceous, lavenderlike odor. It is soluble in most fixed oils and in propylene glycol. It is slightly soluble in glycerin and in mineral oil.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 606, using the same test conditions as specified therein.

Assay Not less than 40.0% and not more than 50.0% of total alcohols, calculated as linalool ($C_{10}H_{18}O$).
Angular Rotation Between $-5°$ and $+5°$.
Esters Not less than 1.5% and not more than 3.0% of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$).
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.463 and 1.468 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.893 and 0.909.

TESTS

Assay Proceed as directed under *Linalool Determination*, page 501, using about 1.5 g of the acetylated oil, accurately weighed, for the saponification.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Esters Weigh accurately about 10 g, and proceed as directed under *Ester Determination*, page 500, using 98.15 as the equivalence factor (*e*) in the calculation.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 3 ml of 70% alcohol. The solution frequently becomes hazy upon further dilution.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, aluminum, tin-lined, or other suitably lined containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Stannous Chloride

$SnCl_2$ Mol wt 189.61

DESCRIPTION

Stannous chloride is anhydrous or contains two molecules of water of hydration. It occurs as white or colorless crystals having no odor or a slight odor of hydrochloric acid. It is very soluble in water, and is soluble in alcohol and in glacial acetic acid.

REQUIREMENTS

Identification

A. To a 1 in 20 solution of the sample in diluted hydrochloric acid TS add mercuric chloride TS dropwise. A white or grayish white precipitate is formed.

B. A 1 in 20 solution of the sample gives positive tests for *Chloride*, page 516.

Assay Not less than 99.0% and not more than the equivalent of 101.0% of $SnCl_2$, or not less than 98.0% and not more than the equivalent of 102.2% of $SnCl_2.2H_2O$.

Arsenic (as As) Not more than 3 ppm.

Other Heavy Metals (as Pb) Not more than 0.01%.

Iron Not more than 0.005%.

Solubility in Hydrochloric Acid Passes test.

Substances Not Precipitated by Sulfide Not more than 0.05%.

Sulfate Passes test (about 0.003%).

TESTS

Assay Transfer about 2 g of the sample, accurately weighed, into a 250-ml volumetric flask, dissolve in 15 ml of hydrochloric acid, dilute to volume with water, and mix. Transfer 50.0 ml of this solution into a 500-ml flask, add 5 g of sodium potassium tartrate, and mix. Make the solution alkaline to litmus with a cold saturated solution of sodium bicarbonate, and titrate at once with 0.1 *N* iodine, using starch TS as the indicator. Each ml of 0.1 *N* iodine is equivalent to 9.48 mg of $SnCl_2$ or 11.28 mg of $SnCl_2.2H_2O$.

Arsenic Determine as directed under *Arsenic Test*, page 464, modifying the *Procedure* as follows: Mix 1.0 g of the sample in the generator flask with 35 ml of water and 4 g of 20-mesh zinc. Add 20 ml of dilute sulfuric acid (1 in 5), but omit the potassium iodide and stannous chloride reagent solutions. *Immediately* connect the generator flask to the lead acetate cotton scrubber unit and the absorber tube containing 3.0 ml of *Silver Diethyldithiocarbamate Solution*, and allow the reaction to continue for 45 min as directed in the *Procedure*.

Other Heavy Metals Dissolve 1 g of the sample in a mixture of 2 ml of hydrochloric acid and 3 ml of nitric acid, and boil until solution is complete and brown fumes are no longer evolved. Cool, and dilute to 50 ml with water. To 10 ml of this solution add 8 ml of sodium hydroxide solution (1 in 10), then cool, and dilute to 40 ml with water. Prepare a control containing 2.0 ml of *Standard Lead Solution* (20 µg Pb), 8 ml of the sodium hydroxide solution, and 30 ml of water. Add 10 ml of hydrogen sulfide TS to each solution. Any color produced in the solution of the sample does not exceed that in the control.

Iron Add 3 ml of dilute hydrochloric acid (1 in 2) to the residue obtained in the test for *Substances Not Precipitated by Sulfide*, cover with a watch glass, and digest on a steam bath for 15 min. Remove the cover, and evaporate to dryness on the steam bath. Dissolve the residue in a few ml of water and 8 ml of hydrochloric acid, dilute to 100 ml with water, and mix. To 2.0 ml of this solution add 2 ml of hydrochloric acid, 46 ml of water, 40 mg of ammonium persulfate crystals, and 3 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 2.0 ml of *Iron Standard Solution* (20 µg of Fe) in an equal volume of solution containing the quantities of the reagents used in the test.

Solubility in Hydrochloric Acid A 5-g portion of the sample dissolves completely in a mixture of 5 ml of hydrochloric acid and 5 ml of water, heating to 40°, if necessary, to effect solution.

Substances Not Precipitated by Sulfide Transfer about 20 g of the sample, accurately weighed, to a 250-ml beaker, and add 50 ml of a solution prepared by adding 75 ml of bromine carefully to 425 ml of 48% hydrobromic acid. Add 1 ml of sulfuric acid, and mix to effect complete solution. Place the beaker on a hot plate, and volatilize the tin slowly, with gentle boiling, to fumes of sulfur trioxide. Cool, add 30 ml of water, and pass hydrogen sulfide gas through the solution for about 5 min. Filter through Whatman No. 42 filter paper, or equivalent, into a weighed platinum dish, and wash with three small portions of a 1% solution of sulfuric acid saturated with hydrogen sulfide. Evaporate carefully to dryness on a hot plate, and heat in a furnace at 800° ± 25° for 13 min. Cool in a desiccator for at least 30 min, and weigh. Calculate the percentage of substances not precipitated by sulfide by the formula $100A/B$, in which A and B are the respective weights of the residue and of the sample taken, in g. Retain the residue for the *Iron* test.

Sulfate Dissolve 5 g of the sample in 5 ml of hydrochloric acid, dilute to 50 ml with water, filter if not clear, and heat the filtrate or clear solution to boiling. Add 5 ml of barium chloride TS, digest in a covered beaker on a steam bath for 2 h, and allow to stand overnight. No precipitate forms.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Reducing agent; antioxidant.

Stearic Acid

Octadecanoic Acid

$C_{18}H_{36}O_2$ Mol wt 284.48

DESCRIPTION

A mixture of solid organic acids obtained from fats consisting chiefly of stearic acid ($C_{18}H_{36}O_2$) and palmitic acid ($C_{16}H_{32}O_2$). It occurs as a hard, white or faintly yellowish, somewhat glossy and crystalline solid, or as a white or yellowish white powder. It has a slight characteristic odor and taste resembling tallow. Stearic acid is practically insoluble in water. One g dissolves in about 20 ml of alcohol, in 2 ml of chloroform, and in about 3 ml of ether. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Acid Value Between 196 and 211.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Iodine Value Not more than 7.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 197 and 212.
Titer (Solidification Point) Between 54.5° and 69°.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.2%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Iodine Value Determine by the *Wijs Method*, page 505.
Residue on Ignition, page 533 Ignite 2 g as directed in the general method.
Saponification Value Determine as directed in the general method, page 509, using about 3 g, accurately weighed.
Titer (Solidification Point) Determine as directed under *Solidification Point*, page 538.
Unsaponifiable Matter, page 509 Determine as directed in the general method.
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Component in the manufacture of other food-grade additives; lubricant; defoaming agent.

Stearyl Monoglyceridyl Citrate

DESCRIPTION

A soft, practically tasteless, off-white to tan, waxy solid having a lardlike consistency. It is insoluble in water, but is soluble in chloroform and in ethylene glycol. It is prepared by a controlled chemical reaction from citric acid, monoglycerides of fatty acids (obtained by the glycerolysis of edible fats and oils or derived from fatty acids), and stearyl alcohol. It conforms to the regulations of the federal Food and Drug Administration pertaining to specifications for fats or fatty acids derived from edible sources.

REQUIREMENTS

Acid Value Between 40 and 52.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Residue on Ignition Not more than 0.1%.
Saponification Value Between 215 and 255.
Total Citric Acid Between 15.0% and 18.0%.
Water Not more than 0.25%.

TESTS

Acid Value Determine as directed under *Method II* in the general procedure, page 504.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Residue on Ignition Ignite 2 g as directed in the general procedure, page 533.
Saponification Value Transfer about 1 g, accurately weighed, into a 250-ml Erlenmeyer flask, and add 25 ml of ethylene glycol, 35.0 ml of 0.5 *N* alcoholic potassium hydroxide and a few glass beads. Reflux for 1 h, using a water condenser, then rinse the condenser with water, and cool. Add 1 ml of phenolphthalein TS, and titrate with 0.5 *N* hydrochloric acid. Perform a blank determination (see page 2), but do not reflux. The difference between the volumes, in ml, of 0.5 *N* hydrochloric acid consumed in the actual test and in the blank titration, multiplied by 28.05 and divided by the weight, in g, of the sample taken, is the saponification value.
Total Citric Acid

Brominating Solution Dissolve 19.84 g of potassium bromide, 5.44 g of potassium bromate, and 12 g of sodium

metavanadate, $NaVO_3$, in water by warming, and dilute to 1000 ml with water. Filter if necessary.

Ferrous Sulfate Solution Dissolve 44 g of ferrous sulfate, $FeSO_4.7H_2O$, in 1 *N* sulfuric acid, dilute to 100 ml with 1 *N* sulfuric acid, and mix. Use within 5 days of preparation.

Sulfide Solution On the day of use, dissolve 4 g of thiourea in 100 ml of a 1 in 50 solution of sodium borate, $Na_2B_4O_7.10H_2O$, and add 2 ml of sodium sulfide TS. Wait 30 min after the addition of the sodium sulfide TS before using.

Standard Solution Transfer about 50 mg of sodium citrate dihydrate, accurately weighed, into a 500-ml volumetric flask, dissolve and dilute to volume with water, and mix. Transfer 15.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Calculate the concentration (*C*), in μg per ml, of citric acid in the final solution by the formula

$$(15 \times 1000 \times 0.6533W)/(100 \times 500),$$

in which *W* is the weight, in mg, of the sodium citrate taken, and 0.6533 is the factor converting sodium citrate dihydrate to citric acid.

Sample Solution Transfer about 250 mg of the sample, accurately weighed, to a 250-ml extraction flask, and add 15 ml of 0.5 *N* sodium hydroxide, 5 ml of alcohol, and a few glass beads. Connect the flask with a water-cooled condenser, and reflux for 3 h. Immediately cool and neutralize to phenolphthalein TS with 0.5 *N* hydrochloric acid, then place the flask in an ice bath and add 5 ml of sulfuric acid TS. Transfer the solution to a 125-ml separator, extract with three 40-ml portions of chloroform, and then extract the combined chloroform extracts in a 250-ml separator with three 10-ml portions of 0.5 *N* sulfuric acid, adding the acid extracts to a second 250-ml separator. Wash the combined acid extracts with two 60-ml portions of chloroform, and discard the chloroform washes. Filter the acid solution into a 500-ml volumetric flask, neutralize slowly with 6 *N* sodium carbonate, and dilute to volume with water. Transfer 10.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Each ml of the final solution contains approximately 10 μg of citric acid.

Procedure Pipet 2 ml each of the *Standard Solution* and of the *Sample solution* into separate 40- or 45-ml glass-stoppered centrifuge tubes, and add 3 ml of water to each tube. Place 5 ml of water in a third tube for the reagent blank. Place the tubes in an ice bath, add 5 ml of sulfuric acid TS, mix thoroughly, and allow to stand for exactly 5 min. Remove the tubes from the ice bath, and allow them to come to room temperature during the next 5 min. To each tube add 5 ml of the *Brominating Solution*, then insert the stoppers, invert the tubes once or twice, and heat in a water bath at 30° for 20 min. Remove the tubes, add 1.5 ml of *Ferrous Sulfate Solution*, invert again, and allow to stand for 5 min, shaking occasionally to ensure complete reduction of the excess free bromine in the tubes. Add 6.5 ml of petroleum ether, shake for 2 or 3 min, and remove the water layer with a syringe. Wash the ether solutions with 15 ml of water, then remove the water and filter the ether extracts into the original centrifuge tubes, which have been previously rinsed with the *Sulfide Solution*. Filter each ether extract through a tight plug of glass wool onto which has been placed a sufficient amount of anhydrous sodium sulfate to remove the last traces of water from the ether. Place 5.0 ml of the filtrate in a clean, dry centrifuge tube, add 3 ml of *Sulfide Solution*, shake vigorously for 1.5 min, and centrifuge. Decant about 0.5 ml of the supernatant ether layer from each tube, then carefully transfer the ether solutions into 1-cm cells and determine the absorbance of the extracts obtained from the *Standard Solution* and the *Sample Solution* at 500 nm with a suitable spectrophotometer, using the reagent blank in the reference cell. Calculate the quantity, in mg, of citric acid in the sample taken by the formula $5C \times A_U/A_S$, in which *C* is the exact concentration, in μg per ml, of citric acid in the *Standard Solution*, A_U is the absorbance of the solution from the *Sample Solution*, and A_S is the absorbance of the solution from the *Standard Solution*.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Emulsion stabilizer.

Succinic Acid

Butanedioic Acid

$$HOOCCH_2CH_2COOH$$

$C_4H_6O_4$ Mol wt 118.09

DESCRIPTION

Colorless or white, odorless crystals having an acid taste. One g dissolves in 13 ml of water at 25°, in 1 ml of boiling water, in 18.5 ml of alcohol, and in 20 ml of glycerin.

REQUIREMENTS

Identification

Place a drop of a saturated solution of the sample in a micro test tube, and add a drop of a 0.5% solution of ammonium chloride and several mg of zinc powder. Cover the mouth of the tube with a disk of filter paper moistened with a solution in hexane of 5% *p*-dimethylaminobenzaldehyde and 20% trichloroacetic acid. Heat with a small flame for about 1 min. A pink to red violet stain appears on the paper.

Assay Not less than 99.0% of $C_4H_6O_4$.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Melting Range Between 185° and 190°.
Residue on Ignition Not more than 0.025%.

TESTS

Assay Dissolve about 250 mg, accurately weighed, in 25 ml of recently boiled and cooled water, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to the first appearance of a faint pink color that persists for at least 30 s. Each ml of 0.1 *N* sodium hydroxide is equivalent to 5.904 mg of $C_4H_6O_4$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 1.5 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 15 µg of lead ion (Pb) in the control (*Solution A*).

Melting Range Determine as directed in the general procedure, page 519.

Residue on Ignition Ignite 8 g as directed in the general method, page 533.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Buffer; neutralizing agent; miscellaneous and general purpose.

Succinylated Monoglycerides

DESCRIPTION

A mixture of succinic acid esters of mono- and diglycerides produced by the succinylation of a product obtained by the glycerolysis of edible fats and oils, or by the direct esterification of glycerol with edible fat-forming fatty acids. It occurs as a waxy solid having an off-white color and a bland taste, melting at about 60°. It is soluble in warm methanol, in ethanol, and in *n*-propanol.

REQUIREMENTS

Acid Value Between 70 and 120.

Arsenic (as As) Not more than 3 ppm.

Bound Succinic Acid Not less than 14.8%.

Free Succinic Acid Not more than 3%.

Heavy Metals (as Pb) Not more than 10 ppm.

Hydroxyl Value Between 138 and 152.

Iodine Value Not more than 3.

Total Succinic Acid Between 14.8% and 25.6%.

TESTS

Acid Value Determine as directed under *Method I* in the general procedure, page 503.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Free and Bound Succinic Acid

0.02 N Sodium Hydroxide in Methanol Dissolve 4.0 g of sodium hydroxide in 1000 ml of anhydrous methanol. Transfer 200.0 ml of this solution to a 1000-ml volumetric flask, dilute to volume with anhydrous methanol, and mix. Standardize the solution against dried succinic acid, using phenolphthalein TS as the indicator.

Procedure Transfer about 125 mg of the sample, accurately weighed, into a 250-ml separator containing 100 ml of benzene, and dissolve the sample by heating the separator with warm water. Treat the sample and a blank, consisting of 100 ml of benzene in another separator, in the same manner as follows: Cool the contents of the separator, add 50 ml of water, and mix by inverting the separator about 20 times. Allow to stand for about 15 min, and then transfer the aqueous layer into a 125-ml Erlenmeyer flask. Add 10 ml of water to the separator, wash the benzene layer by inverting the separator five times, and add the washings to the 125-ml flask. To the flask add five drops of phenolphthalein TS, and titrate with *0.02 N Sodium Hydroxide in Methanol*. Perform a blank determination, and record the net volume of alkali, in ml, as V_1.

Transfer the benzene layer into a 500-ml round-bottom flask, and rinse the separator with 10 ml of benzene. Add a few boiling chips to the flask, and evaporate the benzene, preferably on a thin-film evaporator, under partial vacuum at about 60°. Dissolve the residue in the flask in 10 ml of methanol, add 10 ml of water and five drops of phenolphthalein TS, and titrate with *0.02 N Sodium Hydroxide in Methanol*. Perform a blank determination, and record the net volume of alkali, in ml, as V_2.

Calculate the weight, in mg, of free succinic acid in the sample by the formula

$$118.1 \times N \times V_1/2,$$

and calculate the weight, in mg, of bound succinic acid in the sample by the formula

$$118.1 \times N \times V_2/2,$$

in which N is the exact normality of the sodium hydroxide solution.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Hydroxyl Value Determine as directed under *Method II* in the general procedure, page 504.

Iodine Value Determine by the *Wijs Method*, page 505.

Total Succinic Acid The sum of the *Free Succinic Acid* and the *Bound Succinic Acid* represents the *Total Succinic Acid*.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Emulsifier; dough conditioners.

Sulfur Dioxide

SO_2 Mol wt 64.06

DESCRIPTION

A colorless, nonflammable gas, under normal conditions of temperature and pressure, having a sharp, pungent odor. It is shipped as a liquid under pressure in containers approved by the U.S. Department of Transportation. Its vapor density is 2.26 times that of air at atmospheric pressure and 0°. The specific gravity of the liquid is about 1.436 at 0°/4°. At 20° the solubility is about 10 g of SO_2 per 100 g of solution.

Caution: Sulfur dioxide gas is intensely irritating to the eyes, throat, and upper respiratory system. Liquid sulfur dioxide may cause skin burns, which result from the freezing effect of the liquid on tissue. Safety precautions to be observed in handling of the material are specified in "Pamphlet G-3" published by the Compressed Gas Association, 500 Fifth Avenue, New York, N.Y. 10036.

REQUIREMENTS

Identification

A saturated solution of sulfur dioxide in water gives positive tests for *Sulfite*, page 517.

Assay Not less than 99.9% of SO_2 by weight.
Arsenic (as As) Not more than 3 ppm by weight.
Heavy Metals (as Pb) Not more than 0.003% by weight.
Lead Not more than 10 ppm by weight.
Nonvolatile Residue Not more than 0.05% by weight.
Selenium Not more than 0.002% by weight.
Water Not more than 0.05% by weight.

TESTS

Sampling Samples of sulfur dioxide may be safely withdrawn from a tank or transfer lines, either of which should be equipped with a 3/8-in. nozzle and valve. Samples should be taken in bombs constructed of 316 stainless steel, designed to withstand 1000 psig and equipped with 316 stainless steel needle valves on both ends. To draw a sample, the bomb is first flushed with dry air to remove any sulfur dioxide, remaining from previous sample drawings, and then attached to the tank or transfer lines with a solid pipe connection. A hose is connected to the other end of the bomb and submerged in either a weak caustic solution or water. Any gas in the bomb is discharged into the caustic or water by first opening the valve at the pipe end, followed by slowly opening the valve at the hose end. When all of the gas is dispelled and liquid sulfur dioxide begins to emerge into the solution, the valve at the hose end is blocked off. The other valves are then tightly closed, and the bomb is detached from the pipe connecting it to the tank or transfer line. Approximately 15% of the liquid sulfur dioxide in the bomb is then discharged into the water or caustic solution. The bomb is then capped at its end and transferred to the laboratory for analysis.

Caution: The bomb should never be stored with more than 85% of the total water capacity of the bomb.

Assay Subtract from 100 the percentages of *Nonvolatile Residue* and of *Water*, as determined herein, to obtain the percentage of SO_2.

Sample Solution for the Determination of Arsenic, Heavy Metals, Lead, and Selenium Measure out 100 ml of sulfur dioxide (144 g) into a 125-ml Erlenmeyer flask, and determine the weight of sample taken by the loss in weight of the sample bomb. Evaporate to dryness on a steam bath, add 3 ml of nitric acid and 10 ml of water to the dry flask, and warm gently on a hot plate for 15 min. Transfer the contents of the flask to a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer a 10.0-ml aliquot into a second 100-ml volumetric flask, dilute to volume with water, and mix. (NOTE: The tests in which this solution is to be used will be accurate assuming a 144-g sample has been taken; if not, the weight of sample actually taken must be considered in the calculations.)

Arsenic A 7.0-ml portion of the *Sample Solution*, diluted to 35 ml with water, meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A 5.0-ml portion of the *Sample Solution*, diluted to 25 ml with water, meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A 7.0-ml portion of the *Sample Solution*, diluted to 40 ml with water, meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Nonvolatile Residue Measure out 200 ml of sulfur dioxide (288 g) into a 250-ml Erlenmeyer flask, and determine the weight of sample taken by the loss in weight of the sample bomb. Evaporate to dryness on a steam bath, and displace the residual vapors with dry air. Wipe the flask dry, cool in a desiccator, and weigh.

Selenium A 2.0-ml portion of the *Sample Solution* meets the requirements of the *Selenium Limit Test, Method II*, page 537.

Water Transfer about 50 ml of liquid sulfur dioxide into a Karl Fischer titration jar, determine the weight of sample taken, and determine the water content by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in suitable pressure containers, observing applicable federal regulations pertaining to shipping containers.

Functional Use in Foods Bleaching agent; preservative.

Sulfuric Acid

H_2SO_4 Mol wt 98.07

DESCRIPTION

A clear, colorless, oily liquid. It is very caustic and corrosive. It is miscible with water and with alcohol with the generation of much heat and contraction in volume. When mixed with other liquids, sulfuric acid should be added cautiously to the diluent. Some concentrations of sulfuric acid commercially available are expressed in Baumé degrees (Be°) and others (above 93.0%) as percentage of H_2SO_4. The usually available concentrations are 60° and 66°Be, equivalent to 77.67% and 93.19% of H_2SO_4, respectively, and 98.0% of H_2SO_4. Its specific gravity varies with the concentration of H_2SO_4 (see *Sulfuric Acid Table*, page 546).

REQUIREMENTS

Identification

It responds to the tests for *Sulfate*, page 517.

Assay Not less than the minimum or within the range of Be°, or the percentage of H_2SO_4, claimed or implied by the vendor.

Arsenic (as As) Not more than 3 ppm.

Chloride Not more than 0.005%.

Heavy Metals (as Pb) Not more than 0.002%.

Iron Not more than 0.02%.

Lead Not more than 5 ppm.

Nitrate Not more than 10 ppm.

Reducing Substances (as SO_2) Passes test (approximately 0.004%).

Selenium Not more than 0.002%.

TESTS

Assay Transfer a 1-ml sample into a small, tared, glass-stoppered Erlenmeyer flask, insert the stopper, weigh accurately, and cautiously add about 30 ml of water. Cool the mixture, add methyl orange TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 49.04 mg of H_2SO_4.

For concentrations of sulfuric acid below 93.0%, expressed in Baumé degrees, transfer about 200 ml, previously cooled to a temperature below 15°, into a 250-ml hydrometer cylinder. Insert a suitable Baumé hydrometer graduated at 0.1° intervals, adjust the temperature to exactly 15.6° (60°F), and note the reading at the bottom of the meniscus, estimating it to the nearest 0.05°. The percentage of H_2SO_4 may be obtained by reference to the *Sulfuric Acid Table*, page 546.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464, using as a control a mixture of 3 ml of the *Standard Arsenic Solution* (3 µg As) and 1 g of ACS reagent-grade sulfuric acid.

Chloride, page 471 Transfer a volume equivalent to 5 g of the acid into about 25 ml of water contained in a 50-ml volumetric flask, cool, and dilute to volume. Retain the unused portion for the *Heavy Metals*, *Iron*, and *Lead* tests. Any turbidity produced by 4 ml of this solution (400-mg sample) does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Heavy Metals Dilute 10 ml of the solution (1-g sample) prepared for the *Chloride* test to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Iron Dilute 1 ml of the solution (100-mg sample) prepared for the *Chloride* test to 40 ml. Add about 30 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red color does not exceed that produced by 2.0 ml of *Iron Standard Solution* (20 µg Fe) in an equal volume of solution containing the same quantities of the reagents used in the test.

Lead Dilute 10 ml of the solution (1-g sample) prepared for the *Chloride* test to 40 ml. This solution meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Nitrate

Standard Nitrate Solution Transfer 8.022 g of potassium nitrate, KNO_3, previously dried at 105° for 1 h, into a 500-ml volumetric flask, dissolve it in water, dilute to volume, and mix well. Slowly add from a buret 5.0 ml of this solution to 400 ml of ACS reagent-grade sulfuric acid, previously cooled to 5°, keeping the tip of the buret below the surface of the acid. After the solution has reached room temperature, transfer it into a 500-ml volumetric flask, and dilute to volume with reagent-grade sulfuric acid. Each ml contains 100 µg of HNO_3.

Procedure Into each of two 100-ml Nessler tubes transfer 50 ml of ACS reagent-grade sulfuric acid, add slowly 5 ml of a freshly prepared 1 in 10 solution of ferrous sulfate, $FeSO_4.7H_2O$, mix with a glass rod, and cool in an ice bath to between 10° and 15°. To one tube of the cooled mixture add a 10-ml sample, previously cooled to between 10° and 15°, and dilute to the 100-ml mark with ACS reagent-grade sulfuric acid chilled to about the same temperature. Add the *Standard Nitrate Solution*, dropwise, from a microburet to the other tube, with frequent mixing, until the color of the control nearly matches that of the sample solution. Dilute the control solution to 100 ml and continue adding the *Standard Nitrate Solution* to as exact a match in color intensity as possible when compared with the sample solution by looking down through the solutions against a white background illuminated by diffused light. Compute the weight of H_2SO_4 in the weight of the sample from the specific gravity and the volume taken (see Sulfuric Acid Table, page 546). Not more than 0.1 ml of the *Standard Nitrate Solution* is required for each g of H_2SO_4.

Reducing Substances Carefully dilute 8 g with about 50 ml of ice-cold water, keeping the solution cool during the addition. To the dilution add 0.1 ml of 0.1 *N* potassium permanganate. The solution remains pink for not less than 5 min.

Selenium Determine as directed in *Method II* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in tight containers.
Functional Use in Foods Acid.

Talc

DESCRIPTION

A naturally occurring form of hydrous magnesium silicate containing varying proportions of such associated minerals as alpha-quartz, calcite, chlorite, dolomite, kaolin, magnesite, and phlogopite. *Talc derived from deposits that are known to contain associated asbestiform minerals is not food grade.* It occurs as a white to grayish white, odorless, tasteless, unctuous powder that is insoluble in water and in solutions of alkali hydroxides but is slightly soluble in dilute mineral acids.

REQUIREMENTS

Identification

A. Mix 500 mg of the sample with about 200 mg of anhydrous sodium carbonate and 2 g of anhydrous potassium carbonate, and heat the mixture in a platinum crucible until fusion is complete. Cool, and transfer the fused mixture to a dish or beaker with the aid of about 50 ml of hot water. Add hydrochloric acid to the liquid until effervescence ceases, then add 10 ml more of the acid, and evaporate the mixture on a steam bath to dryness. Cool, add 20 ml of water, boil, and filter. An insoluble residue of silica remains. Dissolve about 2 g of ammonium chloride in the filtrate, and add 5 ml of ammonia TS. Filter, if necessary, and add sodium phosphate TS to the filtrate or clear solution. A white, crystalline precipitate of magnesium ammonium phosphate separates.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample exhibits major peaks at approximately 1015 cm^{-1} and 450 cm^{-1}.

C. Place a few mg of the sample on each of two microscope slides. To slide A add 1 or 2 drops of an immersion liquid standard, such as Cargille immersion liquid, having a refractive index of 1.544 to 1.548. To slide B add 1 or 2 drops of an immersion liquid standard having a refractive index of 1.594 to 1.598. From the level of best microscopic focus for each slide, raise the microscope objective upward. The Becke line moves into the particles with slide A and out of the particles with slide B.

Acid-Soluble Substances (as SO_4) Not more than 2.5%.
Arsenic (as As) Not more than 3 ppm.
Extractable Fluoride Not more than 0.002%.
Free Alkali (as NaOH) Not more than 1%.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Loss on Ignition Not more than 6%.
Soluble Salts Not more than 0.2%.

TESTS

Acid-Soluble Substances Digest 1.00 g with 20 ml of 3 *N* hydrochloric acid at 50° for 15 min, add water to restore the original volume, mix, and filter. To 10 ml of the filtrate add 1 ml of 2 *N* sulfuric acid, evaporate to dryness, and ignite to constant weight. The weight of the residue does not exceed 12.5 mg.

Sample Solution for the Determination of Arsenic, Heavy Metals, and Lead Transfer 10.0 g of the sample into a 250-ml flask, and add 50 ml of 0.5 *N* hydrochloric acid. Attach a reflux condenser to the flask, heat on a steam bath for 30 min, cool, and let the undissolved material settle. Decant the supernatant liquid through Whatman No. 3 filter paper, or equivalent, into a 100-ml volumetric flask, retaining as much as possible of the insoluble material in the beaker. Wash the slurry and beaker with three 10-ml portions of hot water, decanting each washing through the filter into the flask. Finally, wash the filter paper with 15 ml of hot water, cool the filtrate to room temperature, dilute to volume with water, and mix.

Arsenic A 10-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.
Extractable Fluoride Place 1 g of the sample in a 100-ml beaker, add 20 ml of 0.1 *N* hydrochloric acid, and stir at 50° for 2 h, using a combination hot plate–magnetic stirrer. Filter the suspension through acid-washed filter paper, and add the filtrate to a 250-ml distillation flask. Continue as directed in *Method II* under the *Fluoride Limit Test*, page 511, beginning with " . . . cautiously add 20 ml of perchloric acid. . . . "
Free Alkali Add 2 drops of phenolphthalein TS to 20 ml of the diluted filtrate prepared in the test for *Soluble Salts*, representing 1 g of talc. If a pink color is produced, not more than 2.5 ml of 0.1 *N* hydrochloric acid is required to discharge it.
Heavy Metals A 5-ml portion of the *Sample Solution* diluted to 25 ml with water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A 10-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry 10 g at 105° for 1 h.
Loss on Ignition Weigh accurately about 1 g in a tared platinum crucible provided with a cover, initially apply heat gradually, and then ignite to constant weight.
Soluble Salts Boil 10 g of the sample with 150 ml of water for 15 min. Cool to room temperature, and add water to restore the original volume. Allow the mixture to stand for 15 min, and filter until clear. To 75 ml of the clear filtrate add 25 ml of water. Evaporate 50 ml of this solution, representing 2.5 g of talc, in a tared platinum dish on a steam bath to dryness, and ignite gently to constant weight. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Anticaking agent; coating agent; lubricating and release agent; surface-finishing agent; texturizing agent.

Tangerine Oil, Coldpressed

Tangerine Oil, Expressed

DESCRIPTION

The oil obtained by expression from the peels of the ripe fruit of the Dancy tangerine, and from some other closely related varieties. It is a reddish orange to brownish orange liquid with a pleasant orangelike odor. Oils produced from unripe fruit often show a green color. It is soluble in most fixed oils and in mineral oil, slightly soluble in propylene glycol, and relatively insoluble in glycerin. It may contain a suitable antioxidant.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 607, using the same test conditions as specified therein.

Aldehydes Between 0.8% and 1.9% of aldehydes, calculated as decyl aldehyde ($C_{10}H_{22}O$).
Angular Rotation Between +88° and +96°.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Refractive Index Between 1.473 and 1.476 at 20°.
Residue on Evaporation Between 2.3% and 5.8%.
Specific Gravity Between 0.844 and 0.854.

TESTS

Aldehydes Weigh accurately about 10 g, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, using 78.13 as the equivalence factor (*e*) in the calculation. Allow the samples and the blank to stand at room temperature for 30 min after adding the hydroxylamine hydrochloride solution.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Residue on Evaporation Proceed as directed in the general method, page 502. using 5 g, and heat for 5 h.
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass, tin-lined, galvanized, or other suitably lined containers in a cool place protected from light.
Functional Use in Foods Flavoring agent.

Tannic Acid

DESCRIPTION

A tannin usually obtained from nutgalls, the excrescences that form on the young twigs of *Quercus infectoria* Olivier and allied species of *Quercus* L. (Fam. *Fagaceae*), or from the seed pods of Tara (*Caesalpinia spinosa*). It occurs as an amorphous powder, as glistening scales, or as spongy masses, varying in color from yellowish white to light brown. It is odorless or has a faint, characteristic odor and an astringent taste. Tannic acid is very soluble in water, in acetone, and in alcohol, but only slightly soluble in absolute alcohol. It is practically insoluble in benzene, in chloroform, in ether, and in solvent hexane. One g dissolves in about 1 ml of warm glycerin.

REQUIREMENTS

Identification

A. To a 1 in 10 solution add a small quantity of ferric chloride TS. A bluish black color or precipitate forms.
B. A solution of tannic acid when added to a solution of either an alkaloidal salt, albumin, or gelatin produces a precipitate.

Arsenic Not more than 3 ppm.
Gums or Dextrin Passes test.
Heavy Metals Not more than 0.004%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 12%.
Residue on Ignition Not more than 1%.
Resinous Substances Passes test.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Gums or Dextrin Dissolve 1 g in 5 ml of water, filter, and to

the filtrate add 10 ml of alcohol. No turbidity is produced within 15 min.

Heavy Metals Transfer a 500-mg sample into a 150-ml beaker, and cautiously add 15 ml of nitric acid and 5 ml of 70% perchloric acid. Evaporate the mixture to dryness on a hot plate under a suitable hood, cool slightly, add 2 ml of hydrochloric acid, and wash down the sides of the beaker with water. Carefully evaporate the solution to dryness on a hot plate, rotating the beaker to avoid spattering. Repeat the addition of 2 ml of hydrochloric acid, washing down the sides of the beaker with water, and evaporate to dryness. Cool the residue, dissolve it in 10 ml of water, and add 1 drop of phenolphthalein TS and sufficient 1 *N* sodium hydroxide, dropwise, to produce a pink color. To this solution add 1 *N* hydrochloric acid, dropwise, until the pink color just disappears, then add 2 ml of diluted acetic acid TS, and transfer the solution into a 50-ml Nessler tube. Dilute to 25 ml with water, and add 10 ml of hydrogen sulfide TS. After 10 min the color of the solution of the sample is no darker than that produced in a control of equal volume containing 20 µg of lead ion (Pb) and carried through the same procedure as the sample.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2 h.

Residue on Ignition Ignite 1 g as directed in the general method, page 533.

Resinous Substances Dissolve 1 g in 5 ml of water, filter, and dilute the filtrate to 15 ml. No turbidity is produced.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Clarifying agent.

Tarragon Oil

Estragon Oil

DESCRIPTION

The volatile oil obtained by steam distillation from the leaves, stems, and flowers of the plant *Artemesia dracunculus* L. It is a pale yellow to amber liquid having a delicate spicy odor similar to licorice and sweet basil but characteristic of tarragon oil. It is soluble in most fixed oils and in an equal volume of mineral oil, occasionally becoming hazy on further dilution. It is relatively insoluble in propylene glycol, and is insoluble in glycerin.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 607, using the same test conditions as specified therein.

Acid Value Not more than 2.0.

Angular Rotation Between +1.5° and +6.5°.

Heavy Metals (as Pb) Passes test.

Refractive Index Between 1.504 and 1.520 at 20°.

Saponification Value Not more than 18.

Solubility in Alcohol Passes test.

Specific Gravity Between 0.914 and 0.956.

TESTS

Acid Value Proceed as directed in the general method, page 499.

Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.

Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.

Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Saponification Value Proceed as directed in the general method, page 501, using about 5 g, accurately weighed.

Solubility in Alcohol Proceed as directed in the general method, page 502. One ml is soluble in 1 ml of 90% alcohol.

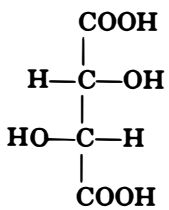
Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight, preferably glass containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Tartaric Acid

L(+)-Tartaric Acid



$C_4H_6O_6$ Mol wt 150.09

DESCRIPTION

Colorless or translucent crystals, or a white, fine to granular, crystalline powder. It is odorless, has an acid taste, and is stable in air. One g dissolves in 0.8 ml of water at 25°, in about 0.5 ml of boiling water, and in about 3 ml of alcohol. Its solutions are dextrorotatory.

REQUIREMENTS

Identification

Its solutions give positive tests for *Tartrate*, page 517.

Assay Not less than 99.7% of $C_4H_6O_6$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Loss on Drying Not more than 0.5%.
Oxalate Passes test.
Residue on Ignition Not more than 0.05%.
Specific Rotation $[\alpha]_D^{25}$: Between +12.0° and +13.0°.
Sulfate Passes test.

TESTS

Assay Weigh accurately about 2 g, previously dried over phosphorus pentoxide for 3 h, dissolve it in 40 ml of water, add phenolphthalein TS, and titrate with 1 *N* sodium hydroxide. Each ml of 1 *N* sodium hydroxide is equivalent to 75.04 mg of $C_4H_6O_6$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry over phosphorus pentoxide for 3 h.

Oxalate Nearly neutralize 10 ml of a 1 in 10 solution with ammonia TS, and add 10 ml of calcium sulfate TS. No turbidity is produced.

Residue on Ignition Ignite 4 g as directed in the general method, page 533.

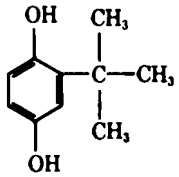
Specific Rotation, page 530 Determine in a solution containing 2 g in each 10 ml.

Sulfate To 10 ml of a 1 in 100 solution add 3 drops of hydrochloric acid and 1 ml of barium chloride TS. No turbidity is produced.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Acid; sequestrant.

TBHQ

tert-Butylhydroquinone; Mono-*tert*-butylhydroquinone



$C_{10}H_{14}O_2$ Mol wt 166.22

DESCRIPTION

A white crystalline solid having a characteristic odor. It is soluble in alcohol and in ether, but is practically insoluble in water.

REQUIREMENTS

Identification

Dissolve a few mg of the sample in 1 ml of methanol, and add a few drops of a 25% solution of dimethylamine in water. A pink to red color is produced.

Assay Not less than 99.0% of $C_{10}H_{14}O_2$.
Arsenic (as As) Not more than 3 ppm.
***t*-Butyl-*p*-benzoquinone** Not more than 0.2%.
2,5-Di-*t*-butylhydroquinone Not more than 0.2%.
Heavy Metals (as Pb) Not more than 10 ppm.
Hydroquinone Not more than 0.1%.
Melting Range Between 126.5° and 128.5°.
Toluene Not more than 0.0025%.
Ultraviolet Absorbance (polynuclear hydrocarbons) Passes test.

TESTS

Assay Transfer about 170 mg of the sample, previously ground to a fine powder and accurately weighed, into a 250-ml wide-mouth Erlenmeyer flask, and dissolve in 10 ml of methanol. Add 150 ml of water, 1 ml of 1 *N* sulfuric acid, and 4 drops of diphenylamine indicator (3 mg of *p*-diphenylaminesulfonic acid sodium salt per ml of 0.1 *N* sulfuric acid), and titrate with 0.1 *N* ceric sulfate to the first complete color change from yellow to red violet. Record the volume, in ml, of 0.1 *N* ceric sulfate required as *V*. Calculate the percentage of $C_{10}H_{14}O_2$ in the sample, uncorrected for hydroquinone (HQ) and 2,5-di-*tert*-butylhydroquinone (DTBHQ), by the formula $8.311N(V - 0.1 \text{ ml})/W$, in which 0.1 ml represents the volume of ceric sulfate solution consumed by the primary oxidation products of *tert*-butylhydroquinone ordinarily present in the sample; *N* is the exact normality of the ceric sulfate solution; and *W* is the weight of the sample taken, in g. Record the uncorrected percentage thus calculated as *A*. If HQ and DTBHQ are present in the sample, they will be

included in the titration. Calculate the corrected percentage of $C_{10}H_{14}O_2$ in the sample by the formula

$$A - (\%\ \text{HQ} \times 1.51) - (\%\ \text{DTBHQ} \times 0.75),$$

using the respective values for percentage of HQ and percentage of DTBHQ as determined under *2,5-Di-t-butylhydroquinone and Hydroquinone*.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

***t*-Butyl-*p*-benzoquinone**

Apparatus Use a suitable double-beam infrared spectrophotometer and matched 0.4-mm liquid sample cells with calcium fluoride windows.

Standard Preparation Transfer about 10 mg of FCC Monotertiary-butyl-*p*-benzoquinone Reference Standard, accurately weighed, into a 10-ml volumetric flask, dissolve in carbon tetrachloride, dilute to volume with the same solvent, and mix.

Sample Preparation Transfer about 1 g of the sample, previously reduced to a fine powder in a high-speed blender and accurately weighed, into a 10-ml volumetric flask, dilute to volume with carbon tetrachloride, and shake for 5 min to extract the *t*-butyl-*p*-benzoquinone. Filter through a Millipore filter (UHWP01300), or equivalent, before use in the *Procedure* below.

Procedure Fill the reference cell with carbon tetrachloride and the sample cell with the *Standard Preparation*, place the cells in the respective reference and sample beams of the spectrophotometer, and record the infrared spectrum from 1600 to 1775 cm^{-1}. On the spectrum draw a background line from 1612 to 1750 cm^{-1}, and determine the net absorbance (A_S) of the *Standard Preparation* at 1659 cm^{-1}. Similarly, obtain the spectrum of the *Sample Preparation*, and determine its net absorbance (A_U) at 1659 cm^{-1}. Calculate the percentage of *t*-butyl-*p*-benzoquinone in the sample by the formula

$$100 \times (A_U/A_S) \times (W_S/W_U),$$

in which W_S is the exact weight, in mg, of the Reference Standard taken, and W_U is the exact weight, in mg, of the sample taken.

2,5-Di-*t*-butylhydroquinone and Hydroquinone

Apparatus Use a suitable gas chromatograph (page 000) equipped with a thermal conductivity detector (F and M Model 810 or equivalent), containing a 0.61-m (2-ft) × 6.35-mm (od) stainless steel column packed with 20% Silicone SE-30, by weight, and 80% Diatoport S (60/80-mesh), or equivalent materials.

Operating Conditions The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: *column temperature*, programmed from 100° to 270°, at 15° per min; *injection port temperature*, 300°; *carrier gas*, helium, flowing at a rate of 100 ml per min; *bridge current*, 140 ma; *sensitivity*, 1× for integrator (Infotronics CRS-100), 2× for recorder.

Stock Solutions Weigh accurately about 50 mg each of hydroquinone (HQ), 2,5-di-*t*-butylhydroquinone (DTBHQ), and methyl benzoate (internal standard), transfer into separate 50-ml volumetric flasks, dilute to volume with pyridine, and mix.

Calibration Standards Into separate 10-ml volumetric flasks add 0.50, 1.00, 2.00, and 3.00 ml of the HQ stock solution, then to each flask add 2.00 ml of the methyl benzoate (internal standard) stock solution, dilute each to volume with pyridine, and mix. In the same manner prepare four DTBHQ calibrating solutions. Prepare the trimethylsilyl derivative of each solution as follows: Add 9 drops of calibration solution to a 2-ml serum vial, cap the vial, evacuate with a 50-ml gas syringe, add 250 µl of *N,O*-bis-trimethylsilylacetamide, and heat at about 80° for 10 min. Chromatograph 10-µl portions of each standard in duplicate, and plot the concentration ratio of HQ to internal standard (X-axis) against the response ratio of HQ to internal standard (Y-axis). Plot the same relationships between DTBHQ and the internal standard.

Sample Preparation and Procedure Transfer about 1 g of the sample, accurately weighed, into a 10-ml volumetric flask, add 2.00 ml of the methyl benzoate internal standard stock solution, dilute to volume with pyridine, and mix. Prepare the trimethylsilyl derivative as described above under *Calibration Standards*, and then chromatograph duplicate 10-µl portions to obtain the chromatogram. The approximate peak times, in min, are: methyl benzoate, 2.5; TMS derivative of HQ, 5.5; TMS derivative of *tert*-butylhydroquinone, 7.3; TMS derivative of DTBHQ, 8.4.

Calculation Determine the peak areas (response) of interest by automatic integration or manual triangulation. Calculate the response ratio of HQ and DTBHQ to internal standard. From the calibration curves determine the concentration ratio of HQ and DTBHQ to internal standard, and calculate the percentage of HQ and the percentage of DTBHQ in the sample by the formula

$$A = Y \times I \times 10/S,$$

in which A is the percentage of HQ or the percentage of DTBHQ in the sample; Y is the concentration ratio (X-axis on calibration curve); I is the percentage (w/v) of internal standard in the *Sample Preparation*; and S is the weight of sample taken, in g.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Melting Range Determine as directed in the general procedure, page 519.

Toluene

Apparatus Use a suitable gas chromatograph (page 475) equipped with a flame ionization detector (F and M Model 810 or equivalent), containing a 3.66-m (12-ft) × 3.18-mm (od) stainless steel column packed with 10% Silicone SE-30, by weight, and 90% Diatoport S (60/80-mesh), or equivalent materials.

Operating Conditions The operating parameters may vary, depending upon the particular instrument used, but a suitable chromatogram may be obtained using the following conditions: *column temperature*, programmed from 70° to 280° at 15° per min and held; *injection port temperature*, 275°;

carrier gas, helium, flowing at a rate of 50 ml per min; *cell temperature*, 300°; *hydrogen and air settings*, 20 psi each; *sensitivity*, 1×10^2.

Standard Solution Prepare a solution of toluene in octyl alcohol containing approximately 50 µg per ml, and calculate the exact concentration (C_R) in percent (w/v).

Sample Solution Transfer about 2 g of the sample, accurately weighed, into a 10-ml volumetric flask, dissolve in octyl alcohol, dilute to volume with the same solvent, and mix. Calculate the exact concentration of the solution (C_S) in percent (w/v).

Procedure Inject a 5-µl portion of the *Standard Solution* into the chromatograph, and measure the height of the toluene peak (H_R) on the chromatogram. The toluene retention time is 3.3 min; other peaks are of no interest in this analysis. Similarly, obtain the chromatogram on a 5-µl portion of the *Sample Solution*, and measure the height of the toluene peak (H_S). Calculate the percentage of toluene in the sample by the formula

$$(H_S/H_R) \times (C_R/C_S) \times 100.$$

Ultraviolet Absorbance Dissolve 1 g of L-ascorbic acid in 100 ml of ethanol and 100 ml of water contained in a 500-ml separator (S-1). Transfer about 50 g of the sample, accurately weighed, into the separator, shake to dissolve, then add 50 ml of isooctane, and extract for 3 min. After the phases have separated, drain the lower, aqueous phase into a second 500-ml separator (S-2), then after 1 min of further separating, drain the lower layer into the separator (S-2). Add a second 50-ml portion of isooctane to the aqueous solution in S-2, and repeat the extraction procedure as previously described, drawing off the lower, aqueous layer into a third 500-ml separator (S-3). Add a third 50-ml portion of isooctane to the aqueous solution in S-3, and repeat the extraction procedure as previously described, drawing off and discarding the lower aqueous layer.

Extract each isooctane solution (i.e., the solutions in S-1, -2, -3) with two 100-ml portions of a 0.5% solution of ascorbic acid in ethanol-water (25/75). Shake each mixture for 1 min, allow the phases to separate, and discard the lower, aqueous layers. Next, extract each isooctane solution with two 100-ml portions of a 5% solution of ethanol in water, and discard the lower, aqueous layers. Finally, wash each solution twice with 100 ml of water, and discard the washes.

Lightly pack a standard-size chromatographic tube with 100 g of anhydrous sodium sulfate, and wash the packed column with 75 ml of isooctane, discarding the wash. Filter the isooctane solution from S-1 through the column, and collect the filtrate in a 500-ml distillation flask. Wash S-1 with the isooctane solution contained in S-2, and then pour the solution onto the column, collecting the filtrate in the flask. Wash S-2 and S-1, successively, with the isooctane solution in S-3, and filter the solution through the column as before. Wash S-3, S-2, and S-1 in that order and in tandem with two successive 25-ml portions of isooctane, and pass the washings individually through the column and into the flask. Let the column drain completely.

Add 2 ml of hexadecane and 2 boiling stones to the 500-ml distillation flask containing the combined isooctane extracts, and attach the flask to a suitable vacuum distillation assembly. Evacuate the assembly to about one-third atmosphere, then immerse the flask in a steam bath, and distil the solvent. When isooctane stops dripping into the receiver, turn off the vacuum, wash down the walls of the flask with 5 ml of isooctane added through the top of the distillation head, then replace the thermometer and again evacuate. The isooctane should distil over in about 1 min. At the end of this distillation, add another 5-ml portion of isooctane, and repeat the stripping procedure.

Quantitatively wash the residue from the distillation flask into a 10-ml volumetric flask with isooctane, dilute to volume with isooctane, and mix. Determine the ultraviolet absorption spectrum of the solution in a 5-cm silica cell from 400 nm to 250 nm, with a suitable spectrophotometer, using isooctane as the blank. Determine the absorbance of a solvent control by following the above procedure in every detail, but with the sample omitted. From the sample spectrum determine the maximum absorbance per cm pathlength in each of the following wavelength intervals: (a) 280 to 289 nm; (b) 290 to 299 nm; (c) 300 to 359 nm; and (d) 360 to 400 nm. Calculate the maximum net absorbance per cm in each interval by subtracting from the sample absorbance the corresponding absorbance per cm of the solvent control. The following net absorbance values are not exceeded at the indicated intervals: (a) 0.15; (b) 0.12; (c) 0.08; and (d) 0.02.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Antioxidant.

Terpene Resin, Natural

DESCRIPTION

A natural terpene occurring in some coal seams. The resin is separated from coal in froth flotation cells. The crude resin is leached with hexane, and the solution produced is freed of suspended matter by pressure filtration. The resin is concentrated in a pre-evaporator, and most of the solvent is removed in a melter-evaporator. The remaining solvent is removed in a spray dryer.

REQUIREMENTS

Acid Value Less than 8.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 3 ppm.
Melting Point Not less than 155°.

TESTS

Acid Value Dissolve about 3 g of the sample, accurately weighed, in 100 ml of a mixture of 75 ml of benzene and 36 ml of alcohol previously neutralized to phenolphthalein TS

with sodium hydroxide. Add 25 ml of a saturated solution of sodium chloride, then add 10 g in addition of sodium chloride and a few drops of phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula

$$56.1 \times V \times N/W,$$

in which *V* is the volume, in ml, and *N* is the normality, respectively, of the alcoholic potassium hydroxide solution, and *W* is the weight of the sample, in g.

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 464. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Melting Point Determine as directed for *Class Ib* substances in the general procedure, page 519.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Terpene Resin, Synthetic

DESCRIPTION

A synthetic resin composed essentially of polymers of alpha-pinene, beta-pinene, and/or dipentene. The polymer is prepared by a batch or continuous process and is usually purified by steam and water washings. It is soluble in benzene, but is insoluble in water. Its color is less than 4 on the Gardner scale (measured in 50% mineral spirit solution).

REQUIREMENTS

Acid Value Less than 5.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 3 ppm.

Saponification Value Less than 5.

TESTS

Acid Value Determine as directed in the general procedure, page 503.

Arsenic Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead Prepare a *Sample Solution* as directed in the general method under *Chewing Gum Base*, page 470. This solution meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

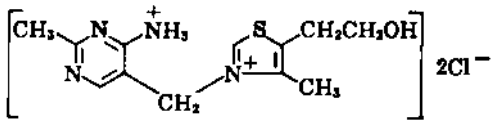
Saponification Value Determine as directed in the general procedure, page 509.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Masticatory substance in chewing gum base.

Thiamin Hydrochloride

Aneurine Hydrochloride; Thiamin Chloride; Vitamin B_1; Vitamin B_1 Hydrochloride; Thiamine Hydrochloride



$C_{12}H_{17}ClN_4OS.HCl$ Mol wt 337.27

DESCRIPTION

Small, white to yellowish white crystals, or crystalline powder, usually having a slight, characteristic odor. When exposed to air, the anhydrous product rapidly absorbs about 4% of water. It melts at about 248° with some decomposition. One g dissolves in about 1 ml of water and in about 100 ml of alcohol. It is soluble in glycerin, and is insoluble in ether and in benzene.

REQUIREMENTS

Identification

A. The infrared absorption spectrum of a potassium bromide dispersion of the sample, previously dried at 105° for 2 h, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Thiamine Hydrochloride Reference Standard.

B. A 1 in 50 solution gives positive tests for *Chloride*, page 517.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{12}H_{17}ClN_4OS.HCl$, calculated on the dried basis.

Color of Solution Passes test.

Loss on Drying Not more than 5%.

Nitrate Passes test.

pH of a 1 in 100 Solution Between 2.7 and 3.4.

Residue on Ignition Not more than 0.2%.

TESTS

Assay

Standard Preparation Prepare as directed for *Standard Preparation* under *Thiamin Assay*, page 548.

Assay Preparation Dissolve about 25 mg of the sample, accurately weighed, in sufficient *Acid Potassium Chloride Solution* (see page 548) to make 500 ml, and mix. Dilute 5 ml of this solution, quantitatively and stepwise, using *Acid Potassium Chloride Solution*, to an estimated concentration of 0.2 µg of the sample per ml. Using this solution as the *Assay Preparation*, proceed as directed for *Procedure* under *Thiamin Assay*, page 548. Calculate the quantity, in mg, of $C_{12}H_{17}ClN_4OS.HCl$ in the sample taken by the formula

$$25(A - b)/(S - d),$$

in which A, b, S, and d are as defined under *Calculation* in the *Thiamin Assay*.

Color of Solution Dissolve 1.0 g in water to make 10 ml. This solution exhibits no more color than a dilution of 1.5 ml of 0.1 *N* potassium dichromate in water to make 1000 ml.

Loss on Drying, page 518 Dry at 105° for 2 h.

Nitrate To 2 ml of a 1 in 50 solution add 2 ml of sulfuric acid, cool, and superimpose 2 ml of ferrous sulfate TS. No brown ring is produced at the junction of the two layers.

pH Determine by the *Potentiometric Method*, page 531.

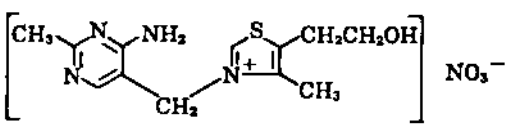
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

Thiamin Mononitrate

Thiamin Nitrate; Vitamin B_1; Vitamin B_1 Mononitrate; Thiamine Mononitrate



$C_{12}H_{17}N_5O_4S$ Mol wt 327.36

DESCRIPTION

White to yellowish white crystals, or crystalline powder, usually having a slight characteristic odor. One g dissolves in about 35 ml of water. It is slightly soluble in alcohol and in chloroform.

REQUIREMENTS

Identification

A. To 2 ml of a 1 in 50 solution add 2 ml of sulfuric acid, cool, and superimpose 2 ml of ferrous sulfate TS. A brown ring is produced at the junction of the two liquids.

B. Dissolve about 5 mg of the sample in a mixture of 1 ml of lead acetate TS and 1 ml of sodium hydroxide solution (1 in 10). A yellow color is produced. Heat the mixture for several min on a steam bath. The color changes to brown, and, on standing, a precipitate of lead sulfide separates.

C. A 1 in 10 solution of the sample yields a white precipitate with mercuric chloride TS, and a red brown precipitate with iodine TS. It is precipitated also by mercuric-potassium iodide TS and by trinitrophenol TS.

D. Dissolve about 5 mg of the sample in 5 ml of 0.5 *N* sodium hydroxide, add 0.5 ml of potassium ferricyanide TS and 5 ml of isobutyl alcohol, shake vigorously for 2 min, and allow the layers to separate. When illuminated from above by a vertical beam of ultraviolet light and viewed at a right angle to this beam, the air-liquid meniscus shows a vivid blue fluorescence, which disappears when the mixture is slightly acidified but reappears when it is again made alkaline.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{12}H_{17}N_5O_4S$, calculated on the dried basis.

Chloride Not more than 0.06%.

Loss on Drying Not more than 1%.

pH of a 1 in 50 Solution Between 6.0 and 7.5.

Residue on Ignition Not more than 0.2%.

TESTS

Assay

Standard Preparation Prepare as directed for *Standard Preparation* under *Thiamin Assay*, page 548.

Assay Preparation Using thiamin mononitrate instead of thiamin hydrochloride, prepare the *Assay Preparation* as directed in the *Assay* under *Thiamin Hydrochloride*, page 324, and proceed as directed for *Procedure* under *Thiamin Assay*, page 548. Calculate the quantity, in mg, of $C_{12}H_{17}N_5O_4S$ in the sample taken by the formula

$$25[0.9706\ (A - b)/(S - d)],$$

in which 0.9706 is the ratio of the molecular weight of thiamin mononitrate to that of thiamin hydrochloride, and A, b, S, and d are defined under *Calculation* in the *Thiamin Assay*.

Chloride, page 471 Any turbidity produced by a 25-mg sample does not exceed that shown in a control containing 15 µg of chloride ion (Cl).

Loss on Drying, page 518 Dry about 500 mg, accurately weighed, at 105° for 2 h.

pH Determine by the *Potentiometric Method*, page 531.

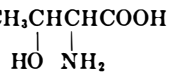
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Threonine

L-2-Amino-3-hydroxybutyric Acid



$C_4H_9NO_3$ Mol wt 119.12

DESCRIPTION

A white, odorless, crystalline powder having a slightly sweet taste. It is freely soluble in water, but insoluble in alcohol, ether, and in chloroform. It melts with decomposition at about 256°.

REQUIREMENTS

Identification

A. To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A reddish purple or purple color is produced.
B. To 5 ml of a 1 in 10 solution add 5 ml of a saturated solution of potassium periodate, and heat. Ammonia is evolved.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_4H_9NO_3$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.2%.
Residue on Ignition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20°}$: Between −26.0° and −29.0°, on the dried basis.

TESTS

Assay Dissolve about 200 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 11.91 mg of $C_4H_9NO_3$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.
Specific Rotation, page 530 Determine in a solution containing 6 g of a previously dried sample in sufficient water to make 100 ml.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Thyme Oil

DESCRIPTION

The volatile oil obtained by distillation from the flowering plant *Thymus vulgaris* L., or *Thymus zygis* L., and its var. *gracilis* Boissier (Fam. *Labiatae*). It is a colorless, yellow, or red liquid with a characteristic pleasant odor and a pungent, persistent taste. It is affected by light.

REQUIREMENTS

Identification

The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 607, using the same test conditions as specified therein.

Assay Not less than 40%, by volume, of phenols.
Angular Rotation Levorotatory, but not more than −3°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.495 and 1.505 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 0.915 and 0.935.
Water-Soluble Phenols Passes test.

TESTS

Assay Proceed as directed under *Phenols*, page 502, allowing the mixture to stand overnight, then adding sufficient potassium hydroxide TS to raise the lower limit of the oily layer into the graduated portion of the neck of the flask. After the solution has become clear, adjust the temperature and read the volume of the residual liquid.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 2 ml of 80% alcohol.
Specific Gravity Determine by any reliable method (see page 3).
Water-Soluble Phenols Shake a 1-ml sample with 10 ml of hot water and, after cooling, pass the water layer through a

moistened filter. Not even a transient blue or violet color is produced in the filtrate upon the addition of 1 drop of ferric chloride TS.

Packaging and Storage Store in full, tight, light-resistant containers in a cool place.

Functional Use in Foods Flavoring agent.

Titanium Dioxide

TiO_2 Mol wt 79.90

DESCRIPTION

Titanium dioxide occurs as a white, odorless, tasteless, amorphous powder that is prepared synthetically. It is insoluble in water, in hydrochloric acid, in dilute sulfuric acid, and in alcohol and other organic solvents. It dissolves slowly in hydrofluoric acid and in hot concentrated sulfuric acid.

REQUIREMENTS

Identification

Add 5 ml of sulfuric acid to 500 mg of the sample, heat gently until fumes of sulfuric acid appear, and cool. Cautiously dilute to about 100 ml with water, and filter. When a few drops of hydrogen peroxide TS are added to 5 ml of the clear filtrate, an orange red color appears immediately.

Assay Not less than 99.0% of TiO_2, after drying, exclusive of any aluminum oxide and/or silicon dioxide added as dispersing aids.

Acid-Soluble Substances Not more than 0.5%.

Aluminum Oxide and/or Silicon Dioxide Not more than 2.0%, either singly or combined.

Antimony Not more than 2 ppm.

Arsenic (as As) Not more than 1 ppm.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

Loss on Ignition Not more than 0.5%, after drying.

Mercury Not more than 1 ppm.

Water-Soluble Substances Not more than 0.25%.

TESTS

Assay Transfer about 300 mg of the sample, previously dried at 105° for 3 h and accurately weighed, into a 250-ml beaker, add 20 ml of sulfuric acid and 7 to 8 g of ammonium sulfate, and mix. Heat on a hot plate until fumes of sulfuric acid appear, and continue heating over a strong flame until the sample dissolves or it is apparent that the undissolved residue is siliceous matter. Cool, cautiously dilute with 100 ml of water, and stir. Heat carefully to boiling while stirring, allow the insoluble matter to settle, and filter. Transfer the entire residue to the filter, and wash thoroughly with cold diluted sulfuric acid TS. Dilute the filtrate with water to 200 ml, and cautiously add about 10 ml of stronger ammonia TS to reduce the acid concentration to about 5%, by volume, of sulfuric acid.

Prepare a zinc amalgam column in a 25-cm Jones reductor tube, placing a pledget of glass wool in the bottom of the tube and filling the constricted portion of the tube with zinc amalgam prepared as follows: Add 20- to 30-mesh zinc to a 2% mercuric chloride solution, using about 100 ml of the solution for each 100 g of zinc. After about 10 min, decant the solution from the zinc, then wash the zinc with water by decantation. Transfer the zinc amalgam to the reductor tube, and wash the column with 100-ml portions of diluted sulfuric acid TS until 100 ml of the washing does not decolorize 1 drop of 0.1 *N* potassium permanganate.

Place 50 ml of ferric ammonium sulfate TS in a 500-ml suction flask, and add 0.1 *N* potassium permanganate until a faint pink color persists for 5 min. Attach the Jones reductor tube, containing the zinc amalgam column, to the neck of the flask, and pass 50 ml of diluted sulfuric acid TS through the tube at a rate of about 30 ml per min. Pass the prepared titanium solution through the column at the same rate, followed by 100 ml each of diluted sulfuric acid TS and water. During these operations, keep the tube filled with solution or water above the upper level of the amalgam column. Gradually release the suction, wash down the outlet tube and the sides of the receiver, and titrate immediately with 0.1 *N* potassium permanganate. Perform a blank determination (see page 2), substituting 200 ml of dilute sulfuric acid (1 in 20) for the sample solution, and make any necessary correction. Each ml of 0.1 *N* potassium permanganate is equivalent to 7.990 mg of TiO_2.

Acid-Soluble Substances Suspend 5 g of the sample in 100 ml of 0.5 *N* hydrochloric acid, and heat on a steam bath for 30 min with occasional stirring. Filter through a Gooch crucible in which the mat has been built up in 3 layers, first using medium-coarse asbestos, then pulped filter paper, and finally fine asbestos. Wash with three 10-ml portions of 0.5 *N* hydrochloric acid, evaporate the combined filtrate and washings to dryness, and ignite at a dull red heat to constant weight.

Aluminum Oxide

0.01 M Zinc Sulfate Dissolve 2.90 g of zinc sulfate, $ZnSO_4.7H_2O$, in sufficient water to make 1000 ml. Standardize the solution as follows: Dissolve 500 mg of high-purity (99.9%) aluminum wire, accurately weighed, in 20 ml of concentrated hydrochloric acid, heating gently to effect solution, then transfer into a 1000-ml volumetric flask, dilute to volume with water, and mix. Transfer a 10.0-ml aliquot of this solution into a 500-ml Erlenmeyer flask containing 90 ml of water and 3 ml of concentrated hydrochloric acid, add 1 drop of methyl orange TS and 25.0 ml of 0.02 *M* disodium EDTA, and continue as directed below under *Sample Solution B*, beginning with "Add dropwise ammonium hydroxide solution (1 in 5) until. . . . " Calculate the titer *T* of the zinc sulfate solution by the formula

$$T = (18.896 \times W)/V,$$

in which *T* is in terms of mg of Al_2O_3 per ml of zinc sulfate solution; *W* is the weight of the aluminum wire taken, in g; *V*

is the volume, in ml, of the zinc sulfate solution consumed in the second titration; and 18.896 is a factor derived as follows:

$$(\text{mol wt } Al_2O_3/\text{mol wt Al}) \times (1000 \text{ mg/g}) \times (10 \text{ ml}/2).$$

Sample Solution A Fuse 1 g of the sample, accurately weighed, with 10 g of sodium bisulfate ($NaHSO_4.H_2O$) contained in a 250-ml high-silica glass Erlenmeyer flask. (*Caution:* Do not use more sodium bisulfate than specified, since an excess concentration of salt will interfere with the EDTA titration later on in the procedure.) Begin heating at low heat on a hot plate, and then gradually raise the temperature until full heat is reached. When spattering has stopped and light fumes of SO_3 appear, heat in the full flame of a Meker burner, with the flask tilted so that the fusion is concentrated at one end of the flask. Swirl constantly until the melt is clear (except for silica content), but guard against prolonged heating to avoid precipitation of titanium dioxide. Cool, add 25 ml of sulfuric acid solution (1 in 2), and then heat until the mass has dissolved and a clear solution results. Cool, and dilute to 120 ml with water.

Sample Solution B Measure out 200 ml of approximately 6.25 *M* sodium hydroxide, and add 65 ml of it to *Sample Solution A* while stirring constantly with a magnetic stirrer; pour the remaining 135 ml of the alkali solution into a 500-ml volumetric flask. Slowly and with constant stirring add the sample mixture to the alkali solution in the 500-ml volumetric flask, then dilute to volume with water, and mix. (NOTE: If the procedure is delayed at this point for more than 2 h, store the contents of the volumetric flask in a polyethylene bottle.) Allow most of the precipitate to settle out (or centrifuge for 5 min), and then filter the supernatant liquid through a very fine filter paper. Label the filtrate *Sample Solution B*.

Sample Solution C Transfer 100.0 ml of *Sample Solution B* into a 500-ml Erlenmeyer flask, add 1 drop of methyl orange TS, acidify with hydrochloric acid solution (1 in 2), and then add about 3 ml in excess. Add 25.0 ml of 0.02 *M* disodium EDTA, and mix. (NOTE: If the approximate Al_2O_3 content is known, calculate the optimum volume of EDTA solution to be added by the formula $[4 \times \% Al_2O_3] + 5$.) Add dropwise ammonium hydroxide solution (1 in 5) until the color is just completely changed from red to orange yellow, then add 10 ml of ammonium acetate buffer solution (77 g of ammonium acetate plus 10 ml of glacial acetic acid, diluted to 1000 ml with water) and 10 ml of dibasic ammonium phosphate solution (150 g of dibasic ammonium phosphate in 700 ml of water, adjusted to pH 5.5 with a 1 in 2 solution of hydrochloric acid, then diluted to 1000 ml with water.) Boil for 5 min, cool quickly to room temperature in a stream of running water, add 3 drops of xylenol orange TS, and mix. If the solution is purple, yellow brown, or pink, bring the pH to 5.3–5.7 by the addition of acetic acid; at the desired pH a pink color indicates that not enough of the EDTA solution has been added, in which case another 100 ml of *Sample Solution B* should be taken and treated as directed from the beginning of this paragraph, except that 50.0 ml, rather than 25.0 ml, of 0.02 *M* disodium EDTA should be used.

Procedure Titrate *Sample Solution C* with 0.01 *M* zinc sulfate to the first yellow brown or pink endpoint color that persists for 5 to 10 s. (*Caution:* This titration should be performed quickly near the endpoint by adding rapidly 0.2-ml increments of the titrant until the first color change occurs; although the color will fade in 5 to 10 s, it is the true endpoint. Failure to observe the first color change will result in an incorrect titration. The fading endpoint does not occur at the second endpoint. This first titration should require more than 8 ml of titrant, but for more accurate work a titration of 10 to 15 ml is desirable.) Add 2 g of sodium fluoride, boil the mixture for 2 to 5 min, and cool in a stream of running water. Titrate the EDTA (which is released by fluoride from its aluminum complex) with 0.01 *M* zinc sulfate to the same fugitive yellow brown or pink endpoint as described above.

Calculation Calculate the percentage of aluminum oxide (Al_2O_3) in the sample taken by the formula

$$(V \times T)/(2 \times S),$$

in which V is the volume, in ml, of 0.01 *M* zinc sulfate consumed in the second titration; T is the titer of the zinc sulfate solution, determined previously, and S is the weight of the sample taken, in g.

Antimony

Stock Standard Solution Transfer 274.28 mg of antimony potassium tartrate, $C_4H_4KO_7Sb.1/2H_2O$, into a 100-ml volumetric flask, dissolve in 6 *N* hydrochloric acid, dilute to volume with the same solvent, and mix. Each ml contains 1 mg of Sb.

Diluted Standard Solution Pipet 2.00 ml of the *Stock Standard Solution* into a 100-ml volumetric flask, dilute to volume with 6 *N* hydrochloric acid, and mix. Each ml contains 20 μg of Sb. Prepare this solution fresh weekly.

Standard Preparation Transfer 1.00 ml of the *Diluted Standard Solution* into a 250-ml separator, and add 25 ml of mercuric chloride solution (6% in 12 *N* hydrochloric acid) and 25 ml of 12 *N* hydrochloric acid.

Sample Preparation Transfer 10.00 g of the sample into a 250-ml beaker, add 50 ml of hot 0.5 *N* hydrochloric acid, cover the beaker, and boil the slurry for 15 min with occasional stirring. Remove from heat, allow to settle for a few seconds, and decant through a double Whatman No. 42 filter paper plus a No. 12 fluted paper, all previously washed with 0.5 *N* hydrochloric acid. Evaporate the filtrate slowly on a hot plate until the volume is slightly less than 20 ml, then cool, and transfer to a 25-ml graduate. Rinse the beaker with 5 ml of 0.5 *N* hydrochloric acid, and add to the graduate. Dilute to the 25-ml mark with 0.5 *N* hydrochloric acid, and transfer into a 250-ml separator. Rinse the beaker and graduate with a total of 25 ml of mercuric chloride solution (6% in 12 *N* hydrochloric acid) and with a total of 25 ml of 12 N hydrochloric acid, adding the washings to the separator.

Procedure To the *Sample Preparation* contained in the separator, add 1.0 ml of 0.1 *N* ceric sulfate [prepared by dissolving 16.5 g of $Ce(SO_4)_2$ in 500 ml of aqueous solution containing 15 ml of 36 *N* sulfuric acid], and start a stopwatch at the moment of first addition. Mix the solutions together and pass a stream of clean air over the mixture. At exactly 1.0 min, add 75 ml of water, mix, and continue passing air over the solution. At 2.0 min, add 8 drops of a 1% solution of

hydroxylamine hydrochloride, mix, and continue passing air over the solution. At 3.0 min, add 5.0 ml of a 0.2% solution of rhodamine B. Pipet 50.00 ml of benzene into the separator, shake for 1 min, and allow the layers to separate for 90 s. Discard the aqueous layer and a small portion of the organic phase. Transfer about 15 ml of the organic phase to a centrifuge tube, and centrifuge at high speed for 1 min. Determine the absorbance of the clarified solution in a 1-cm cell at 565 nm, with a suitable spectrophotometer, referring to water after having compared benzene in the sample cell to water in the reference cell. The color is stable for several min; it should be measured within 15 to 20 min after starting the stopwatch. (NOTE: The colloidal antimony color complex may resist rinsing from the cell with benzene, in which case the cell should be rinsed in succession with dilute nitric acid, hot water, acetone, and benzene. Check the absorbance of the cell with benzene against water contained in the reference cell.) The absorbance produced by the solution from the *Sample Preparation*, after correction for a reagent blank, is not greater than that produced by the *Standard Preparation*, treated in the same manner in the above procedure as the *Sample Preparation*, beginning with " . . . add 1.0 ml of 0.1 *N* ceric sulfate. . . . "

Sample Solution for the Determination of Arsenic and Lead Transfer 10.00 g of the sample into a 250-ml beaker, add 50 ml of 0.5 *N* hydrochloric acid, cover with a watch glass, and heat to boiling on a hot plate. Boil gently for 15 min, then pour the slurry into a 100- to 150-ml centrifuge bottle, and centrifuge for 10 to 15 min, or until undissolved material settles. Decant the supernatant extract through a Whatman No. 4 filter paper, collecting the filtrate in a 100-ml volumetric flask and retaining as much as possible of the undissolved material in the centrifuge bottle. Add 10 ml of hot water to the original beaker, washing off the watch glass with the water, and pour the slurry into the centrifuge bottle. Form a slurry, using a glass stirring rod, and centrifuge. Decant through the same filter paper, and collect the washings in the volumetric flask containing the initial extract. Repeat the entire washing process with two additional 10-ml portions of hot water. Finally, wash the filter paper with 10 to 15 ml of hot water. Cool the contents of the flask to room temperature, dilute to volume with water, and mix.

Arsenic A 30-ml portion of the *Sample Solution* meets the requirements of the *Arsenic Test*, page 464.

Lead A 10-ml portion of the *Sample Solution* meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Loss on Ignition Ignite a 2-g sample, previously dried at 105° for 3 h, at 800° ± 25° to constant weight.

Mercury

Apparatus The apparatus consists of a source of nitrogen (supplied through a regulator or flowmeter capable of measuring a flow rate of 1 L per min) connected to a suitable quartz combustion tube contained in a hinged furnace (Type 70 T, Arthur H. Thomas Co., or equivalent), in which the sample is pyrolyzed at 650°. The exit end of the combustion tube is connected to the optical cell of a suitable mercury vapor meter (Beckman Model K-23 or equivalent), the microammeter of which is connected in parallel through an attenuator to a 1-mV strip chart recorder. The quartz combustion tube (48.3 cm in length and 18.3 mm in outside diameter) is fitted at each end with Pyrex ball-joint adaptors and is packed near the exit end with 40 g of copper oxide (held in place by small wads of quartz wool), the inlet end being used to hold an 88- × 12- × 8-mm combustion boat for the sample.

Standard Mercury Solution Prepare a stock solution by dissolving 1.353 g of mercuric chloride in water and diluting to volume in a 1000-ml volumetric flask. Pipet 1.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix to obtain a working solution containing 0.01 µg of Hg per µl.

Standardization of Mercury Vapor Meter Preheat the combustion tube furnace to 650° and adjust the nitrogen flow to 1 L per min. Standardize the meter in accordance with the manufacturer's instructions, using the internal standard with which the instrument is equipped. Adjust the attenuator so that the scale on the recorder is 200 mV. Under these conditions, a meter reading of 0.078 mg/m^3, obtained with the internal standard, is 50% full scale on the recorder. Check the standardization of the instrument periodically and adjust as necessary.

Calibration of Mercury Vapor Meter Prepare a set of mercury standards containing 0.01, 0.02, and 0.03 µg of Hg by pipetting the required amount of the working standard onto 1- × 0.5- × 0.1-cm pieces of asbestos, previously ignited at 800° for 1 h, contained in separate combustion boats. Cover the asbestos pads with 1 to 2 g of fine granular anhydrous sodium carbonate, previously checked for absence of mercury by this procedure. Place a standard in the tube furnace, and close the inlet with a ball joint sealed at one end and held in place by a clamp. After 1 min, start the gas flow by connecting the nitrogen supply tube to the inlet port of the combustion tube. Record the maximum response from either the observed meter deflection or the chart record for each standard, and prepare a standard curve of the response versus amount of mercury added. The mercury vapor meter should be calibrated each time a series of samples is run, and the calibration should be checked periodically by running a single standard.

Sample Analysis Place 25 mg of the sample in a combustion boat, and cover the sample with 1 to 2 g of the sodium carbonate as described above. Ignite the sample as described above for the standards, record the maximum response, and determine the amount of mercury in the sample by reference to the standard curve.

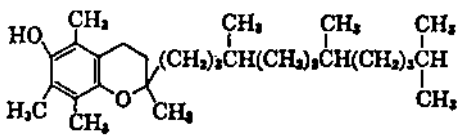
Silicon Dioxide Fuse 1 g of the sample, accurately weighed, with 10 g of sodium bisulfate ($NaHSO_4.H_2O$) contained in a 250-ml high-silica glass Erlenmeyer flask. Heat gently over a Meker burner, while swirling the flask, until decomposition and fusion are complete and the melt is clear, except for the silica content, and then cool. (*Caution:* Do not overheat the contents of the flask at the beginning, and heat cautiously during fusion to avoid spattering.) To the cold melt add 25 ml

of sulfuric acid solution (1 in 2), and heat very carefully and very slowly until the melt is dissolved. Cool, and carefully add 150 ml of water, pouring very small portions down the sides of the flask, with frequent swirling, to avoid overheating and spattering. Allow the contents of the flask to cool, and then filter through fine ashless filter paper, using a 60-degree gravity funnel. Wash out all of the silica from the flask onto the filter paper with sulfuric acid solution (1 in 10). Transfer the filter paper and its contents into a platinum crucible, dry in an oven at 120°, and then heat the partly covered crucible over a Bunsen burner. To prevent flaming of the filter paper, heat first the cover from above, and then the crucible from below. When the filter paper is consumed, transfer the crucible to a muffle furnace and ignite at 1000° for 30 min. Cool in a desiccator, and weigh. Add 2 drops of sulfuric acid (1 in 2) and 5 ml of concentrated hydrofluoric acid (sp. gr. 1.15), and carefully evaporate to dryness, first on a low-heat hot plate (to remove the HF) and then over a Bunsen burner (to remove the H_2SO_4). Take precautions to avoid spattering, especially after removal of the HF. Ignite at 1000° for 10 min, cool in a desiccator, and weigh again. Record the difference between the two weights as the content of SiO_2 in the sample.

Water-Soluble Substances Suspend 4 g of the sample in 50 ml of water, mix, and allow to stand overnight. Transfer to a 200-ml volumetric flask, add 2 ml of ammonium chloride TS, and mix. If the titanium dioxide does not settle, add another 2-ml portion of ammonium chloride TS, then allow the suspension to settle, dilute to volume with water, and mix. Filter through a double thickness of filter paper, discarding the first 10 ml of filtrate, and collect 100 ml of the subsequent clear filtrate. Transfer into a tared platinum dish, evaporate on a hot plate to dryness, and ignite at a dull red heat to constant weight.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Color.

dl-α-Tocopherol



$C_{29}H_{50}O_2$ Mol wt 430.71

DESCRIPTION

A form of vitamin E. It occurs as a yellow to amber, nearly odorless, clear viscous oil that oxidizes and darkens in air and on exposure to light. It is insoluble in water, is freely soluble in alcohol, and is miscible with acetone, chloroform, ether, and vegetable oils.

REQUIREMENTS

Identification

A. Dissolve about 10 mg of the sample in 10 ml of absolute alcohol, add with swirling 2 ml of nitric acid, and heat at about 75° for 15 min. A bright red to orange color develops.
B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the internal standard, as obtained in the *Assay*.
C. If the isomeric form is not otherwise known, determine the optical rotation (see page 530) on a 1 in 10 solution of the sample in chloroform. The specific rotation is not appreciable (approximately ±0.05°).

Assay Not less than 96.0% and not more than 102.0% of $C_{29}H_{50}O_2$.
Acidity Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.

TESTS

NOTE: In the following *Assay*, use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Prepare a solution in *n*-hexane containing 3 mg of hexadecyl hexadecanoate, accurately weighed, in each ml.

Standard Preparation Dissolve about 30 mg of USP Alpha Tocopherol Reference Standard, accurately weighed, in 10.0 ml of the *Internal Standard Solution*.

Assay Preparation Dissolve about 30 mg of the sample, accurately weighed, in 10.0 ml of the *Internal Standard Solution*.

Chromatographic System Use a gas chromatograph equipped with a flame-ionization detector and a glass-lined sample-introduction system or on-column injection. Under typical conditions, the instrument contains a 2-m × 4-mm borosilicate glass column packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh acid-base washed silinized chromatographic diatomaceous earth. The column is maintained isothermally between 240° and 260°, the injection port at about 290°, and the detector block at about 300°. The flow rate of dry carrier gas is adjusted to obtain a hexadecyl hexadecanoate peak approximately 18 to 20 min after sample introduction when a 2% column is used, or 30 to 32 min when a 5% column is used. [NOTE: Cure and condition the column as necessary (see page 475).]

System Suitability Chromatograph a sufficient number of injections of a mixture in *n*-hexane of 1 mg per ml each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard, as directed under *Calibration*, to assure that the resolution factor, *R*, is not less than 1.0 (see page 476).

Calibration Chromatograph successive 2- to 5-µl portions of the *Standard Preparation* until the relative response

factor, F, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 0.51 (α-tocopherol) and 1.00 (hexadecyl hexadecanoate), and record the values as A_S and A_I, respectively. Calculate the relative response factor, F, by the formula $(A_S/A_I) \times (C_I/C_S)$, in which C_I and C_S are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the *Standard Preparation*, respectively.

Procedure Inject a suitable portion (2 to 5 µl) of the *Assay Preparation* into the chromatograph, and record the chromatogram. Measure the areas under the major peaks occurring at relative retention times of approximately 0.51 (α-tocopherol) and 1.00 (hexadecyl hexadecanoate), and record the values as a_U and a_I, respectively. Calculate the weight, in mg, of *dl*-α-tocopherol in the sample by the formula $(10C_I/F) \times (a_U/a_I)$.

Acidity Dissolve 1.0 g of the sample in 25 ml of a mixture of equal volumes of alcohol and ether that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Not more than 1.0 ml of 0.1 *N* sodium hydroxide is required.

Heavy Metals Place a 500-mg sample in a silica crucible, and proceed as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Packaging and Storage Store in tight containers blanketed by inert gas and protected from heat and light.

Labeling Label claims in terms of International Units (IU) should be based on the following: 1 mg *dl*-α-tocopherol = 1.1 IU.

Functional Use in Foods Nutrient; dietary supplement; antioxidant.

d-α-Tocopherol Concentrate

DESCRIPTION

A form of vitamin E obtained by the vacuum steam distillation of edible vegetable oil products, comprising a concentrated form of *d*-α-tocopherol. It occurs as a red, nearly odorless, clear viscous oil. It oxidizes and darkens slowly in air and on exposure to light. It may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the content of *d*-α-tocopherol may be adjusted by suitable physical and chemical means. It is insoluble in water, is soluble in alcohol, and is miscible with acetone, chloroform, ether, and vegetable oils.

REQUIREMENTS

Identification

A. Dissolve about 50 mg of the sample in 10 ml of absolute alcohol, add with swirling 2 ml of nitric acid, and heat at about 75° for 15 min. A bright red to orange color develops.
B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the internal standard, as obtained in the *Assay*.

Assay Not less than 40.0% of total tocopherols, of which not less than 95.0% consists of *d*-α-tocopherol ($C_{29}H_{50}O_2$).

Acidity Passes test.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Specific Rotation $[\alpha]_D^{25°}$: Not less than +24°.

TESTS

Assay, Acidity, Heavy Metals, Lead, and Specific Rotation Proceed as directed under *Tocopherols Concentrate, Mixed*, this page.

Packaging and Storage Store in tight containers blanketed by inert gas and protected from heat and light.

Labeling Label to indicate the mg per g of *d*-α-tocopherol present. Label claims in terms of International Units (IU) should be based on the following: 1 mg *d*-α-tocopherol = 1.49 IU.

Functional Use in Foods Nutrient; dietary supplement; antioxidant.

Tocopherols Concentrate, Mixed

DESCRIPTION

This monograph establishes specifications for two types of mixed tocopherols concentrate. Both types are obtained by the vacuum steam distillation of edible vegetable oil products, and both contain a specified minimum amount of total tocopherols (see REQUIREMENTS), differing only in the levels of the *d*-tocopherol forms.

The *high-alpha* type contains a relatively high proportion of *d*-α-tocopherol and is recognized as a form of vitamin E and also as an antioxidant. The *low-alpha* type contains a relatively high proportion of *d*-β-, *d*-γ-, and *d*-δ-tocopherols, with a minor level of *d*-α-tocopherol, and thus is not considered to be a form of vitamin E but rather an antioxidant. Both types may contain an edible vegetable oil added to adjust the required amount of total tocopherols, and the tocopherol forms may be adjusted by suitable physical or chemical means.

Mixed tocopherols concentrate occurs as a brownish red to red, clear viscous oil having a mild, characteristic odor and taste. It may show a slight separation of waxlike constituents in microcrystalline form. It oxidizes and darkens slowly in air and on exposure to light, particularly when in alkaline media. It is insoluble in water, is soluble in alcohol, and is miscible with acetone, chloroform, ether, and vegetable oils.

REQUIREMENTS

Identification

A. Dissolve about 50 mg of the sample in 10 ml of absolute alcohol, add with swirling 2 ml of nitric acid, and heat at about 75° for 15 min. A bright red to orange color develops.

B. *High-alpha type:* The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the internal standard, as obtained in the *Assay*. *Low-alpha type:* The retention time of the third major peak (i.e., the peak occurring just before that of the internal standard) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the internal standard, as obtained in the *Assay*.

Assay *High-alpha type:* not less than 50.0% of total tocopherols, of which not less than 50.0% consists of *d*-α-tocopherol ($C_{29}H_{50}O_2$) and not less than 20.0% consists of *d*-β- plus *d*-γ- ($C_{28}H_{48}O_2$) plus *d*-δ-tocopherols ($C_{27}H_{46}O_2$). *Low-alpha type:* not less than 50.0% of total tocopherols, of which not less than 80.0% consists of *d*-β- plus *d*-γ- plus *d*-δ-tocopherols.

Acidity Passes test.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Specific Rotation $[\alpha]_D^{25}$ *High-alpha type:* not less than +24°. *Low-alpha type:* not less than +20°.

TESTS

NOTE: In the tests for *Assay* and for *Specific Rotation*, use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Transfer about 600 mg of hexadecyl hexadecanoate, accurately weighed, to a 200-ml volumetric flask, dissolve in a solution containing 2 parts of pyridine and 1 part of propionic anhydride, dilute to volume with the solution, and mix.

Standard Preparations Transfer 12-, 25-, 37-, and 50-mg portions of USP Alpha Tocopherol Reference Standard, accurately weighed, to separate 50-ml Erlenmeyer flasks having 19/38 standard-taper ground-glass necks. Pipet 25.0 ml of the *Internal Standard Solution* into each flask, mix, and reflux for 10 min under water-cooled condensers.

Assay Preparation Transfer about 60 mg of the sample, accurately weighed, to another 50-ml Erlenmeyer flask, pipet 10.0 ml of the *Internal Standard Solution* into the flask, mix, and reflux for 10 min under a water-cooled condenser.

Chromatographic System Use the *System* described in the *Assay* under *dl-α-Tocopherol*, page 330.

System Suitability Chromatograph a suitable number of injections of the *Assay Preparation*, as directed under *Calibration*, to assure that the resolution factor, *R*, between the major peaks occurring at retention times of approximately 0.50 (δ-tocopheryl propionate) and 0.63 (β- plus γ-tocopheryl propionates), relative to hexadecyl hexadecanoate at 1.00, is not less than 2.5 (see page 476).

Calibration Chromatograph successive 2- to 5-µl portions of each *Standard Preparation* until the relative response factor, *F*, for each is constant (i.e., within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the first (α-tocopheryl propionate) and second (hexadecyl hexadecanoate) major peaks (excluding the solvent peak), and record the values as A_S and A_I, respectively. Calculate the factor, *F*, for each concentration of *Standard Preparation* by the formula $(A_S/A_I) \times (C_I/C_S)$, in which C_I and C_S are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopherol Reference Standard in the *Standard Preparation*, respectively. Prepare a relative response factor curve by plotting area of α-tocopheryl propionate versus relative response factor.

Procedure Inject a suitable portion (2 to 5 µl) of the *Assay Preparation* into the chromatograph, and record the chromatogram. Measure the areas under the four major peaks occurring at relative retention times of 0.50, 0.63, 0.76, and 1.00, and record the values as a_δ, $a_{\beta+\gamma}$, a_α, and a_I, corresponding to δ-tocopheryl propionate, β- plus γ-tocopheryl propionates, α-tocopheryl propionate, and hexadecyl hexadecanoate, respectively. Calculate the weight, in mg, of each tocopherol form in the sample by the following formulas:

$$\delta\text{-tocopherol} = (10C_I/F) \times (a_\delta/a_I);$$

$$\beta\text{- plus } \gamma\text{-tocopherols} = (10C_I/F) \times (a_{\beta+\gamma}/a_I);$$

$$\alpha\text{-tocopherol} = (10C_I/F) \times (a_\alpha/a_I),$$

in which *F* is obtained from the relative response factor curve (see *Calibration*) for each of the corresponding areas under the δ-, β- plus γ-, and α-tocopheryl propionate peaks produced by the *Assay Preparation*. (NOTE: The relative response factor for δ-tocopheryl propionate and for β- plus γ-tocopheryl propionates has been determined empirically to be the same as for α-tocopheryl propionate.)

Acidity Dissolve 1 g of the sample in 25 ml of a mixture of equal volumes of alcohol and ether that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Not more than 1.0 ml of 0.1 *N* sodium hydroxide is required.

Heavy Metals Place a 500-mg sample in a silica crucible, and proceed as directed in *Method II* under the *Heavy Metals*

Test, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

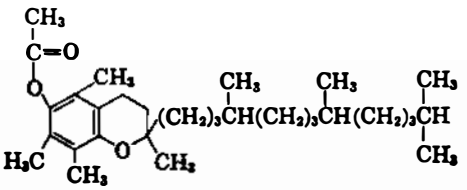
Specific Rotation Transfer an accurately weighed amount of sample, equivalent to about 100 mg of total tocopherols, to a separator, and dissolve it in 50 ml of ether. To the separator add 20 ml of a 10% solution of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-ml portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 ml of isooctane, and determine the optical rotation. Calculate the specific rotation (see page 530), using as *c* the concentration expressed as the number of g of total tocopherols, determined in the *Assay*, in 100 ml of the solution.

Packaging and Storage Store in tight containers blanketed by inert gas and protected from heat and light.

Labeling *High-alpha type:* Label to indicate the mg per g of total tocopherols and of *d*-α-tocopherol present. Label claims in terms of International Units (IU) should be based on the following: 1 mg *d*-α-tocopherol = 1.49 IU. *Low-alpha type:* Label to indicate the mg per g of total tocopherols and of *d*-β- plus *d*-γ- plus *d*-δ-tocopherols present.

Functional Use in Foods *High-alpha type:* nutrient; dietary supplement; antioxidant. *Low-alpha type:* antioxidant.

d-α-Tocopheryl Acetate



$C_{31}H_{52}O_3$ Mol wt 472.75

DESCRIPTION

A form of vitamin E obtained by the vacuum steam distillation and acetylation of edible vegetable oil products. It occurs as a colorless to yellow, nearly odorless, clear viscous oil. It may solidify on standing, and melts at about 25°. It is unstable in the presence of alkalies. It is insoluble in water, is freely soluble in alcohol, and is miscible with acetone, chloroform, ether, and vegetable oils.

REQUIREMENTS

Identification

A. To 10 ml of the *Test Solution* obtained as directed under *Specific Rotation* add with swirling 2 ml of nitric acid, and heat at about 75° for 15 min. A bright red to orange color develops.

B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the internal standard, as obtained in the *Assay*.

Assay Not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Acidity Passes test.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

Specific Rotation $[\alpha]_D^{25}$: Not less than +24°.

TESTS

NOTE: In the tests for *Assay* and for *Specific Rotation*, use low-actinic glassware for all solutions containing tocopherols.

Assay

Internal Standard Solution Prepare a solution in *n*-hexane containing about 3 mg of hexadecyl hexadecanoate, accurately weighed, in each ml.

Standard Preparation Dissolve about 30 mg of USP Alpha Tocopheryl Acetate Reference Standard, accurately weighed, in 10.0 ml of the *Internal Standard Solution*.

Assay Preparation Dissolve about 30 mg of the sample, accurately weighed, in 10.0 ml of the *Internal Standard Solution*.

Chromatographic System Use the *System* described in the *Assay* under *dl-α-Tocopherol*, page 330.

System Suitability Chromatograph a suitable number of injections of a mixture in *n*-hexane of 1 mg per ml each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard, as directed under *Calibration*, to assure that the resolution factor, *R*, is not less than 1.0 (see page 476).

Calibration Chromatograph successive 2- to 5-µl portions of the *Standard Preparation* until the relative response factor, *F*, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain at least 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 0.60 (α-tocopheryl acetate) and 1.00 (hexadecyl hexadecanoate), and record the values as A_S and A_I, respectively. Calculate the relative response factor, *F*, by the formula $(A_S/A_I) \times (C_I/C_S)$, in which C_I and C_S are the exact concentrations, in mg per ml,

of hexadecyl hexadecanoate and of USP Alpha Tocopheryl Acetate Reference Standard in the *Standard Preparation*, respectively.

Procedure Inject a suitable portion (2 to 5 µl) of the *Assay Preparation* into the chromatograph, and record the chromatogram. Measure the areas under the major peaks occurring at relative retention times of approximately 0.60 (α-tocopheryl acetate) and 1.00 (hexadecyl hexadecanoate), and record the values as a_U and a_I, respectively. Calculate the weight, in mg, of *d*-α-tocopheryl acetate in the sample by the formula $(10C_I/F) \times (a_U/a_I)$.

Acidity Dissolve 1.0 g of the sample in 25 ml of a mixture of equal volumes of alcohol and ether that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Not more than 1.0 ml of 0.1 *N* sodium hydroxide is required.

Heavy Metals Place a 500-mg sample in a silica crucible, and proceed as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Specific Rotation

Test Solution Transfer an accurately weighed amount of the sample, equivalent to about 200 mg of α-tocopherol, to a 150-ml round-bottom glass-stoppered flask, and dissolve it in 25 ml of absolute alcohol. Add 20 ml of diluted sulfuric acid TS in alcohol (1 in 7), and reflux in an all-glass apparatus for 3 h, protected from sunlight. Cool, transfer to a 200-ml volumetric flask, dilute to volume with diluted sulfuric acid TS in alcohol (1 in 72), and mix.

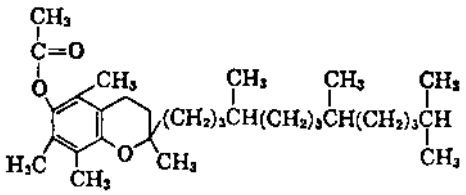
Procedure Transfer an accurately measured volume of the *Test Solution*, equivalent to about 100 mg of α-tocopherol, to a separator, and add 200 ml of water. Extract first with 75 ml, then with two 25-ml portions of ether, and combine the ether extracts in another separator. To the ether solution add 20 ml of a 10% solution of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-ml portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 ml of isooctane, and determine the optical rotation. Calculate the specific rotation (see page 530), using as *c* the concentration of α-tocopheryl acetate, determined in the *Assay*, in 100 ml of solution.

Packaging and Storage Store in tight, light-resistant containers.

Labeling Label claims in terms of International Units (IU) should be based on the following: 1 mg *d*-α-tocopheryl acetate = 1.36 IU.

Functional Use in Foods Nutrient; dietary supplement.

dl-α-Tocopheryl Acetate



$C_{31}H_{52}O_3$ Mol wt 472.75

DESCRIPTION

A form of vitamin E. It occurs as a colorless to yellow or greenish yellow, nearly odorless, clear viscous oil. It is unstable in the presence of alkalies. It is insoluble in water, is freely soluble in alcohol, and is miscible with acetone, chloroform, ether, and vegetable oils.

REQUIREMENTS

Identification

It meets the requirements of *Identification Tests A* and *B* under *d-α-Tocopheryl Acetate*, page 333, and of *Identification Test C* under *dl-α-Tocopherol*, page 330.

Assay Not less than 96.0% and not more than 102.0% of $C_{31}H_{52}O_3$.

Acidity Passes test.

Heavy Metals (as Pb) Not more than 0.004%.

Lead Not more than 10 ppm.

TESTS

NOTE: In the following *Assay*, use low-actinic glassware for all solutions containing tocopherols.

Assay Proceed as directed in the *Assay* under *d-α-Tocopheryl Acetate*, page 333. Calculate the weight, in mg, of *dl*-α-tocopheryl acetate in the sample by the formula $(10C_I/F) \times (a_U/a_I)$.

Acidity Proceed as directed under *d-α-Tocopheryl Acetate*, this page.

Heavy Metals Place a 500-mg sample in a silica crucible, and proceed as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion in the control.

Packaging and Storage Store in tight, light-resistant containers.

Labeling Label claims in terms of International Units (IU) should be based on the following: 1 mg *dl*-α-tocopheryl acetate = 1 IU.

Functional Use in Foods Nutrient; dietary supplement.

d-α-Tocopheryl Acetate Concentrate

d-α-Tocopheryl Acetate Preparation

DESCRIPTION

A form of vitamin E obtained by the vacuum steam distillation and acetylation of edible vegetable oil products. The content of *d*-α-tocopheryl acetate may be adjusted by suitable physical or chemical means. It occurs as a light brownish yellow, nearly odorless, clear viscous oil. It is unstable in the presence of alkalies. It is insoluble in water, is soluble in alcohol, and is miscible with acetone, chloroform, ether, and vegetable oils.

REQUIREMENTS

Identification

It meets the requirements of *Identification Tests A* and *B* under *d-α-Tocopheryl Acetate*, page 330.

Assay Not less than 40.0% of *d*-α-tocopheryl acetate ($C_{31}H_{52}O_3$).
Acidity Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Specific Rotation $[\alpha]_D^{25}$: Not less than +24°.

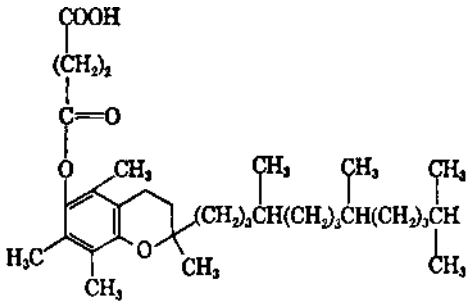
TESTS

NOTE: In the tests for *Assay* and for *Specific Rotation*, use low-actinic glassware for all solutions containing tocopherols.

Assay Proceed as directed in the *Assay* under *d-α-Tocopheryl Acetate*, page 330, using the following as the *Assay Preparation*: Dissolve an accurately weighed amount of the sample, equivalent to about 30 mg of *d*-α-tocopheryl acetate, in 10.0 ml of the *Internal Standard Solution*.
Acidity, Heavy Metals, and Lead Proceed as directed under *d-α-Tocopheryl Acetate*, page 334.
Specific Rotation Proceed as directed under *d-α-Tocopheryl Acetate*, page 334, using for the *Test Solution* an accurately weighed sample equivalent to about 200 mg of α-tocopherol.

Packaging and Storage Store in tight, light-resistant containers.
Labeling Label to indicate the mg per g of *d*-α-tocopheryl acetate present. Label claims in terms of International Units (IU) should be based on the following: 1 mg *d*-α-tocopheryl acetate = 1.36 IU.
Functional Use in Foods Nutrient; dietary supplement.

d-α-Tocopheryl Acid Succinate



$C_{33}H_{54}O_5$ Mol wt 530.79

DESCRIPTION

A form of vitamin E obtained by the vacuum steam distillation and succinylation of edible vegetable oil products. It occurs as a white to off-white crystalline powder having little or no taste or odor. It is stable in air, but is unstable to alkali and to heat. It is insoluble in water; soluble in acetone, alcohol, ether, and vegetable oils; and very soluble in chloroform. It melts at about 75°.

REQUIREMENTS

Identification

A. To 10 ml of the *Test Solution*, obtained as directed under *Specific Rotation*, add with swirling 2 ml of nitric acid, and heat at about 75° for 15 min. A bright red to orange color develops.
B. The retention time of the major peak (excluding the solvent peak) in the chromatogram of the *Assay Preparation* is the same as that of the *Standard Preparation*, both relative to the internal standard, as obtained in the *Assay*.

Assay Not less than 96.0% and not more than 102.0% of $C_{33}H_{54}O_5$.
Acidity Passes test.
Heavy Metals (as Pb) Not more than 0.004%.
Lead Not more than 10 ppm.
Specific Rotation $[\alpha]_D^{25}$: Not less than +24°.

TESTS

Assay

Internal Standard Solution Prepare a solution in *n*-hexane containing about 3 mg of hexadecyl hexadecanoate, accurately weighed, in each ml.

Standard Preparation Transfer about 30 mg of USP Alpha Tocopheryl Acid Succinate Reference Standard, accurately weighed, to a 4-dram (approximately 15 ml) screw-cap vial. Pipet 2.0 ml of absolute methanol, 1.0 ml of 2,2-dimethoxypropane, and 0.1 ml of concentrated hydrochloric acid into the vial, cap, mix well, and allow to stand for 1 h in the dark. Evaporate just to dryness on a steam bath with the

aid of a stream of nitrogen. Pipet 10.0 ml of the *Internal Standard* into the vial, cap, and shake vigorously.

Assay Preparation Prepare as directed for the *Standard Preparation*, using an accurately weighed amount of the sample equivalent to about 30 mg of *d*-α-tocopheryl acid succinate.

Chromatographic System Use a gas chromatograph equipped with a flame-ionization detector and a glass-lined sample-introduction system or on-column injection. Under typical conditions, the instrument contains a 2-m × 4-mm borosilicate glass column packed with 2% to 5% methylpolysiloxane gum on 80- to 100-mesh acid-base washed silinized chromatographic diatomaceous earth. The column is maintained isothermally between 260° and 280°, the injection port at about 290°, and the detector block at about 300°. The flow rate of dry carrier gas is adjusted to obtain a hexadecyl hexadecanoate peak 12 to 14 min after sample introduction. [NOTE: Cure and condition the column as necessary (see page 475).]

System Suitability Chromatograph a suitable number of injections of a mixture in *n*-hexane of 1 mg per ml each of USP Alpha Tocopherol Reference Standard and USP Alpha Tocopheryl Acetate Reference Standard, as directed under *Calibration*, to assure that the resolution factor, *R*, is not less than 1.0 (see page 476).

Calibration Chromatograph successive 2- to 5-µl portions of the upper layer of the *Standard Preparation* until the relative response factor, *F*, is constant (i.e., within a range of approximately 2%) for three consecutive injections. If graphic integration is used, adjust the instrument to obtain 70% maximum recorder response for the hexadecyl hexadecanoate peak. Measure the areas under the major peaks occurring at relative retention times of approximately 1.00 (hexadecyl hexadecanoate) and 1.99 (methyl α-tocopheryl succinate), and record the values as A_I and A_S, respectively. Calculate the relative response factor, *F*, by the formula $(A_S/A_I) \times (C_I/C_S)$, in which C_I and C_S are the exact concentrations, in mg per ml, of hexadecyl hexadecanoate and of USP Alpha Tocopheryl Acid Succinate Reference Standard in the *Standard Preparation*, respectively.

Procedure Inject a suitable portion (2 to 5 µl) of the *Assay Preparation* into the chromatograph, and record the chromatogram. Measure the areas under the major peaks occurring at relative retention times of approximately 1.00 (hexadecyl hexadecanoate) and 1.99 (methyl α-tocopheryl succinate), and record the values as a_I and a_U, respectively. Calculate the weight, in mg, of *d*-α-tocopheryl acid succinate in the sample by the formula $(10C_I/F) \times (a_U/a_I)$.

Acidity Dissolve 1.0 g of the sample in 25 ml of a mixture of equal volumes of alcohol and ether that has been neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide, add 0.5 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 30 s. Between 18.0 ml and 19.3 ml of 0.1 *N* sodium hydroxide is required.

Heavy Metals Place a 500-mg sample in a silica crucible, and proceed as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Specific Rotation

NOTE: Use low-actinic glassware.

Test Solution Transfer an accurately weighed amount of the sample, equivalent to about 200 mg of α-tocopherol, to a 250-ml round-bottom glass-stoppered flask, dissolve in 50 ml of absolute alcohol, and reflux for 1 min. While the solution is boiling, add through the condenser 1 g of potassium hydroxide pellets, one at a time to avoid overheating.

Caution: Wear safety goggles.

Continue refluxing for 20 min, and then, without cooling, add 2 ml of hydrochloric acid dropwise through the condenser. (This technique is essential to prevent oxidative action by air while the sample is in an alkaline medium.) Cool, and transfer the contents of the flask to a 500-ml separator, rinsing the flask with 100 ml each of water and of ether and adding the rinsings to the separator. Shake vigorously, allow the layers to separate, and collect each of the two layers in separate separators. Extract the aqueous layer with two 50-ml portions of ether, and add these extracts to the main ether extract. Wash the combined ether extracts with four 100-ml portions of water, and then evaporate the solutions on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain. Complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in diluted sulfuric acid TS in alcohol (1 in 72), transfer to a 200-ml volumetric flask, dilute to volume with the alcoholic sulfuric acid, and mix.

Procedure Transfer an accurately measured volume of the *Test Solution*, equivalent to about 100 mg of α-tocopherol, to a separator, and add 200 ml of water. Extract first with 75 ml, then with two 25-ml portions of ether, and combine the ether extracts in another separator. To the ether solution add 20 ml of a 10% solution of potassium ferricyanide in sodium hydroxide solution (1 in 125), and shake for 3 min. Wash the ether solution with four 50-ml portions of water, discard the washings, and dry over anhydrous sodium sulfate. Evaporate the dried ether solution on a water bath under reduced pressure or in an atmosphere of nitrogen until about 7 or 8 ml remain, and then complete the evaporation, removing the last traces of ether without the application of heat. Immediately dissolve the residue in 5.0 ml of isooctane, and determine the optical rotation. Calculate the specific rotation (see page 530), using as *c* the concentration expressed as the number of g of α-tocopheryl acid succinate, determined in the *Assay*, in 100 ml of solution.

Packaging and Storage Store in tight, light-resistant containers.

Labeling Label claims in terms of International Units (IU) should be based on the following: 1 mg *d*-α-tocopheryl acid succinate = 1.21 IU.

Functional Use in Foods Nutrient; dietary supplement.

Tragacanth

DESCRIPTION

A dried gummy exudation obtained from *Astragalus gummifer* Labillardiere, or other Asiatic species of *Astragalus* (Fam. *Leguminosae*). *Unground Tragacanth* occurs as flattened, lamellated, frequently curved fragments or straight or spirally twisted linear pieces from 0.5 to 2.5 mm in thickness. It is white to weak yellow in color, translucent, horny in texture, and having a short fracture. It is odorless and has an insipid, mucilaginous taste. It is rendered more easily pulverizable if heated to a temperature of 50°. *Powdered Tragacanth* is white to yellowish white in color.

REQUIREMENTS

Identification

When examined microscopically in water mounts, it shows numerous angular fragments with circular or irregular lamellae, and starch grains up to 25 μm in diameter. It should show very few or no fragments of lignified vegetable tissue. One g in 50 ml of water swells to form a smooth, stiff, opalescent mucilage free from cellular fragments.

Arsenic (as As) Not more than 3 ppm.
Ash (Total) Not more than 3.0%.
Ash (Acid-Insoluble) Not more than 0.5%.
Heavy Metals (as Pb) Not more than 0.004%.
Karaya Gum Passes test.
Lead Not more than 10 ppm.
Viscosity of a 1% Solution Not less than 250 centipoises.

TESTS

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash (Total) Determine as directed in the general method, page 466.

Ash (Acid-Insoluble) Determine as directed in the general method, page 466.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Karaya Gum Boil 1 g with 20 ml of water until a mucilage is formed, add 5 ml of hydrochloric acid, and again boil the mixture for 5 min. No permanent pink or red color develops.

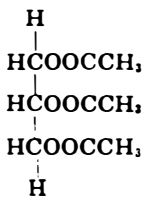
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Viscosity Transfer a 4.0-g sample, finely powdered, into the container of a stirring apparatus equipped with blades capable of revolving at 10,000 rpm. Add 10 ml of alcohol to the sample, swirl to wet the gum uniformly, and then add 390 ml of water, avoiding the formation of lumps. Immediately stir the mixture for 7 min, pour the resulting dispersion into a 500-ml bottle, insert a stopper, and allow to stand for about 24 h in a water bath at 25°. Determine the apparent viscosity at this temperature with a Model LVF Brookfield or equivalent type viscometer (see *Viscosity of Sodium Carboxymethylcellulose*, page 550) using Spindle No. 2 at 30 rpm and a factor of 10.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Stabilizer; thickener; emulsifier.

Triacetin

Glyceryl Triacetate



$C_9H_{14}O_6$ Mol wt 218.21

DESCRIPTION

A colorless, somewhat oily liquid with a slight, fatty odor and a bitter taste. It is soluble in water, and is miscible with alcohol, with ether, and with chloroform. It distils between 258° and 270°.

REQUIREMENTS

Identification

A. Heat a few drops in a test tube with about 500 mg of potassium bisulfate. Pungent vapors of acrolein are evolved.
B. The solution resulting from the *Assay* gives positive tests for *Acetate*, page 515.

Assay Not less than 98.5% of $C_9H_{14}O_6$.
Acidity Passes test.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Refractive Index Between 1.429 and 1.431 at 25°.
Specific Gravity Between 1.154 and 1.158.
Unsaturated Compounds Passes test.
Water Not more than 0.2%.

TESTS

Assay Transfer about 1 g of the sample, accurately weighed, into a suitable pressure bottle, add 25.0 ml of 1 *N* potassium hydroxide and 15 ml of isopropanol, stopper the bottle, and wrap securely in a canvas bag. Place in a water bath maintained at 98° ± 2°, and heat for 1 h, allowing the water

in the bath to just cover the liquid in the bottle. Remove the bottle from the bath, cool in air to room temperature, then loosen the wrapper, uncap the bottle to release any pressure, and remove the wrapper. Add 6 to 8 drops of phenolphthalein TS, and titrate the excess alkali with 0.5 *N* sulfuric acid just to the disappearance of the pink color. Perform a blank determination (see page 2). Each ml of 0.5 *N* sulfuric acid is equivalent to 36.37 mg of $C_9H_{14}O_6$.

Acidity Transfer about 25 g of the sample, accurately weighed, into a 125-ml conical flask, add 50 ml of toluene and 2 drops of thymol blue TS, and titrate rapidly with 0.02 *M* sodium methoxide in toluene. Swirl the flask continuously until the yellow color changes to a dark color, and then continue the titration without stopping but slowing the addition of titrant until a single drop changes the solution to a clear blue color. The endpoint is stable for about 8 to 15 s. Not more than 1.0 ml of 0.02 *M* sodium methoxide is required.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Refractive Index, page 533 Determine at 25° with an Abbé or other refractometer of equal or greater accuracy.

Specific Gravity Determine by any reliable method (see page 3).

Unsaturated Compounds To 10 ml of the sample in a glass-stoppered tube add, dropwise, a solution of bromine in carbon tetrachloride (1 ml in 100 ml) until a permanent yellow color is produced, and allow to stand in a dark place for 18 h. No turbidity or precipitate appears.

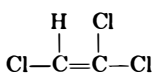
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers, and do not permit contact with metal.

Functional Use in Foods Humectant; solvent.

Trichloroethylene

Ethylene Trichloride; Trichloroethene; 1,1,2-Trichloroethylene



C_2HCl_3 Mol wt 131.39

DESCRIPTION

A clear, colorless, mobile liquid having a sweet, chloroformlike odor. It is immiscible with water, but is miscible with alcohol, ether, acetone, and carbon tetrachloride. Its refractive index at 20° is about 1.477. It may contain a suitable stabilizer.

REQUIREMENTS

Acidity (as HCl) Not more than 10 ppm.
Alkalinity (as NaOH) Not more than 10 ppm.
Distillation Range Between 86° and 88°.
Free Halogens Passes test.
Heavy Metals (as Pb) Not more than 1 ppm.
Nonvolatile Residue Not more than 10 ppm.
Specific Gravity Between 1.454 and 1.458.
Water Not more than 0.05%.

TESTS

Acidity or Alkalinity Transfer 25 ml of water and 2 drops of phenolphthalein TS to a 250-ml glass-stoppered flask, and add 0.01 *N* sodium hydroxide to the first appearance of a slight pink color. Add 25 ml (about 36 g) of the sample, and shake for 30 s. If the pink color persists, titrate with 0.01 *N* hydrochloric acid, shaking repeatedly, until the pink color just disappears; not more than 0.9 ml is required. If the pink color is discharged when the sample is added, titrate with 0.01 *N* sodium hydroxide until the faint pink color is restored; not more than 1.0 ml is required.

Distillation Range Determine as directed in the general method, page 478.

Free Halogens Shake 10 ml of the sample vigorously for 2 min with 10 ml of potassium iodide solution (1 in 10) and 1 ml of starch TS. A blue color does not appear in the water layer.

Heavy Metals Evaporate 14 ml (about 20 g) of the sample to dryness (*Caution:* use hood) on a steam bath in a glass evaporating dish. Cool, add 2 ml of hydrochloric acid, and slowly evaporate to dryness again on the steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Filter if necessary through a small filter, wash the evaporating dish and the filter with about 10 ml of water, and dilute to 25 ml with water. This solution meets the requirements of the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Nonvolatile Residue Evaporate 69 ml (about 100 g) of the sample to dryness (*Caution:* use hood) in a tared dish on a steam bath, dry the residue at 105° for 30 min, cool, and weigh.

Specific Gravity Determine by any reliable method (see page 3).

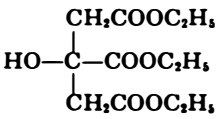
Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in tight containers.

Functional Use in Foods Extraction solvent.

Triethyl Citrate

Ethyl Citrate



$C_{12}H_{20}O_7$ Mol wt 276.29

DESCRIPTION

An odorless, practically colorless, oily liquid. It is slightly soluble in water, but is miscible with alcohol and with ether. Its specific gravity is approximately 1.137 at 20°.

REQUIREMENTS

Assay Not less than 99.0% of $C_{12}H_{20}O_7$.
Acidity (as citric acid) Not more than 0.02%.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 10 ppm.
Refractive Index Between 1.439 and 1.441.
Water Not more than 0.25%.

TESTS

Assay Weigh accurately about 1.5 g of the sample into a 500-ml flask equipped with a standard-taper ground joint, and add 25 ml of isopropyl alcohol and 25 ml of water. Pipet 50 ml of 0.5 *N* sodium hydroxide into the mixture, add a few boiling chips, and attach a suitable water-cooled condenser. Reflux for 1.5 h, then cool, wash down the condenser with about 20 ml of water, add 5 drops of phenolphthalein TS, and titrate the excess alkali with 0.5 *N* sulfuric acid. Perform a blank determination (see page 2). Each ml of 0.5 *N* sulfuric acid is equivalent to 46.05 mg of $C_{12}H_{20}O_7$.

Acidity Dissolve 32 g, accurately weighed, in 30 ml of neutralized alcohol, add phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide. Not more than 1.0 ml is required.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 2-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

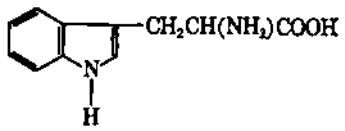
Refractive Index, page 533 Determine with an Abbé or other refractometer of equal or greater accuracy.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Sequestrant.

DL-Tryptophan

DL-α-Amino-3-indolepropionic Acid



$C_{11}H_{12}N_2O_2$ Mol wt 204.23

DESCRIPTION

White, odorless crystals or a crystalline powder. It is soluble in water and in dilute acids and alkalies. It is sparingly soluble in alcohol. It is optically inactive.

REQUIREMENTS

Identification

To a solution of DL-tryptophan in water add bromine TS. A red color appears that may be extracted with amyl alcohol.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{11}H_{12}N_2O_2$ after drying.
Ammonium Salts (as NH_3) Not more than 0.03%.
Arsenic (as As) Not more than 3 ppm.
Chloride Not more than 0.02%.
Heavy Metals (as Pb) Not more than 0.004%.
Indole Passes test.
Iron Not more than 0.005%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Nitrogen (Total) Between 13.4% and 13.9%.
Residue on Iguition Not more than 0.1%.
Sulfate Not more than 0.04%.

TESTS

Assay Dissolve about 300 mg of the sample, previously dried at 105° for 3 h and accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

Ammonium Salts

Ammonium Chloride Standard Solution Dissolve 78.5 mg of ammonium chloride in sufficient water to make exactly 250 ml, and dilute 10.0 ml of this solution to 100.0 ml. The dilute solution contains the equivalent of 10 µg of NH_3 in each ml.

Procedure Place 5 g of sodium hydroxide pellets and 300 ml of water in a 500-ml distillation flask. Remove ammonia from the solution by distilling until 25 ml of the distillate gives no color with 0.5 ml of alkaline mercuric-potassium iodide TS. Allow the solution in the distillation flask to cool, and add 50 mg of tryptophan. Distil, collecting two 25-ml fractions in 50-ml Nessler tubes. Add 0.5 ml of alkaline

mercuric-potassium iodide TS to the distillates and to a series of standards, prepared by dilution of the *Ammonium Chloride Standard Solution*, containing the equivalent of 0, 5, 10, 15, and 20 μg of ammonia in 25 ml of solution. Stopper the tubes, and invert them several times to mix their contents. After allowing 10 min for color development, determine the amount of ammonia present in the distillates by comparing their color intensities with those of the standards. If the second 25 ml of distillate from the tryptophan sample contains appreciable ammonia, distil and collect additional 25-ml fractions and determine their ammonia content.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements for the *Arsenic Test*, page 464.

Chloride, page 471 Any turbidity produced by a 100-mg sample does not exceed that shown in a control containing 20 μg of chloride ion (Cl).

Heavy Metals A solution of 500 mg in 20 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Indole Dissolve 500 mg in 1 ml of diluted hydrochloric acid TS, and dilute to 50 ml. Transfer 10 ml of this solution to a test tube, add 2 ml of *n*-amyl or isoamyl alcohol, and shake vigorously. The amyl alcohol layer has no more than a light yellow color (an orange or pink color indicates the presence of indole or other color-producing intermediates). Allow the remaining 40 ml of the solution to stand overnight. A slightly pink color develops, but no precipitate forms.

Iron To the ash obtained in the test for *Residue on Ignition* add 2 ml of dilute hydrochloric acid (1 in 12), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid, and dilute with water to 50 ml. Dilute 10 ml of this solution to 40 ml with water, and add 30 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 μg of Fe) in an equal volume of a solution containing the quantities of reagents used in the test.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Nitrogen (Total) Determine as directed under *Nitrogen Determination*, page 521, using about 300 mg of the sample, previously dried and accurately weighed.

Residue on Ignition Ignite 2 g as directed in the general method, page 533.

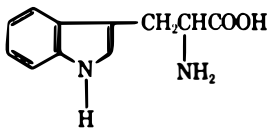
Sulfate, page 471 Any turbidity produced by a 500-mg sample does not exceed that shown in a control containing 200 μg of sulfate (SO_4).

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

L-Tryptophan

L-α-Amino-3-indoleproprionic Acid



$C_{11}H_{12}N_2O_2$ Mol wt 204.23

DESCRIPTION

White to yellowish white crystals or a crystalline powder. It is odorless and has a slightly bitter taste. One g dissolves in about 100 ml of water. It is soluble in hot alcohol, in dilute hydrochloric acid, and in alkali hydroxide solutions.

REQUIREMENTS

Identification

Dissolve about 1 g in 100 ml of hydrochloric acid solution (1 in 5). To 1 ml of this solution add 1 ml of sodium sulfite solution (1 in 20). A yellow color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_{11}H_{12}N_2O_2$, calculated on the dried basis.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 0.002%.

Lead Not more than 10 ppm.

Loss on Drying Not more than 0.3%.

Residue on Ignition Not more than 0.1%.

Specific Rotation $[\alpha]_D^{20}$: Between −30.0° and −33.0°, on the dried basis.

TESTS

Assay Dissolve about 300 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 20.42 mg of $C_{11}H_{12}N_2O_2$.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of *Arsenic Test*, page 464.

Heavy Metals Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

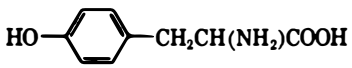
Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution containing 1 g of a previously dried sample in sufficient water to make 100 ml.

Packaging and Storage Store in well-closed, light-resistant containers.
Functional Use in Foods Nutrient; dietary supplement.

L-Tyrosine

L-β-(*p*-Hydroxyphenyl)alanine



$C_9H_{11}NO_3$ Mol wt 181.19

DESCRIPTION

Colorless, silky needles, or a white crystalline powder. One g is soluble in about 230 ml of water. It is soluble in dilute mineral acids and in alkaline solutions. It is very slightly soluble in alcohol.

REQUIREMENTS

Identification

Heat 5 ml of a 1 in 1000 solution with 1 ml of triketohydrindene hydrate TS. A reddish purple color is produced.

Assay Not less than 98.5% of $C_9H_{11}NO_3$ after drying.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.003%.
Iron Not more than 0.005%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Nitrogen Between 7.5% and 7.8%.
Residue on Iguition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{25°}$: Between −9.8° and −11.2°; $[\alpha]_D^{20°}$: between −11.3° and −12.3°.

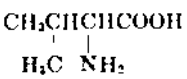
TESTS

Assay Transfer about 400 mg, previously dried at 105° for 2 h and accurately weighed, into a 250-ml flask. Dissolve the sample in about 50 ml of acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a bluish green endpoint. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.1 *N* perchloric acid is equivalent to 18.12 mg of $C_9H_{11}NO_3$.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Heavy Metals Prepare and test a 670-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 μg of lead ion (Pb) in the control (*Solution A*).
Iron To the ash obtained in the test for *Residue on Ignition* add 2 ml of dilute hydrochloric acid (1 in 2), and evaporate to dryness on a steam bath. Dissolve the residue in 1 ml of hydrochloric acid and dilute with water to 50 ml. Dilute 10 ml of this solution to 50 ml with water, and add 40 mg of ammonium persulfate crystals and 10 ml of ammonium thiocyanate TS. Any red or pink color does not exceed that produced by 1.0 ml of *Iron Standard Solution* (10 μg Fe) in an equal volume of a solution containing the quantities of the reagents used in the test.
Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 μg of lead ion (Pb) in the control.
Loss on Drying, page 518 Dry at 105° for 3 h.
Nitrogen (Total) Determine as directed under *Nitrogen Determination*, page 521, using about 300 mg of the sample, previously dried and accurately weighed.
Residue on Ignition Ignite 2 g as directed in the general method, page 533.
Specific Rotation, page 530 $[\alpha]_D^{25°}$: Determine in a solution containing 4 g in sufficient 1 *N* hydrochloric acid to make 100 ml; $[\alpha]_D^{20°}$: determine in a solution containing 5 g in sufficient 1 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

L-Valine

L-2-Amino-3-methylbutyric Acid



$C_5H_{11}NO_2$ Mol wt 117.15

DESCRIPTION

A white, odorless, crystalline powder having a characteristic taste. It is freely soluble in water, but is practically insoluble in alcohol and in ether. The pH of a 1 in 20 solution is between 5.5 and 7.0. In a closed capillary tube it melts at about 315°.

REQUIREMENTS

Identification

To 5 ml of a 1 in 1000 solution add 1 ml of triketohydrindene hydrate TS. A reddish purple or bluish color is produced.

Assay Not less than 98.0% and not more than the equivalent of 102.0% of $C_5H_{11}NO_2$, calculated on the dried basis.
Arsenic (as As) Not more than 3 ppm.
Heavy Metals (as Pb) Not more than 0.002%.
Lead Not more than 10 ppm.
Loss on Drying Not more than 0.3%.
Residue on Iguition Not more than 0.1%.
Specific Rotation $[\alpha]_D^{20°}$: Between +26.5° and +29.0°, on the dried basis.

TESTS

Assay Dissolve about 200 mg, accurately weighed, in 3 ml of formic acid and 50 ml of glacial acetic acid, add 2 drops of crystal violet TS, and titrate with 0.1 *N* perchloric acid to a green endpoint or until the blue color disappears completely. Each ml of 0.1 *N* perchloric acid is equivalent to 11.72 mg of $C_5H_{11}NO_2$.

Arsenic (as As) A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals (as Pb) Prepare and test a 1-g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 3 h.

Residue on Ignition, page 533 Ignite 1 g as directed in the general method.

Specific Rotation, page 530 Determine in a solution containing 8 g of a previously dried sample in sufficient 6 *N* hydrochloric acid to make 100 ml.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Vitamin A

DESCRIPTION

A suitable form or derivative of retinol ($C_{20}H_{30}O$; vitamin A alcohol). It usually consists of retinol or esters of retinol formed from edible fatty acids, principally acetic and palmitic acids, or mixtures of these. It may be diluted with edible oils, or it may be incorporated in solid edible carriers, extenders, or excipients. It may contain suitable preservatives, dispersants, and antioxidants, providing it is not to be used in foods in which such substances are prohibited. In liquid form it is a light yellow to red oil that may solidify on refrigeration. In solid form it may have the appearance of the diluent that has been added to it. It may be nearly odorless, or have a mild fishy odor, but no rancid odor or taste. In liquid form it is very soluble in chloroform and in ether, it is soluble in absolute alcohol and in vegetable oils, but it is insoluble in glycerin and in water. In solid form it may be dispersible in water. It is unstable to air and light.

REQUIREMENTS

Identification

A. Dissolve an amount equivalent to about 6 µg of retinol in 1 ml of chloroform, and add 10 ml of antimony trichloride TS. A transient blue color appears at once.

B. Assemble an apparatus for *Thin-layer Chromatography* (see page 474), using chromatographic silica gel as the adsorbent and a mixture of 4 parts of cyclohexane and 1 part of ether as the solvent system. Prepare a *Standard Solution* by dissolving the contents of 1 capsule of USP Vitamin A Reference Solution in sufficient chloroform to make 25.0 ml.

If the vitamin A is in liquid form, dissolve a volume representing approximately 15,000 International Units in sufficient chloroform to make 10 ml. If the vitamin A is in solid form, weigh a quantity representing approximately 15,000 International Units, place in a separator, add 75 ml of water, heat, if necessary, to dissolve the carrier, and cool. Shake vigorously for 1 min, extract with 10 ml of chloroform by shaking for 1 min, and centrifuge to clarify the chloroform extract.

Apply at the starting point of the chromatogram 0.015 ml of the *Standard Solution* and 0.01 ml of the chloroform solution of the vitamin A sample. Develop the chromatogram in the chromatographic chamber, lined with filter paper dipping into the solvent mixture. When the solvent has ascended for a distance of 10 cm, remove the plate, allow it to dry in air, and spray it with antimony trichloride TS. The blue spot formed is indicative of the presence of retinol. The approximate R_f values of the predominant spots, corresponding to the different forms of retinol, are 0.1 for the alcohol, 0.45 for the acetate, and 0.7 for the palmitate.

Assay Not less than 95.0% of the vitamin A activity declared on the label.

Absorbance Ratio (corrected/observed at 325 nm) Not less than 0.85.

TESTS

Assay Proceed as directed under *Vitamin A Assay*, page 551.

Absorbance Ratio Determine by the formula $[A_{325}]/A_{325}$, the terms of which are defined under *Calculation* in the *Vitamin A Assay*, page 551.

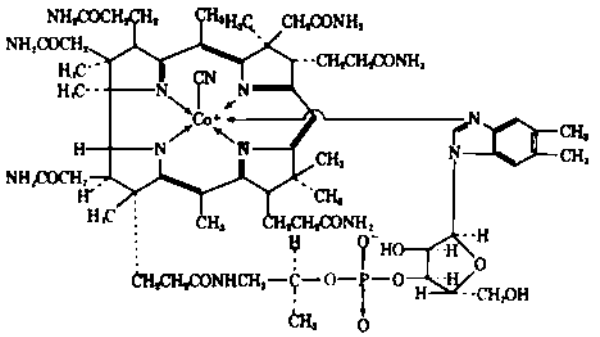
Packaging and Storage Store in a cool place in tight containers, preferably under an atmosphere of an inert gas, protected from light.

Labeling Label to indicate the form of the vitamin A, to declare the presence of any preservative, dispersant, antioxidant, or other added substance, and to declare the vitamin A activity in terms of the equivalent amount of retinol in mg per g and in International Units. (NOTE: One International Vitamin A Unit is the specific biologic activity of 0.3 µg of the all-*trans* isomer of retinol.)

Functional Use in Foods Nutrient; dietary supplement.

Vitamin B_{12}

Cyanocobalamin



$C_{63}H_{88}CoN_{14}O_{14}P$ Mol wt 1355.38

DESCRIPTION

Dark red crystals, or amorphous or crystalline powder. In the anhydrous form it is very hygroscopic, and when exposed to air it may absorb about 12% of water. It is sparingly soluble in water, soluble in alcohol, and insoluble in acetone, in chloroform, and in ether.

REQUIREMENTS

Identification

A. The absorption spectrum of the solution of the sample employed for measurement of absorbance in the *Assay* exhibits maxima within ±1 nm at 278 nm and 361 nm, and within ±2 nm at 550 nm. The ratio A_{361}/A_{550} is between 3.15 and 3.40.

B. Fuse about 1 mg of the sample with about 50 mg of potassium pyrosulfate in a porcelain crucible, cool, and break up the mass with a glass rod. Add 3 ml of water, and dissolve by boiling. Add 1 drop of phenolphthalein TS, mix, and then add sodium hydroxide solution (1 in 10), dropwise, until just pink. Add 500 mg of sodium acetate, 0.5 ml of diluted acetic acid, and 0.5 ml of nitroso R salt solution (1 in 500). A red or orange red color appears at once. Add 0.5 ml of hydrochloric acid, and boil for 1 min. The red color persists.

C. Dissolve about 5 mg of the sample in 5 ml of water in a 50-ml distilling flask connected with a short water-cooled vertical condenser, the tip of which dips into a test tube containing 1 ml of sodium hydroxide solution (1 in 50). To the flask add 2.5 ml of hypophosphorous acid, then close the flask, heat at simmering for 10 min, and distil 1 ml into the test tube. To the tube add 4 drops of cold saturated ferrous ammonium sulfate solution, shake gently, then add about 30 mg of sodium fluoride, and bring the contents to a boil. Immediately add, dropwise, dilute sulfuric acid (1 in 7) until a clear solution results, and then add 3 to 5 drops more of the acid. A blue or blue green color develops within a few min.

Assay Not less than 96.0% of $C_{63}H_{88}CoN_{14}O_{14}P$, calculated on the dried basis.

Loss on Drying Not more than 12%.

Pseudo Cyanocobalamin Passes test.

TESTS

Assay With the aid of water, transfer about 30 mg of the sample, accurately weighed, to a 1000-ml volumetric flask, dilute with water to volume, and mix. Dissolve an accurately weighed quantity of USP Cyanocobalamin Reference Standard in water, and dilute quantitatively and stepwise with water to obtain a standard solution having a known concentration of about 30 µg per ml. Concomitantly determine the absorbances of both solutions in 1-cm cells at the wavelength of maximum absorption at about 361 nm, with a suitable spectrophotometer, using water as the blank. Calculate the quantity, in mg, of $C_{63}H_{88}CoN_{14}O_{14}P$ in the sample taken by the formula $C \times A_U/A_S$, in which C is the concentration of the reference standard solution, in µg per ml, and A_U and A_S are the absorbances of the sample solution and the reference standard solution, respectively.

Loss on Drying, page 518 Dry about 25 mg at 105° in a vacuum at a pressure of not more than 5 mm of Hg for 2 h.

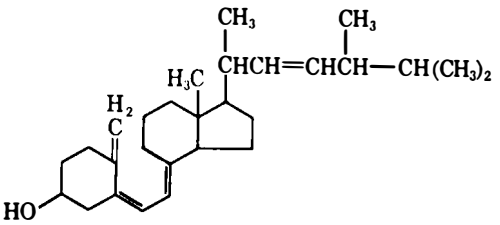
Pseudo Cyanocobalamin Dissolve 1.0 mg of the sample in 20 ml of water contained in a small separator, add 5 ml of a mixture of equal volumes of carbon tetrachloride and cresol, and shake well for about 1 min. Allow the layers to separate, and draw off the lower layer into a second small separator. Add 5 ml of dilute sulfuric acid (1 in 7), shake well, and allow to separate completely, centrifuging if necessary. The separated upper layer is colorless or has no more color than a mixture of 0.15 ml of 0.1 *N* potassium permanganate in 250 ml of water.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement.

Vitamin D_2

Ergocalciferol; Vitamin D



$C_{28}H_{44}O$ Mol wt 396.66

DESCRIPTION

White, odorless crystals. It is affected by air and by light. It is insoluble in water, but is soluble in alcohol, in chloroform, in ether, and in fatty oils.

REQUIREMENTS

Identification

A. To a solution of about 0.5 mg of the sample in 5 ml of chloroform add 0.3 ml of acetic anhydride and 0.1 ml of sulfuric acid, and shake vigorously. A bright red color is produced that rapidly changes through violet and blue to green.

B. Prepare without heating, and handle without delay, a 1 in 100 solution of squalane in chloroform containing 50 mg of the sample per ml, and prepare a solution of USP Ergocalciferol Reference Standard in the same solvent and of the same concentration. Prepare a 1 in 100 solution of squalane in chloroform eontaining 100 μg of USP Ergosterol Reference Standard per ml. Spot 10 μl each of the sample solution, the ergocalciferol standard solution, and the ergosterol standard solution on a line parallel to and about 2.5 cm from the bottom edge of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel containing a suitable fluorescing substance. Place the plate in a developing chamber containing, and equilibrated with, a mixture of equal volumes of cyclohexane and ether. Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Perform the development and subsequent operations in the dark. Remove the plate from the chamber, allow the solvent to evaporate, and spray with a 1 in 50 solution of acetyl chloride in antimony trichloride TS. The chromatogram obtained with the sample solution shows a yellowish orange area (ergocalciferol) having the same R_f value as the area of the ergocalciferol standard and may show a violet area below the ergocalciferol area (see also *Ergosterol* under TESTS below).

C. The infrared absorption spectrum of a potassium bromide dispersion of the sample, in the range of 2 to 12 μm, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Ergocalciferol Reference Standard.

D. The ultraviolet absorption spectrum of the sample in alcohol solution exhibits inflections at the same wavelengths as that of USP Ergocalciferol Reference Standard, similarly measured, and the respective absorptivities at 265 nm do not differ by more than 3.0%.

Assay Not less than 97.0% and not more than the equivalent of 103.0% of $C_{28}H_{44}O$.

Ergosterol Passes test.

Melting Range Between 115° and 119°.

Reducing Substances Passes test.

Specific Rotation $[\alpha]_D^{25°}$: Between +103° and +106°.

TESTS

Assay

Standard Preparation (NOTE: Use low-actinic glassware, and prepare solutions fresh daily.) Transfer about 30 mg of USP Ergocalciferol Reference Standard, accurately weighed, into a 50-ml volumetric flask, dissolve without heating in toluene, add toluene to volume, and mix. Pipet 10 ml of this solution into a second 50-ml volumetric flask, dilute to volume with the *Mobile Phase*, and mix.

Assay Preparation (NOTE: Use low-actinic glassware, and prepare solutions fresh daily.) Transfer about 30 mg of the sample, accurately weighed, into a 50-ml volumetric flask, dissolve without heating in toluene, add toluene to volume, and mix. Pipet 10 ml of this solution into a second 50-ml volumetric flask, dilute to volume with the *Mobile Phase*, and mix.

Mobile Phase Prepare a 3 in 1000 mixture of *n*-amyl alcohol in ACS Reagent-Grade Hexanes (suitable for use in ultraviolet spectrophotometry) that has been dried by passing through a 60- × 8-cm column containing 500 g of 50- to 250-μm chromatographic siliceous earth. The ratio of components and the flow rate may be varied to meet the requirements of the *System Suitability Test*.

Chromatographic System Use a suitable high-pressure liquid chromatograph, operated at room temperature, fitted with a 25-cm × 4.6-mm stainless steel column packed with porous silica microparticles, 5 to 10 μm in diameter, and an ultraviolet detector that monitors absorption at 254 nm.

System Suitability Preparation Dissolve about 250 mg of USP Vitamin D Assay System Suitability Reference Standard in 10 ml of a mixture of equal volumes of toluene and the *Mobile Phase*. Reflux this solution at 90° for 45 min, and cool. (This solution contains cholecalciferol, pre-cholecalciferol, and *trans*-cholecalciferol.)

System Suitability Test Chromatograph five injections of the *System Suitability Preparation*, and measure the peak responses as directed under the *Procedure*. The relative standard deviation for the peak response does not exceed 2.0%, and the resolution between *trans*-cholecalciferol and pre-cholecalciferol is not less than 1.0. The chromatograms obtained in this test exhibit relative retention times of approximately 0.4, 0.5, and 1.0, for pre-cholecalciferol, *trans*-cholecalciferol, and cholecalciferol, respectively.

Procedure By means of a suitable sampling valve, introduce equal volumes (5 to 10 µl) of the *Standard Preparation* and the *Assay Preparation* into the chromatograph. Measure the peak responses for the major peaks, at corresponding retention times, obtained with the *Assay Preparation* and the *Standard Preparation*. Calculate the quantity, in mg, of $C_{28}H_{44}O$ in the sample taken by the formula $0.25C \times A_U/A_S$, in which C is the exact concentration, in µg per ml, of USP Ergocalciferol Reference Standard in the *Standard Preparation*, and A_U and A_S are the peak responses for ergocalciferol obtained with the *Assay Preparation* and the *Standard Preparation*, respectively.

Ergosterol The color of any violet area in the chromatogram of the sample solution, obtained as directed under *Identification Test B*, is not more intense than that of the violet area in the chromatogram of the ergosterol standard.

Melting Range, page 519 Proceed as directed for *Class Ib*.

Reducing Substances To 10 ml of a 1 in 100 solution of the sample in absolute alcohol add 0.5 ml of a 1 in 200 solution of blue tetrazolium in absolute alcohol. Add 0.5 ml of a solution prepared by diluting 1 volume of 10% tetramethylammonium hydroxide with 9 volumes of absolute alcohol. Allow the mixture to stand for 5 min, then add 1 ml of glacial acetic acid, and mix. Prepare a blank by treating 10 ml of absolute alcohol in the same manner. The absorbance of the sample solution, determined at 525 nm with a suitable spectrophotometer against the blank, is not greater than that obtained with a solution containing 0.2 µg per ml of hydroquinone in absolute alcohol, similarly treated.

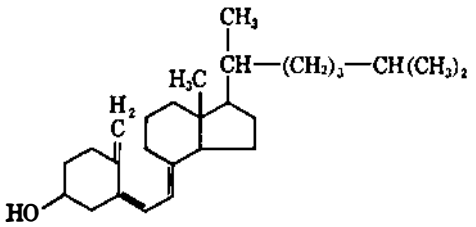
Specific Rotation, page 530 Determine in a solution in alcohol containing 150 mg in each 10 ml. Prepare the solution without delay, using a sample from a container opened not longer than 30 min, and determine the rotation within 30 min after the solution has been prepared.

Packaging and Storage Store in hermetically sealed containers under nitrogen, in a cool place, and protected from light.

Functional Use in Foods Nutrient; dietary supplement.

Vitamin D_3

Cholecalciferol; Vitamin D



$C_{27}H_{44}O$ Mol wt 384.65

DESCRIPTION

White, odorless crystals. It is affected by air and by light. It is insoluble in water. It is soluble in alcohol, in chloroform, and in fatty oils.

REQUIREMENTS

A. To a solution of about 0.5 mg in 5 ml of chloroform add 0.3 ml of acetic anhydride and 0.1 ml of sulfuric acid, and shake vigorously. A bright red color, which rapidly changes through violet and blue to green, is produced.

B. Prepare without heating, and handle without delay, a 1 in 100 solution of squalane in chloroform containing 50 mg of the sample per ml, and prepare a solution of USP Cholecalciferol Reference Standard in the same solvent and having the same concentration. Spot 10 µl each of the sample solution and of the standard solution on a line parallel to and about 2.5 cm from the bottom edge of a thin-layer chromatographic plate coated with a 0.25-mm layer of chromatographic silica gel containing a suitable fluorescing substance. Place the plate in a developing chamber containing, and equilibrated with, a mixture of equal volumes of cyclohexane and ether. Develop the chromatogram until the solvent front has moved about 15 cm above the line of application. Perform the development and subsequent operations in the dark. Remove the plate from the chamber, allow the solvent to evaporate, and spray with a 1 in 50 solution of acetyl chloride in antimony trichloride TS. The chromatogram obtained with the sample solution shows a yellowish orange area (cholecalciferol) having the same R_f value as the area of the cholecalciferol standard and may show a violet area, attributed to 7-dehydrocholesterol, below the cholecalciferol area.

C. The infrared absorption spectrum of a potassium bromide dispersion of the sample, in the range of 2 to 12 µm, exhibits maxima only at the same wavelengths as that of a similar preparation of USP Cholecalciferol Reference Standard.

D. The ultraviolet absorption spectrum of the sample in a solution in alcohol exhibits inflections at the same wavelengths as that of USP Cholecalciferol Reference Standard, similarly measured, and the respective absorptivities at the

point of maximum absorbance occurring at about 265 nm do not differ by more than 3.0%.

Assay Not less than 97.0% and not more than the equivalent of 103.0% of $C_{27}H_{44}O$.
Melting Range Between 84° and 89°.
Specific Rotation $[\alpha]_D^{25°}$: Between +105° and +112°.

TESTS

Assay

Standard Preparation (NOTE: Use low-actinic glassware, and prepare solutions fresh daily.) Transfer about 30 mg of USP Cholecalciferol Reference Standard, accurately weighed, into a 50-ml volumetric flask, dissolve without heating in toluene, add toluene to volume, and mix. Pipet 10 ml of this solution into a second 50-ml volumetric flask, dilute to volume with the *Mobile Phase*, and mix.

Assay Preparation (NOTE: Use low-actinic glassware, and prepare solutions fresh daily.) Transfer about 30 mg of the sample, accurately weighed, into a 50-ml volumetric flask, dissolve without heating in toluene, add toluene to volume, and mix. Pipet 10 ml of this solution into a second 50-ml volumetric flask, dilute to volume with the *Mobile Phase*, and mix.

Mobile Phase, Chromatographic System, System Suitability Preparation, and *System Suitability Test* Proceed as directed in the *Assay* under *Vitamin D_2*, page 344.

Procedure By means of a suitable sampling valve, introduce equal volumes (5 to 10 µl) of the *Standard Preparation* and the *Assay Preparation* into the chromatograph. Measure the peak responses for the major peaks, at corresponding retention times, obtained with the *Assay Preparation* and the *Standard Preparation*. Calculate the quantity, in mg, of $C_{27}H_{44}O$ in the sample taken by the formula $0.25C \times A_U/A_S$, in which C is the exact concentration, in µg per ml, of USP Cholecalciferol Reference Standard in the *Standard Preparation*, and A_U and A_S are the peak responses for cholecalciferol obtained with the *Assay Preparation* and the *Standard Preparation*, respectively.

Melting Range, page 519 Proceed as directed for *Class Ib*.
Specific Rotation, page 530 Determine in a solution in alcohol containing 50 mg in each 10 ml. Prepare the solution without delay, using a sample from a container opened not longer than 30 min, and determine the rotation within 30 min after the solution has been prepared.

Packaging and Storage Store in hermetically sealed containers under nitrogen, in a cool place, and protected from light.
Functional Use in Foods Nutrient; dietary supplement.

Wintergreen Oil

Gaultheria Oil

DESCRIPTION

Wintergreen oil is obtained by maceration and subsequent distillation with steam from the leaves of *Gualtheria procumbens* L. (Fam. *Ericaceae*) or from the bark of *Betula lenta* L. (Fam. *Betulaceae*). It is a colorless, yellowish, or reddish liquid having the characteristic odor and taste of wintergreen. It boils, with decomposition, between 219° and 224°. It is soluble in alcohol and in glacial acetic acid, and it is very slightly soluble in water.

REQUIREMENTS

Identification

A. Shake 1 drop with about 5 ml of water, and add 1 drop of ferric chloride TS. A deep violet color is produced.
B. The infrared absorption spectrum of the sample exhibits relative maxima (that may vary in intensity) at the same wavelengths (or frequencies) as those shown in the respective spectrum under *Infrared Spectra of Essential Oils*, page 612, using the same test conditions as specified therein.

Assay Not less than 98.0% of methyl salicylate ($C_8H_8O_3$).
Acid Value Not more than 1.0.
Angular Rotation Slightly levorotatory, exhibiting a rotation of not more than −1.5°.
Heavy Metals (as Pb) Passes test.
Refractive Index Between 1.535 and 1.538 at 20°.
Solubility in Alcohol Passes test.
Specific Gravity Between 1.176 and 1.182.

TESTS

Assay Weigh accurately about 2 g, and proceed as directed under *Ester Determination*, page 500, using 76.08 as the equivalence factor (*e*) in the calculation. Modify the procedure by using 50.0 ml of 0.5 *N* alcoholic potassium hydroxide and by refluxing on the steam bath for 2 h.
Acid Value Determine as directed in the general method, page 499, using bromocresol purple TS instead of phenolphthalein TS as the indicator.
Angular Rotation Determine in a 100-mm tube as directed under *Optical Rotation*, page 530.
Heavy Metals Shake 10 ml of the oil with an equal volume of water to which 1 drop of hydrochloric acid has been added, and pass hydrogen sulfide through the mixture until it is saturated. No darkening in color is produced in either the oil or the water.
Refractive Index, page 530 Determine with an Abbé or other refractometer of equal or greater accuracy.
Solubility in Alcohol Proceed as directed in the general method, page 502. One ml dissolves in 7 ml of 70% alcohol. The solution may have not more than a slight cloudiness.

Specific Gravity Determine by any reliable method (see page 3).

Packaging and Storage Store in full, tight containers in a cool place protected from light.

Functional Use in Foods Flavoring agent.

Xanthan Gum

DESCRIPTION

A high-molecular-weight polysaccharide gum produced by a pure-culture fermentation of a carbohydrate with *Xanthomonas campestris*, purified by recovery with isopropyl alcohol, dried, and milled. It contains D-glucose and D-mannose as the dominant hexose units, along with D-glucuronic acid, and is prepared as the sodium, potassium, or calcium salt. It occurs as a cream-colored powder that is readily soluble in hot or cold water. Its solutions are neutral.

REQUIREMENTS

Identification

To 300 ml of water, previously heated to 80° and stirred rapidly with a mechanical stirrer in a 400-ml beaker, add, at the point of maximum agitation, a dry blend of 1.5 g of the sample and 1.5 g of locust bean gum. Stir until the mixture goes into solution, and then continue stirring for 30 min longer. Do not allow the water temperature to drop below 60° during stirring. Discontinue stirring, and allow the mixture to cool at room temperature for at least 2 h. A firm rubbery gel forms after the temperature drops below 40°, but no such gel forms in a 1% control solution of the sample prepared in the same manner but omitting the locust bean gum.

Assay It yields, on the dry basis, not less than 4.2% and not more than 5.0% of carbon dioxide (CO_2), corresponding to between 91.0% and 108.0% of xanthan gum.

Arsenic (as As) Not more than 3 ppm.

Ash Between 6.5% and 16%.

Heavy Metals (as Pb) Not more than 0.003%.

Isopropyl Alcohol Not more than 0.075%.

Lead Not more than 5 ppm.

Loss on Drying Not more than 15%.

Pyruvic Acid Not less than 1.5%.

Viscosity Passes test.

TESTS

Assay Proceed as directed under *Alginates Assay*, page 463, but use an undried sample of about 1.2 g, accurately weighed.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Ash Weigh accurately about 3 g, previously dried at 105° for 4 h, in a tared crucible, and incinerate at about 650° until free from carbon. Cool the crucible and its contents in a desiccator, weigh, and determine the weight of the ash.

Heavy Metals Prepare and test a 500-mg sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using a platinum crucible for the ignition and 15 µg of lead ion (Pb) in the control (*Solution A*).

Isopropyl Alcohol

IPA Standard Solution Transfer 500.0 mg of chromatographic quality isopropyl alcohol into a 50-ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

TBA Standard Solution Transfer 500.0 mg of chromatographic quality *tert*-butyl alcohol into a 50-ml volumetric flask, dilute to volume with water, and mix. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with water, and mix.

Mixed Standard Solution Pipet 4 ml each of the *IPA Standard Solution* and of the *TBA Standard Solution* into a 125-ml graduated Erlenmeyer flask, dilute to about 100 ml with water, and mix. This solution contains approximately 40 µg each of isopropyl alcohol and of *tert*-butyl alcohol per ml.

Sample Preparation Disperse 1 ml of a suitable antifoam emulsion, such as Dow-Corning G-10 or equivalent, in 200 ml of water contained in a 1000-ml 24/40 round-bottom distilling flask. Add about 5 g of the sample, accurately weighed, and shake for 1 h on a wrist-action mechanical shaker. Connect the flask to a fractionating column, and distil about 100 ml, adjusting the heat so that foam does not enter the column. Add 4.0 ml of *TBA Standard Solution* to the distillate to obtain the *Sample Preparation*.

Procedure Inject about 5 µl of the *Mixed Standard Solution* into a suitable gas chromatograph equipped with a flame-ionization detector and a 1.8-m × 3.2-mm stainless steel column packed with 80/100-mesh Porapak QS or equivalent. The carrier is helium flowing at 80 ml per min. The injection port temperature is 200°, the column temperature is 165°, and the detector temperature is 200°. The retention time of isopropyl alcohol is about 2 min, and that of *tert*-butyl alcohol about 3 min.

Determine the areas of the IPA and TBA peaks, and calculate the response factor, f, by the formula A_{IPA}/A_{TBA}, in which A_{IPA} is the area of the isopropyl alcohol peak, and A_{TBA} is the area of the *tert*-butyl alcohol peak.

Similarly, inject about 5 µl of the *Sample Preparation*, and determine the peak areas, recording the area of the isopropyl alcohol peak as a_{IPA}, and that of the *tert*-butyl alcohol peak as a_{TBA}. Calculate the isopropyl alcohol content, in ppm, in the sample taken by the formula

$$(a_{IPA} \times 4000)/(f \times a_{TBA} \times W),$$

in which W is the weight of the sample taken, in g.

Lead A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 5 µg of lead ion (Pb) in the control.

Loss on Drying, page 518 Dry at 105° for 2.5 h.

Pyruvic Acid

Sample Preparation Dissolve 600.0 mg of the sample, accurately weighed, in sufficient water to make 100.0 ml, and transfer 10.0 ml of the solution into a 50-ml glass-stoppered flask. Pipet 20 ml of 1 *N* hydrochloric acid into the flask, weigh the flask, and reflux for 3 h, taking precautions to prevent loss of vapors. Cool to room temperature, and add water to make up for any weight loss during refluxing. Pipet 1.0 ml of a 1 in 200 solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid into a 30-ml separator, then add 2.0 ml of the sample solution, mix, and allow to stand at room temperature for 5 min. Extract the mixture with 5 ml of ethyl acetate, and discard the aqueous layer. Extract the hydrazone from the ethyl acetate with three 5-ml portions of sodium carbonate TS, collecting the extracts in a 50-ml volumetric flask. Dilute to volume with sodium carbonate TS, and mix.

Standard Preparation Transfer 45.0 mg of pyruvic acid, accurately weighed, into a 500-ml volumetric flask, dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a 50-ml glass-stoppered flask, and continue as directed under *Sample Preparation*, beginning with "Pipet 20 ml of 1 *N* hydrochloric acid into the flask. . . . "

Procedure Determine the absorbance of each solution with a suitable spectrophotometer in 1-cm cells at the maximum at about 375 nm, using sodium carbonate TS as the blank. The absorbance of the *Sample Preparation* is equal to or greater than that of the *Standard Preparation*.

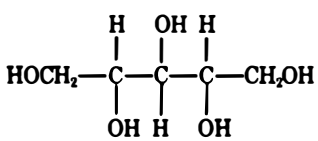
Viscosity Prepare two identical solutions, each containing 1% of the sample and 1% of potassium chloride in water, and stir for 2 h. Determine the viscosity of one solution at 23.9° (75°F) as directed under *Viscosity of Sodium Carboxymethylcellulose*, page 550, using a No. 3 spindle rotating at 60 rpm. The viscosity (V_1) thus determined is not less than 600 centipoises. Determine the viscosity (V_2) of the other solution in the same manner, but maintain the temperature at 65.6° (150°F). The ratio of the viscosities, V_1/V_2, is between 1.02 and 1.45.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Stabilizer; thickener; emulsifier; suspending agent; bodying agent; foam enhancer.

Xylitol

1,2,3,4,5-Pentahydroxypentane



$C_5H_{12}O_5$ Mol wt 152.15

DESCRIPTION

White crystals or crystalline powder. It has a sweet taste and produces a cooling sensation in the mouth. One g dissolves in about 0.65 ml of water. It is sparingly soluble in alcohol. Crystalline xylitol has a melting range between 92° and 96°.

REQUIREMENTS

Identification

A. Dissolve 5 g of the sample in 10 ml of a mixture of equal volumes of hydrochloric acid and formalin, and allow to react at 50° for 2 h. Add 25 ml of ethanol, collect the crystals produced, and dissolve them in 10 ml of water by heating. Add 50 ml of ethanol in order to crystallize, collect the separated crystals by filtration, recrystallize them from ethanol twice, and dry at 105° for 2 h. The crystals melt between 195° and 201°.

B. The infrared absorption spectrum of a potassium bromide dispersion of the sample, at a concentration of 1 mg per 300 mg, exhibits maxima only at the same wavelengths as that of a similar preparation of FCC Xylitol Reference Standard. If a difference appears, dissolve portions of both the sample and the reference standard in a suitable solvent, evaporate the solutions to dryness, and repeat the test on the residues.

Assay Not less than 98.5% and not more than the equivalent of 101.0% of $C_5H_{12}O_5$ after drying.

Arsenic (as As) Not more than 3 ppm.

Heavy Metals (as Pb) Not more than 10 ppm.

Loss on Drying Not more than 0.5%.

Other Polyols Not more than 2.0%.

Reducing Sugars (as glucose) Not more than 0.2%.

Residue on Ignition Not more than 0.5%.

TESTS

Assay

Internal Standard Solution Transfer about 500 mg of octadecane (C_{18} hydrocarbon), accurately weighed, into a 25-ml volumetric flask, dissolve in and dilute to volume with heptane, and mix.

Standard Preparation Transfer about 50 mg of FCC Xylitol Reference Standard, accurately weighed, into a 25-ml volumetric flask, add 1 ml of pyridine, and dissolve the xylitol by heating on a steam bath. Cool to room temperature, add 0.2 ml of hexamethyldisilazane (HMDS) and 0.1 ml of trimethylchlorosilane (TMCS), and allow to stand at room temperature for 30 min. Add 5.0 ml of *Internal Standard Solution*, dilute to volume with heptane, and mix.

Sample Preparation Transfer about 50 mg of the sample, previously dried in vacuum over phosphorus pentoxide at 60° for 4 h and accurately weighed, into a 25-ml volumetric flask, add 1 ml of pyridine, and dissolve the sample by heating on a steam bath. Cool to room temperature, add 0.2 ml of HMDS and 0.1 ml of TMCS, and allow to stand at room temperature for 30 min. Add 5.0 ml of *Internal Standard Solution*, dilute to volume with heptane, and mix.

Chromatographic System Under typical conditions, the gas chromatograph is equipped with a flame-ionization

detector and contains a 6-ft × 1/8-in. od stainless steel column packed with 20% methylpolysiloxane gum on 60/80-mesh acid-base washed silanized chromatographic diatomaceous earth, utilizing on-column injection. The column is maintained isothermally at 190°, and the flow rate of the carrier gas is adjusted to obtain the xylitol TMS peak about 13 min after sample injection. The column is conditioned by making several 10-µl injections of the *Standard Preparation* until constant detector response is obtained.

Procedure Inject a 10-µl portion of the *Standard Preparation* into the chromatograph, and record the areas of the xylitol TMS and octadecane peaks as A_x and A_o, respectively. Calculate the xylitol response ratio, *RR*, by the formula

$$(A_x \times C_o)/(A_o \times C_x),$$

in which C_o and C_x are the respective concentrations, in mg per ml, of octadecane and FCC Xylitol Reference Standard in the *Standard Preparation*. Similarly, inject a 10-µl portion of the *Sample Preparation*, and record the areas of the xylitol TMS and octadecane peaks as a_x and a_o, respectively. Calculate the quantity, in mg, of xylitol in the sample taken by the formula

$$(a_x \times C_o \times 25)/(a_o \times RR).$$

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

Heavy Metals A solution of 2 g in 25 ml of water meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

Loss on Drying, page 518 Dry about 0.5 g in vacuum over phosphorus pentoxide at 60° for 4 h.

Other Polyols

Standard Mixture Weigh accurately about 25 mg each of mannitol, dulcitol, arabitol, and sorbitol, and 100 mg of FCC Xylitol Reference Standard, and transfer into separate 10-ml volumetric flasks. Into each flask pipet 0.2 ml of anhydrous pyridine and 1 ml of acetic anhydride, and heat the flasks on a hot plate for 30 min at the lowest temperature so that the contents do not boil over. Allow the flasks to cool, and then dilute the mannitol, dulcitol, arabitol, and sorbitol flasks to volume with acetone. Accurately transfer 0.2 ml each of the mannitol and sorbitol solutions, and 0.08 ml of the arabitol and dulcitol solutions, into the FCC Xylitol Reference Standard flask, dilute to volume with acetone, and mix.

Test Preparation Transfer about 100 mg of the sample, accurately weighed, into a 10-ml volumetric flask, add 0.2 ml of anhydrous pyridine and 1 ml of acetic anhydride, and heat on a hot plate at low temperature for 30 min. Allow to cool, then dilute to volume with acetone, and mix.

Chromatographic System Under typical conditions, the gas chromatograph is equipped with a flame-ionization detector and contains a 3-ft × 1/8-in. od stainless steel column packed with 5% ethylenesuccinate-cyanoethylsilicone copolymer or 10% cyanoethylsilicone on 80/100-mesh acid-base washed silanized chromatographic diatomaceous earth, utilizing either a glass-lined sample introduction system or on-column injection. The column is maintained isothermally at a temperature of 170° or is adjusted accordingly to obtain a retention time of 13 min for xylitol pentaacetate. The flow rate of the carrier gas is adjusted to obtain the xylitol pentaacetate peak about 13 min after sample introduction. Condition the column by making several 10-µl injections of the *Standard Mixture* until a constant response is obtained.

Procedure Inject a suitable portion (i.e., 10 µl) of the *Standard Mixture* into the chromatograph, and record the chromatogram so as to obtain at least 20% of the maximum recorder response for mannitol at the 0.5% level. The approximate retention times for the components of the *Standard Mixture* are: arabitol hexaacetate, 8 min; xylitol pentaacetate, 13 min; mannitol hexaacetate, 30 min; dulcitol hexaacetate, 22 min; and sorbitol hexaacetate, 26 min. Measure the peak area, A_S, for each of the polyols in the *Standard Mixture*. Similarly, inject a suitable portion of the *Test Preparation*, and record the chromatogram. Measure the peak area of each known polyol as A_K, and of each unknown polyol as A_U. Calculate the percentage of each known polyol by the formula

$$(A_K \times W \times 100D)/(A_S \times w),$$

in which W is the weight, in mg, of mannitol, dulcitol, sorbitol, or arabitol in the *Standard Mixture*; w is the weight, in mg, of the sample taken; and D is the dilution factor: 0.02 for mannitol and sorbitol, and 0.008 for dulcitol and arabitol. Calculate the percentage of each unknown polyol versus the mannitol peak by the formula

$$(A_U \times W \times 2)/(A_S \times w).$$

Reducing Sugars Dissolve about 500 mg of the sample, accurately weighed, in 2 ml of water in a 10-ml conical flask, and add 2 ml of a dextrose solution, containing 0.5 mg per ml, to another conical flask. Add 1 ml of Fehling's Solution A and of Fehling's Solution B (see *Cupric Tartrate TS, Alkaline*, page 570) to each flask, heat to boiling, and cool. The sample solution is less turbid than the dextrose solution, which forms a reddish brown precipitate.

Residue on Ignition, page 533 Ignite 2 g as directed in the general method.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutritive sweetener.

Zinc Gluconate

$$[CH_2OH(CHOH)_4COO]_2Zn$$

$C_{12}H_{22}O_{14}Zn$ Mol wt 455.68

DESCRIPTION

Zinc gluconate is anhydrous or contains three molecules of water of hydration. It occurs as a white or nearly white, granular or crystalline powder. It is freely soluble in water and very slightly soluble in alcohol.

REQUIREMENTS

Identification

A. A 1 in 10 solution gives positive tests for *Zinc*, page 517.

B. To 5 ml of a warm solution (1 in 10) add 0.7 ml of glacial acetic acid and 1 ml of freshly distilled phenylhydrazine, heat on a steam bath for 30 min, and allow to cool. Induce crystallization by scratching the inner surface of the container with a glass stirring rod. Crystals of gluconic acid phenylhydrazide form.

Assay Not less than 97.0% and not more than 102.0% of $C_{12}H_{22}O_{14}Zn$, calculated on the anhydrous basis.

Arsenic (as As) Not more than 3 ppm.

Cadmium Not more than 5 ppm.

Chloride Not more than 0.05%.

Lead Not more than 10 ppm.

Reducing Substances Not more than 1.0%.

Sulfate Not more than 0.05%.

Water *Anhydrous:* not more than 9.0%; *trihydrate:* not more than 11.6%.

TESTS

Assay Dissolve about 700 mg of the sample, accurately weighed, in 100 ml of water, warming if necessary, and add 5 ml of ammonia-ammonium chloride buffer TS and 0.1 ml of eriochrome black TS. Titrate with 0.05 *M* disodium EDTA until the solution is blue in color. Each ml of 0.05 *M* disodium EDTA is equivalent to 22.78 mg of $C_{12}H_{22}O_{14}Zn$.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Cadmium Determine as directed in the test for *Cadmium* under *Zinc Sulfate*, page 351.

Chloride, page 471 Any turbidity produced by a 40-mg sample does not exceed that shown in a control containing 20 µg of chloride ion (Cl).

Lead The following method has been found to be satisfactory when the particular atomic absorption spectrophotometer specified is used. The method may be modified as necessary for use with other suitable atomic absorption spectrophotometers capable of determining lead in the sample at the limit specified.

Standard Preparation Transfer 10.0 ml of *Lead Nitrate Stock Solution* (see page 512) into a 500-ml volumetric flask, dilute to volume with water, and mix. This solution should be prepared on the day of use. Each ml contains the equivalent of 2 µg of lead ion (Pb).

Sample Preparation Transfer about 10 g of the sample, accurately weighed, into a 100-ml volumetric flask, previously rinsed with nitric acid and water. Dissolve in 75 ml of water, warming if necessary, add 2 ml of nitric acid, dilute to volume with water, and mix.

Procedure Using a Perkin-Elmer 403 atomic absorption spectrophotometer equipped with a deuterium arc background corrector, a digital readout device, and a burner head capable of handling 10% solids, blank the instrument with water following the manufacturer's operating instructions. Aspirate a portion of the *Standard Preparation*, and record the absorbance as A_S; then aspirate a portion of the *Sample Preparation*, and record the absorbance as A_U. Calculate the lead content, in ppm, of the sample taken by the formula

$$100 \times (C/W) \times (A_U/A_S),$$

in which C is the exact concentration of Pb in the *Standard Preparation*, in µg per ml, and W is the weight of the sample taken, in g.

Reducing Substances Transfer about 1 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, dissolve in 10 ml of water, and add 25 ml of alkaline cupric citrate TS. Cover the flask with a small beaker, boil gently for exactly 5 min, and cool rapidly to room temperature. Add 25 ml of a 1 in 10 solution of acetic acid, 10.0 ml of 0.1 *N* iodine, 10 ml of diluted hydrochloric acid TS, and 3 ml of starch TS, and titrate with 0.1 *N* sodium thiosulfate to the disappearance of the blue color. Calculate the weight, in mg, of reducing substances (as D-glucose) by the formula

$$27 \times (V_1N_1 - V_2N_2),$$

in which 27 is an empirically determined equivalence factor for D-glucose; V_1 and N_1 are the volume, in ml, and the normality of the iodine solution, respectively; and V_2 and N_2 are the volume, in ml, and the normality of the sodium thiosulfate solution, respectively.

Sulfate, page 471 Any turbidity produced by a 500-mg sample does not exceed that in a control containing 250 µg of sulfate ion (SO_4).

Water Determine by the *Karl Fischer Titrimetric Method*, page 552.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Nutrient; dietary supplement.

Zinc Oxide

ZnO Mol wt 81.38

DESCRIPTION

A fine, white, odorless amorphous powder. It gradually absorbs carbon dioxide from the air. It is insoluble in water and in alcohol, and is soluble in dilute acids and in strong bases.

REQUIREMENTS

Identification

A. When strongly heated, it assumes a yellow color that disappears on cooling.

B. A solution of the sample in a slight excess of 3 *N* hydrochloric acid gives positive tests for *Zinc*, page 517.

Assay Not less than 99.0% of ZnO after ignition.

Alkalinity Passes test.

Arsenic (as As) Not more than 3 ppm.
Cadmium Not more than 10 ppm.
Lead Not more than 10 ppm.
Loss on Ignition Not more than 1.0%.
Substances Not Precipitated by Sulfide Not more than 0.5%.

TESTS

Assay Dissolve about 1.5 g of freshly ignited sample, accurately weighed, and 2.5 g of ammonium chloride in 50.0 ml of 1 *N* sulfuric acid with the aid of gentle heat, if necessary. When solution is complete, add methyl orange TS, and titrate the excess sulfuric acid with 1 *N* sodium hydroxide. Each ml of 1 *N* sulfuric acid is equivalent to 40.69 mg of ZnO.

Alkalinity Suspend 2 g in 20 ml of water, boil for 1 min, filter, and add 0.1 ml of phenolphthalein TS to the filtrate. No red color is produced.

Arsenic A suspension of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Cadmium Determine as directed in the test for *Cadmium* under *Zinc Sulfate*, this page, using the following as the *Sample Solution*: Transfer 5 g of the sample, accurately weighed, into a 50-ml volumetric flask, dissolve in a minimum volume of dilute hydrochloric acid (2 in 3), dilute to volume with water, and mix.

Lead Suspend 2 g in 5 ml of water, add just enough hydrochloric acid to dissolve, and add a few drops of nitric acid. Add ammonium hydroxide dropwise until a faint but permanent precipitate forms. Clear the turbidity with a few drops of 10% nitric acid, and dilute to 40 ml with water. Add 20 ml of this solution to 25 ml of a 10% sodium cyanide solution, stirring constantly during the addition. If a precipitate persists, add solid sodium cyanide until the solution remains clear. Dilute to 50 ml with 10% sodium cyanide solution, add 0.2 ml of sodium sulfide TS, mix, and transfer into a Nessler tube. After 5 min, any color does not exceed that produced by 1.0 ml of *Standard Lead Solution* (10 µg Pb ion), page 512, in an equal volume of solution containing the quantities of reagents used in the test.

Loss on Ignition Weigh accurately about 2 g, and ignite at 800° ± 25° to constant weight.

Substances Not Precipitated by Sulfide Transfer 2 g, accurately weighed, to a 200-ml volumetric flask, dissolve in 20 ml of dilute acetic acid (1 in 4), dilute to volume with water, and mix. Precipitate the zinc completely with ammonium sulfide TS, dilute to volume with water, and mix. Filter through a dry filter, discarding the first portion of filtrate, and collect 100 ml of the subsequent filtrate. Add a few drops of sulfuric acid, and evaporate to dryness on a steam bath in a tared dish. Ignite cautiously until the ammonium salts are volatilized, ignite to constant weight at 800° ± 25°, cool, and weigh. The weight of the residue does not exceed 5 mg.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Nutrient; dietary supplement.

Zinc Sulfate

$ZnSO_4.xH_2O$ Mol wt (anhydrous) 161.44

DESCRIPTION

Zinc sulfate contains one or seven molecules of water of hydration. It occurs as colorless, transparent prisms or small needles, or as a granular, crystalline powder. It is odorless. The monohydrate loses water at temperatures above 238°, whereas the heptahydrate effloresces in dry air at room temperature. Its solutions are acid to litmus. The monohydrate is soluble in water and practically insoluble in alcohol. One g of the heptahydrate dissolves in about 0.6 ml of water and in about 2.5 ml of glycerin; it is insoluble in alcohol.

REQUIREMENTS

Identification

A 1 in 20 solution gives positive tests for *Zinc*, page 517, and for *Sulfate*, page 517.

Assay *Monohydrate:* not less than 99.0% and not more than 100.5% of $ZnSO_4.H_2O$; *heptahydrate:* not less than 99.0% and not more than 108.7% of $ZnSO_4.7H_2O$.
Acidity Passes test.
Alkalies and Alkaline Earths Not more than 0.5%.
Arsenic (as As) Not more than 3 ppm.
Cadmium Not more than 5 ppm.
Lead Not more than 10 ppm.
Mercury Not more than 5 ppm.
Selenium Not more than 0.003%.

TESTS

Assay Dissolve about 175 mg of the monohydrate, or about 300 mg of the heptahydrate, accurately weighed, in 100 ml of water, add 5 ml of ammonia-ammonium chloride buffer TS and 0.1 ml of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA until the solution is deep blue in color. Each ml of 0.05 *M* disodium EDTA is equivalent to 8.072 mg of $ZnSO_4.H_2O$, or 14.38 mg of $ZnSO_4.7H_2O$.

Acidity A 1 in 20 solution is not colored pink by methyl orange TS.

Alkalies and Alkaline Earths Transfer a 2-g sample to a 200-ml volumetric flask, dissolve in about 150 ml of water, and precipitate the zinc completely with ammonium sulfide TS. Dilute to volume with water, and mix. Filter through a dry filter, rejecting the first portion of the filtrate, and add a few drops of sulfuric acid to 100 ml of the subsequent filtrate. Evaporate to dryness in a tared dish, ignite to constant weight, cool, and weigh. The weight of the residue does not exceed 5 mg.

Arsenic A solution of 1 g in 35 ml of water meets the requirements of the *Arsenic Test*, page 464.

Cadmium

Spectrophotometer Use any suitable atomic absorption

spectrophotometer equipped with a Boling-type burner, an air-acetylene flame, and a hollow-cathode cadmium lamp. The instrument should be capable of operating within the sensitivity necessary for the determination.

Standard Solution Transfer 100 mg of cadmium chloride crystals ($CdCl_2.2\text{-}1/2H_2O$), accurately weighed, into a 1000-ml volumetric flask, dissolve in and dilute to volume with water, and mix. Pipet 25 ml of this solution into a 100-ml volumetric flask, add 1 ml of hydrochloric acid, dilute to volume with water, and mix. Each ml contains 12.5 µg of Cd.

Sample Solution Transfer 10 g of the sample, accurately weighed, into a 50-ml volumetric flask, dissolve in and dilute to volume with water, and mix.

Procedure Transfer 5.0 ml of the *Sample Solution* into each of five separate 25-ml volumetric flasks. Dilute *Flask 1* to volume with water, and mix. To *Flasks 2*, *3*, *4*, and *5* add 1.00, 2.00, 3.00, and 4.00 ml of the *Standard Solution*, respectively, then dilute each flask to volume with water, and mix. Determine the absorbance of each solution at 228.8 nm, setting the instrument to previously established optimum conditions. Plot absorbance versus µg added Cd per 25 ml, and extrapolate to zero absorbance. Read the (negative) concentration of Cd in *Flask 1*, which equals the ppm of Cd in the sample taken.

Lead Dissolve 500 mg of the sample in 5 ml of water, transfer to a color-comparison tube (*A*), add 10 ml of potassium cyanide solution (1 in 10), mix, and allow the mixture to become clear. In a similar matched color-comparison tube (*B*) place 5 ml of water, and add 0.50 ml of *Standard Lead Solution* (5 µg of Pb) and 10 ml of potassium cyanide solution (1 in 10). To each solution add 0.1 ml of sodium sulfide TS, mix, and allow to stand for 5 min. When viewed downward over a white surface, the solution in tube *A* is no darker than that in tube *B*.

Mercury Determine as directed under *Mercury Limit Test*, page 520, using the following as the *Sample Preparation*: Dissolve 400 mg of the sample in 10 ml of water in a small beaker, add 1 ml of dilute sulfuric acid solution (1 in 5) and 1 ml of potassium permanganate solution (1 in 25), cover the beaker, boil for a few seconds, and cool.

Selenium Determine as directed in *Method I* under the *Selenium Limit Test*, page 537, using 200 mg of sample.

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutrient; dietary supplement.

3 / *Specifications for Flavor Aromatic Chemicals and Isolates*

As indicated in the preface to this edition, page xvii, specifications for flavoring agents, other than the essential oils, are presented in tabular form rather than as separate monographs. Specifications for all such ingredients from the *Food Chemicals Codex*, Second Edition, together with a number of new specifications, are provided on the following pages of this section.

EXPLANATORY NOTES TO TABULAR SPECIFICATIONS

The FCC title of the substance is followed by its synonym(s) in parentheses and, where available, by the number assigned to the substance by the Flavor and Extract Manufacturers Association (FEMA) in brackets. The explanatory notes to the specifications in this section apply throughout the tabular series and are as follows:

Note 1 (Solubility) Approximate solubilities (see page 4) are indicated by the abbreviations: *vs* = very soluble; *s* = soluble; *ss* = slightly soluble; *ins* = insoluble or practically insoluble; *m* = miscible. Other abbreviations are: alc = alcohol; gly = glycerin; org = organic; prop = propylene (as in propylene glycol); veg = vegetable (as in vegetable oil); vol = volatile.

Note 2 (B.P.) Boiling points (B.P.) are expressed in °C. They are approximate values given for information only and not as requirements.

Note 3 (GLC Profile) The notation "provided" in this column indicates that gas-liquid chromatographic analysis data have been developed for the particular substance by the Essential Oil Association (EOA) and that the results of such analyses are provided in *Section 5*, beginning on page 439. These data are for information only and are not intended to be, nor should they be regarded as, requirements.

Note 4 (I.D.) The notation "IR" in the identification (I.D.) column indicates that an infrared absorption spectrum is provided for the particular substance in *Section 9*, beginning on page 583. Where the IR requirement is specified, the infrared absorption spectrum of the sample shall exhibit maxima at the same wavelengths (or frequencies) as those shown in the respective spectrum in *Section 9*, using the test conditions as specified therein.

Note 5 (Assay) Assay requirements are specified as *minimum* values (unless a range of assay values is given) and are stated in weight percent, unless otherwise indicated. References to assay methods are indicated by citations in parentheses, e.g., "(M-5a)," to methods provided in *Section 4*, beginning on page 421.

Note 6 (A.V.) Unless otherwise indicated, determine the acid value (A.V.) as directed in the general method, page 499, in *Section 6* under *Essential Oils and Flavors*, using phenolphthalein TS as indicator unless another indicator is specified for an individual substance. Where *Method II* is specified, determine the acid value as directed in the general method on page 504 under *Fats and Related Substances*.

Note 7 (Ref. Index) Refractive index determinations are made at 20°, unless another temperature is specified, according to the general method, page 533.

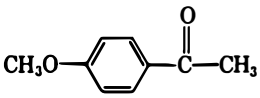
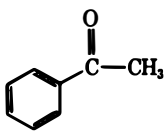
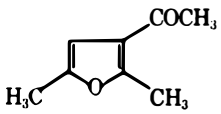
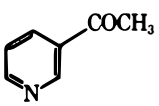
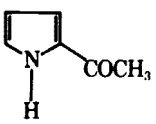
Note 8 (Solubility in Alcohol) Determine the solubility in alcohol at 25° as directed in the general method, page 502.

Note 9 (Sp. Gr.) Determine the specific gravity (Sp. Gr.) at 25°, unless otherwise specified, by any reliable method (see page 3).

Note 10 (Other Requirements) Numerical limits for other requirements are specified as *maximum* values, unless otherwise indicated (max = maximum; NLT = not lower than or not less than, as appropriate). Test methods are indicated by citations in parentheses, which refer either to methods given in *Section 4*, e.g., "M-15b," or to general methods in *Section 6* (indicated by appropriate page references to the chapter on *Essential Oils and Flavors*, beginning on page 499).

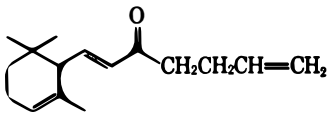
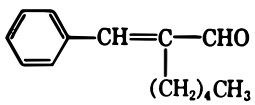
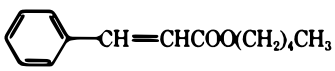
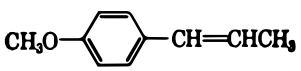
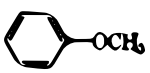
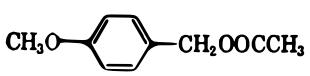
Unless specifically prohibited by a notation in this column, the flavor aromatic chemicals and isolates listed herein may contain a suitable antioxidant (see Added Substances, page 5). If an antioxidant is used, it shall be named on the label of the substance to which it is added, and its percentage shall be indicated.

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility [1]/B.P.[2]	GLC Profile [3]
Acetaldehyde (Ethanal) [FEMA No. 2003]	44.05/C_2H_4O/ CH_3CHO	flammable, colorless liq/pungent, ethereal	*m*—water, alc, org solvents/21°	
Acetanisole (*p*-Methoxyacetophenone) [FEMA No. 2005]	150.18/$C_9H_{10}O_2$/ 	colorless to pale yel, fused solid/hawthorn-like	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Acetic Acid [see p. 8]				
Acetoin (Acetyl Methyl Carbinol; Dimethylketol; 3-Hydroxy-2-butanone) [FEMA No. 2008]	88.11/$C_4H_8O_2$/ $CH_3CH(OH)COCH_3$	colorless to pale yel liq (monomer), or white cryst powder (dimer)/buttery	*m*—alc, prop glycol, water; *ins*—veg oils/148°	
Acetophenone [FEMA No. 2009]	120.15/C_8H_8O/ 	practically colorless liq above 20°/very sweet, pungent	*vs*—prop glycol, most fixed oils; *s*—alc, chloroform, ether; *ss*—water; *ins*—gly	provided
3-Acetyl-2,5-dimethyl Furan (2,5-Dimethyl-3-acetylfuran) [FEMA No. 3391]	138.16/$C_8H_{10}O_2$/ 	yel liq/powerful, slightly roasted, nutlike	*s*—alc, prop glycol, most fixed oils; *ss*—water	
3-Acetylpyridine [FEMA No. 3424]	121.14/C_7H_7NO/ 	colorless to yel liq/ sweet, nutty, like popcorn	*s*—acids, alc, ether, water/230°	
2-Acetylpyrrole (Methyl 2-Pyrrolyl Ketone) [FEMA No. 3202]	109.12/C_6H_7NO/ 	light beige to yellowish, fine crystals/bready		
Allyl Cyclohexanepropionate (Allyl-3-cyclohexanepropionate) [FEMA No. 2026]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ pineapplelike	*m*—alc, chloroform, ether; *ins*—gly, water	provided
Allyl Hexanoate (Allyl Caproate) [FEMA No. 2032]	156.22/$C_9H_{16}O_2$/ $CH_3(CH_2)_4COOCH_2CH=CH_2$	colorless to light yel liq/strong, pineapple-like	*m*—alc, most fixed oils; *ins*—prop glycol, water/185°	provided

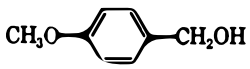
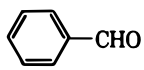
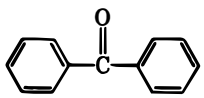
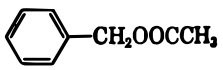
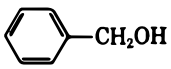
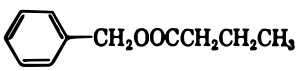
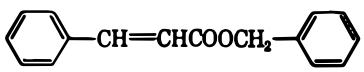
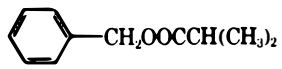
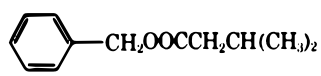
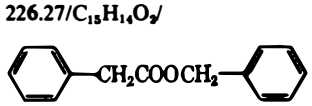
	Requirements					
I.D. Test [4]	Assay Min, % [5]	A.V. Max [6]	Ref. Index [7]	Solubility in Alcohol [8]	Sp. Gr. [9]	Other Requirements [10]
IR	99.0% of C_2H_4O (M-5a)				0.804–0.811 (0°/20°)	**Acidity**—0.1% (M-15b); **Res. on Evap.**—0.006% (p. 502, 80-g samp)
IR	98.0% of $C_9H_{10}O_2$ (M-3)			1 g in 5 ml 50% alc		**Arsenic**—3 ppm (M-18); **Chlorinated Cmpds.**—passes test (p. 500); **Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27)
IR	96.0% of $C_4H_8O_2$ (M-8c)		1.417–1.420		1.005–1.019	
IR	98.0% of C_8H_8O (M-3)		1.533–1.535	1 ml in 5 ml 50% alc	1.025–1.028	**Chlorinated Cmpds.**—passes test (p. 500); **Solidification Pt.**—NLT 19° (p. 538)
IR	99.8% of $C_8H_{10}O_2$ (M-8a)		1.475–1.496 (25°)		1.027–1.048	**Angular Rotation**—between −1° and +1° (p. 530)
IR	98.0% of C_7H_7NO (M-8a)		1.530–1.540 (25°)		1.100–1.115 (20°)	**Water**—0.5% (p. 552, *KF*)
	98.0% of C_8H_7NO (M-8a)					**Arsenic**—3 ppm (M-18); **Heavy Metals**—10 ppm (M-24a); **Melting Range**—85° to 90° (p. 519); **Res. on Ignit.**—0.3% (p. 533); **Water**—0.5% (p. 552, *KF*)
IR	98.0% of $C_{12}H_{20}O_2$ (M-6)	5.0	1.457–1.462	1 ml in 4 ml 80% alc	0.945–0.950	
IR	98.0% of $C_9H_{16}O_2$ (M-6)	1.0	1.422–1.426	1 ml in 3.5 ml 70% alc	0.884–0.890	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Allyl α-Ionone (Allyl Ionone) [FEMA No. 2033]	232.37/$C_{16}H_{24}O$/ 	colorless to yel liq/fruity, woody	*s*—alc; *ins*—water/ 265°	
Allyl Isothiocyanate (Mustard Oil, Volatile) [FEMA No. 2034]	99.15/C_3H_5NCS/ $CH_2=CH-CH_2-N=C=S$	colorless to pale yel, strongly refractive liq/very pungent irritating odor, acrid taste, mustardlike [*Caution:* lachrymator]	*m*—alc, carbon disulfide, ether	
α-Amylcinnamaldehyde (Amylcinnamaldehyde) [FEMA No. 2061]	202.30/$C_{14}H_{18}O$/ 	yel liq/strong, floral, like jasmine on dilution, spicy	*s*—most fixed oils; *ins*—gly, prop glycol	provided
Amyl Cinnamate (Isoamyl Cinnamate; Isoamyl 3-Phenyl Propenate) [FEMA No. 2063]	218.28/$C_{14}H_{18}O_2$ 	colorless to pale yel liq/faint, balsamic, cocoalike	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/310°	provided
Amyl Octanoate (Isoamyl Octanoate; Isoamyl Caprylate) [FEMA No. 2079]	214.34/$C_{13}H_{26}O_2$/ $CH_3(CH_2)_6COOC_5H_{11}$	colorless liq/ fruity	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/260°	
Amyl Propionate (Isoamyl Propionate) [FEMA No. 2082]	144.21/$C_8H_{16}O_2$/ $CH_3CH_2COOC_5H_{11}$	colorless liq/fruity, apricot-pineapple	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/160°	provided
Anethole (*p*-Propenylanisole) [FEMA No. 2086]	148.20/$C_{10}H_{12}O$/ 	colorless to faintly yel liq at or above 23°; sweet taste; affected by light/ aniselike	*m*—chloroform, ether; *ss*—water	
Anisole (Methylphenyl Ether) [FEMA No. 2097]	108.14/C_7H_8O/ 	colorless liq/ phenolic, aniselike	*s*—alc, ether; *ins*—water	
Anisyl Acetate (*p*-Methoxybenzyl Acetate) [FEMA No. 2098]	180.20/$C_{10}H_{12}O_3$/ 	colorless to slightly yel liq/floral, fruity, balsamic	*s*—alc, most fixed oils; *ins*—gly, prop glycol	provided

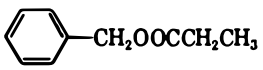
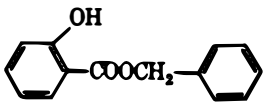
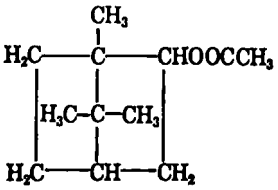
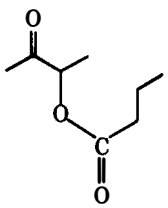
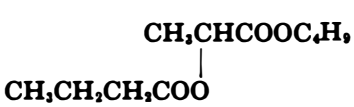
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	88.0% of $C_{16}H_{24}O$ (M-3)		1.503–1.507	1 ml in 8 ml 70% alc gives clear soln	0.928–0.935	
IR	93.0% of C_3H_5NCS (M-11e)		1.527–1.531		1.013–1.020	**Dist. Range**—148° to 154° (p. 478); **Phenols**—passes test (M-33c)
IR	97.0% of $C_{14}H_{18}O$ (M-3)	5.0	1.554–1.559	1 ml in 4.5 ml 80% alc	0.963–0.968	**Chlorinated Cmpds.**—passes test (p. 500)
IR	96.0% of $C_{14}H_{18}O_2$ (M-6)	1.0	1.535–1.539	1 ml in 7 ml 80% alc (may be opalescent)	0.992–0.997	
IR	98.0% of $C_{13}H_{26}O_2$ (M-6)	1.0	1.425–1.429	1 ml in 7 ml 80% alc, remains clear to 10 ml	0.855–0.861	
IR	98.0% of $C_8H_{16}O_2$ (M-6)	1.0	1.405–1.409	1 ml in 3 ml 70% alc	0.866–0.871	
IR			1.557–1.561	1 ml in 2 ml alc	0.983–0.988	**Aldehydes and Ketones**—passes test (M-16c); **Angular Rotation**—between −0.15° and +0.15° (p. 530, 100-mm tube); **Dist. Range**—231° to 237° (p. 478); **Phenols**—passes test (M-33c); **Solidification Pt.**—NLT 20° (p. 538)
IR			1.515–1.518		0.990–0.993	**Dist. Range**—within a 2° range (p. 478); **Phenols**—passes test (M-33c)
IR	97.0% of $C_{10}H_{12}O_2$ (M-6)	1.0	1.511–1.516	1 ml in 6 ml 60% alc, remains in soln to 10 ml	1.104–1.107	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Anisyl Alcohol (Anisic Alcohol; *p*-Methoxybenzyl Alcohol) [FEMA No. 2099]	138.17/$C_8H_{10}O_2$/ 	colorless to slightly yel liq/floral	*s*—most fixed oils; *ss*—gly	provided
Benzaldehyde [FEMA No. 2127]	106.12/C_7H_6O/ 	colorless liq, burning taste/bitter almond oil	*ss*—water; *m*—alc, ether, fixed or vol oils	
Benzophenone (Diphenyl Ketone; Benzoylbenzene) [FEMA No. 2134]	182.22/$C_{13}H_{10}O$/ 	white rhombic cryst or flaky solid; m.p. 48.5°/delicate, persistent, roselike	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly	provided
Benzyl Acetate [FEMA No. 2135]	150.18/$C_9H_{10}O_2$/ 	colorless liq/sweet, floral, fruity	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/214°	provided
Benzyl Alcohol (Phenyl Carbinol) [FEMA No. 2137]	108.14/C_7H_8O/ 	colorless liq with a sharp burning taste/faint, aromatic	*m*—alc, chloroform, ether; 1 ml in 30 ml water/206° (decomp)	
Benzyl Benzoate [FEMA No. 2138]	212.25/$C_{14}H_{12}O_2$/ 	colorless, oily liq/slight, aromatic odor	*m*—alc, chloroform, ether; *ins*—gly, water	
Benzyl Butyrate (Benzyl *n*-Butyrate) [FEMA No. 2140]	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/floral, fruity, plumlike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/239°	provided
Benzyl Cinnamate [FEMA No. 2142]	238.29/$C_{16}H_{14}O_2$/ 	white to pale yel solid/sweet, balsamic	*s*—most fixed oils; *ins*—gly, prop glycol	
Benzyl Isobutyrate (Benzyl 2-Methyl Propionate) [FEMA No. 2141]	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/floral, fruity, jasminelike	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly/229°	provided
Benzyl Isovalerate (Benzyl 3-Methyl Butyrate) [FEMA No. 2152]	192.25/$C_{12}H_{16}O_2$/ 	colorless liq/fruity, herbaceous, applelike	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/246°	provided
Benzyl Phenylacetate [FEMA No. 2149]	226.27/$C_{15}H_{14}O_2$/ 	colorless liq/sweet, floral, honey undertone	*m*—alc, chloroform, ether	

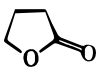
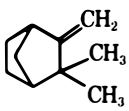
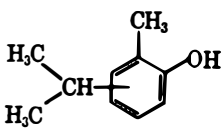
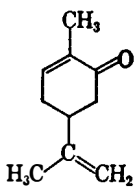
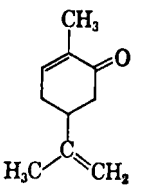
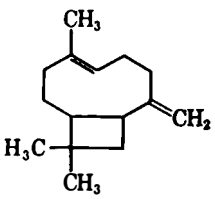
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_8H_{10}O_2$ (M-12)	1.0	1.543–1.545	1 ml in 1 ml 50% alc, remains in soln to 10 ml	1.110–1.115	**Aldehydes**—1.0% (M-16a); **Solidification Pt.**—min. 23.5° (p. 538)
IR	98.0% of C_7H_8O (M-2a)		1.544–1.547		1.041–1.046	**Chlorinated Cmpds.**—passes test (p. 500); **Hydrocyanic Acid**—passes test (M-26); **Solubility in Sulfite**—passes test (M-39)
IR				1 g in 10 ml 80% alc		**Arsenic**—3 ppm (M-18); **Chlorinated Cmpds.**—passes test (p. 500); **Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27); **Solidification Pt.**—NLT 47° (p. 538)
IR	98.0% of $C_9H_{10}O_2$ (M-6)	1.0	1.501–1.504	1 ml in 5 ml 60% alc	1.052–1.056	**Chlorinated Cmpds.**—passes test (p. 500)
IR			1.539–1.541		1.042–1.047	**Aldehydes**—0.2% (M-16b); **Chlorinated Cmpds.**—passes test (p. 500); **Dist. Range**—NLT 95% between 202.5° and 206.5° (p. 478)
IR	99.0% of $C_{14}H_{12}O_2$ (M-6)	1.0	1.568–1.570		1.116–1.120	**Chlorinated Cmpds.**—passes test (p. 500); **Solidification Pt.**—NLT 18° (p. 538)
IR	98.0% of $C_{11}H_{14}O_2$ (M-6)	1.0	1.492–1.496	1 ml in 2 ml 80% alc	1.006–1.009	
IR	99.0% of $C_{16}H_{14}O_2$ (M-6)	1.0		1 g in 8 ml 90% alc		**Arsenic**—3 ppm (M-18); **Chlorinated Cmpds.**—passes test (p. 500); **Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27); **Solidification Pt.**—between 33.0° and 34.5° (p. 538)
IR	97.0% of $C_{11}H_{14}O_2$ (M-6)	1.0	1.489–1.492	1 ml in 6 ml 70% alc	1.001–1.005	
IR	98.0% of $C_{12}H_{16}O_2$ (M-6)	1.0	1.486–1.490	1 ml in 3 ml 80% alc, remains in soln on dilution	0.985–0.991	
IR	98.0% of $C_{15}H_{14}O_2$ (M-6)	1.0	1.553–1.558	1 ml in 3 ml 90% alc gives clear soln	1.095–1.099	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Benzyl Propionate [FEMA No. 2150]	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/sweet, floral fruity	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water	provided
Benzyl Salicylate [FEMA No. 2151]	228.25/$C_{14}H_{12}O_3$/ 	almost colorless liq/faint, sweet odor	*s*—most fixed oils; *ins*—gly, prop glycol	provided
Bornyl Acetate (*levo*-Bornyl Acetate) [FEMA No. 2159]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq, semicryst mass, or white cryst solid/sweet, herbaceous, piney	*s*—alc, most fixed oils; *ss*—water; *ins*—gly, prop glycol/226°	provided
2-Butanone (Methyl Ethyl Ketone) [FEMA No. 2170]	72.11/C_4H_8O/ $CH_3COCH_2CH_3$	colorless, mobile liq/ethereal, nauseating	*m*—alc, ether, most fixed oils; 1 ml in 4 ml water/78.6°–80°	
Butan-3-one-2-yl Butyrate [FEMA No. 3332]	158.19/$C_8H_{14}O_3$/ 	white to slightly yel liq/sweet, red berry character	*s*—alc, prop glycol, most fixed oils; *ins*—water	
Butyl Acetate (*n*-Butyl Acetate) [FEMA No. 2174]	116.16/$C_6H_{12}O_2$/ $CH_3COO(CH_2)_3CH_3$	colorless, mobile liq/strong, fruity	*m*—alc, ether, prop glycol; 1 ml in 145 ml water/126°	
Butyl Alcohol (1-Butanol) [FEMA No. 2178]	74.12/$C_4H_{10}O$/ $CH_3(CH_2)_2CH_2OH$	colorless, mobile liq/vinous	*m*—alc, ether, other org solvents; 1 ml in 15 ml water/117.7°	
Butyl Butyrate (*n*-Butyl *n*-Butyrate) [FEMA No. 2186]	144.21/$C_8H_{16}O_2$/ $CH_3CH_2CH_2COOC_4H_9$	colorless liq/fruity, pineapplelike on dilution	*m*—alc, ether, most veg oils; *ss*—prop glycol, water; 1 ml in 3 ml 70% alc/165°	
Butyl Butyryllactate (Butyryllactic Acid, Butyl Ester; Lactic Acid Butyl Ester, Butyrate) [FEMA No. 2190]	216.28/$C_{11}H_{20}O_4$/ 	colorless liq/ mild, buttery, creamlike	*m*—alc, most fixed oils; *s*—prop glycol; *ins*—water	

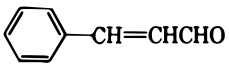
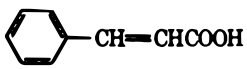
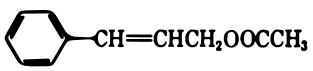
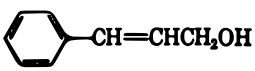
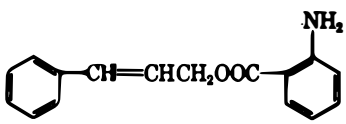
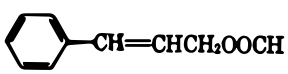
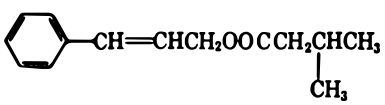
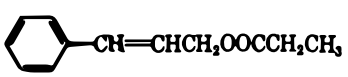
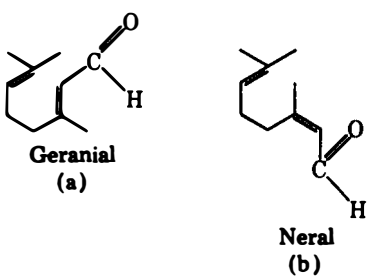
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{10}H_{12}O_2$ (M-6)	1.0	1.496–1.500	1 ml in 3 ml 70% alc, remains clear to 10 ml	1.028–1.032	
IR	98.0% of $C_{14}H_{12}O_2$ (M-6)	1.0	1.579–1.582	1 ml in 9 ml 90% alc	1.176–1.180	Solidification Pt.—NLT 23.5° (p. 538)
IR	98.0% of $C_{12}H_{20}O_2$ (M-6)	1.0	1.462–1.466	1 ml in 3 ml 70% alc, remains in soln to 10 ml	0.981–0.985	Angular Rotation—between −39.5° and −45.0° (p. 530, 100-mm tube); Solidification Pt.—NLT 25° (p. 538)
IR	99.5% of C_4H_8O (M-8c)				0.801–0.803	Acidity—0.003% (M-15a); Dist. Range—within a 1.5° range (p. 478); Water—0.2% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_8H_{14}O_3$ (M-8a)		1.408–1.429		0.972–0.992	
IR	98.0% of $C_8H_{12}O_2$ (M-11c)		1.393–1.395		0.876–0.880	Acidity—0.01% (M-15a); Dist. Range—120° to 128° (p. 478)
IR					0.807–0.809	Acidity—0.005% (M-15a); Aldehydes—passes test (M-16c); Butyl Ether—0.2% (M-19); Dist. Range—max. 1.5° between beginning and end (p. 478)
IR	98.0% of $C_8H_{16}O_2$ (M-6)	1.0	1.405–1.407		0.867–0.871	
IR	95.0% of $C_{11}H_{20}O_4$ (M-6)	1.0	1.420–1.423	1 ml in 3 ml 70% alc	0.970–0.974	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Butyl Isobutyrate [FEMA No. 2188]	144.22/$C_8H_{16}O_2$/ $(CH_3)_2CHCOOC_4H_9$	colorless liq/ fresh, fruity, apple-pineapple	*m*—alc, ether, most fixed oils; *ins*—gly, prop glycol, water/ 166°	provided
Butyraldehyde (Butyl Aldehyde) [FEMA No. 2219]	72.11/C_6H_8O/ $CH_3(CH_2)_2CHO$	colorless, mobile liq/ pungent, nutty	1 ml in 15 ml water; *m*—alc, ether/74.8°	
Butyric Acid [FEMA No. 2221]	88.11/$C_4H_8O_2$/ $CH_3(CH_2)_2COOH$	colorless liq/strong, rancid, butterlike	*m*—alc, most fixed oils, prop glycol, water/164°	
γ-Butyrolactone [FEMA No. 3291]	86.09/$C_4H_6O_2$/ 	colorless to slightly yel liq/faint, sweet, caramellike	*s*—water; *m*—alc	
Camphene [FEMA No. 2229]	136.23/$C_{10}H_{16}$/ 	colorless cryst mass; m.p. 52°/ camphoraceous-oily odor	*s*—alc; *m*—most fixed oils; *ins*—water	
Carvacrol [FEMA No. 2245]	150.22/$C_{10}H_{14}O$/ 	colorless to pale yel liq/pungent, spicy, thymollike	*s*—alc, ether; *ins*—water	
***d*-Carvone** (Dextro-Carvone; *d*-1-Methyl-4-isopropenyl-6-cyclohexen-2-one) [FEMA No. 2249]	150.22/$C_{10}H_{14}O$/ 	colorless to light yel liq/carawaylike	*s*—prop glycol, fixed oils; *m*—alc; *ins*—gly	provided
***l*-Carvone** (Levo-Carvone; *l*-1-Methyl-4-isopropenyl-6-cyclohexen-2-one) [FEMA No. 2249]	150.22/$C_{10}H_{14}O$/ 	colorless to pale straw-colored liq/ spearmintlike	*s*—prop glycol, fixed oils; *m*—alc; *ins*—gly	provided
β-Caryophyllene [FEMA No. 2252]	204.36/$C_{15}H_{24}$/ 	colorless to slightly yel, oily liq/clove-like	*s*—alc, ether; *ins*—water	

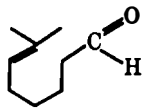
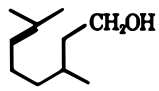
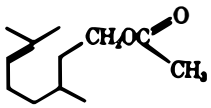
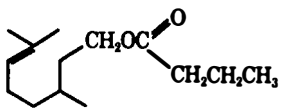
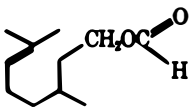
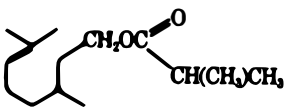
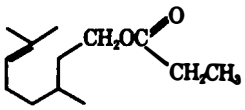
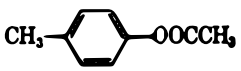
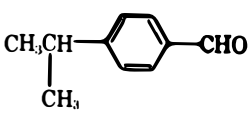
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_8H_{16}O_2$ (M-6)	1.0	1.401–1.404	1 ml in 7 ml 60% alc	0.859–0.864	
IR	98.0% of C_4H_8O (M-5a)				0.797–0.802	**Acidity**—0.75% (M-15b); **Dist. Range**—72° to 80° (first 95%, p. 478); ***para*-Butyraldehyde**—2.5% (M-20); **Water**—0.5% (p. 552, *KF*)
IR	99.0% of $C_4H_8O_2$ (M-11a)		1.397–1.399		0.952–0.956	**Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27); **Reducing Subs.**—passes test (M-36)
IR	98.0% of $C_4H_8O_2$ (M-8a)		1.434–1.454 (25°)		1.120–1.130	
	80.0% of $C_{10}H_{16}$ (M-8a)		1.452–1.456 (55°)		0.836–0.841 (60°/15.5°)	**Solidification Pt.**—>45° (p. 538)
IR	98.0% of phenols by vol (M-10)		1.521–1.526	1 ml in 4 ml 60% alc gives clear soln	0.974–0.979	
IR	*Natural:* 95.0% of $C_{10}H_{14}O$; *synthetic:* 97.0% of $C_{10}H_{14}O$ (M-3)		1.496–1.499	1 ml in 5 ml 60% alc	*Natural:* 0.956–0.960; *synthetic:* 0.955–0.960	**Angular Rotation**—*Natural:* between +56° and + 60°; *synthetic:* between +50° and +60° (p. 530, 100-mm tube)
IR	97.0% of $C_{10}H_{14}O$ (M-3)		1.495–1.499	1 ml in 2 ml 70% alc	0.956–0.960	**Angular Rotation**—between −57° and −62° (p. 530, 100-mm tube)
IR			1.498–1.504	1 ml in 4 ml 95% alc gives clear soln	0.897–0.910	**Angular Rotation**—between −5° and −10° (p. 530, 100-mm tube); **Phenols**—3.0% (M-33b)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Cinnamaldehyde (Cinnamic Aldehyde; Cinnamal) [FEMA No. 2286]	132.16/C_9H_8O/ 	yellow, strongly refractive liq/ cinnamonlike, burning aromatic taste	*m*—alc, chloroform, ether, fixed and vol oils; 1 g in 700 ml water	provided
Cinnamic Acid (3-Phenylpropionic Acid [FEMA No. 2288]	148.16/$C_9H_8O_2$/ 	white cryst scales; m.p. 133°/honey-floral	*s*—acetic acid, acetone, benzene, most fixed oils; 1 g in 2000 ml water, 6 ml alc, 12 ml chloroform	
Cinnamyl Acetate [FEMA No. 2293]	176.22/$C_{11}H_{12}O_2$/ 	colorless to slightly yel liq/sweet, balsamic, floral	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, water/264°	
Cinnamyl Alcohol, Synthetic [FEMA No. 2294]	134.14/$C_9H_{10}O$/ 	white to slightly yel cryst solid; m.p. 33°/balsamic	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Cinnamyl Anthranilate [FEMA No. 2295]	253.30/$C_{16}H_{15}NO_2$/ 	reddish yel powder; m.p. 60°/balsamic, fruity	*s*—alc, chloroform, ether; *ins*—water	
Cinnamyl Formate [FEMA No. 2299]	162.19/$C_{10}H_{10}O_2$/ 	colorless to slightly yel liq/ green, herbaceous, balsamic odor	*m*—alc, chloroform, ether, most fixed oils; *ins*—water/250°	
Cinnamyl Isovalerate [FEMA No. 2302]	218.30/$C_{14}H_{18}O_2$/ 	colorless to slightly yel liq/spicy, floral, fruity	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, prop glycol, water/313°	
Cinnamyl Propionate [FEMA No. 2301]	190.24/$C_{12}H_{14}O_2$/ 	colorless to pale yel liq/spicy, fruity, balsamic	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, prop glycol, water/289°	
Citral [Mixture of Geranial (*trans*-3,7-dimethyl-2,6-octadien-1-al) and Neral (the *cis* isomer)] [FEMA No. 2303]	152.24/$C_{10}H_{16}O$/  Geranial (a) Neral (b)	pale yel liq/strong, lemonlike	*s*—fixed oils, min oil, prop glycol; *ins*—gly	provided

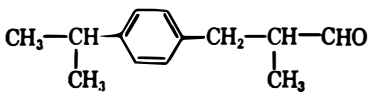
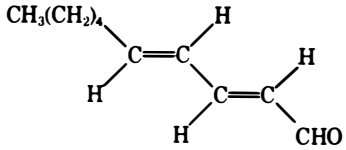
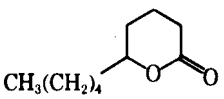
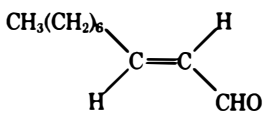
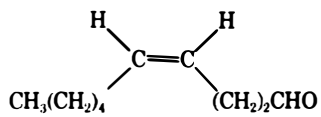
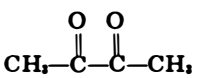
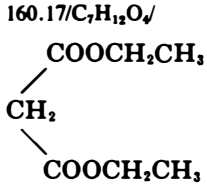
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of C_9H_8O (M-2a)	5.0	1.619–1.623	1 ml in 5 ml 60% alc	1.046–1.050	**Chlorinated Cmpds.**—passes test (p. 500); **Solubility in Bisulfite**—passes test (M-39)
IR	99.0% of $C_9H_8O_2$ after drying (M-11b)					**Arsenic**—3 ppm (M-18); **Chlorinated Cmpds.**—0.005% (M-22); **Heavy Metals**—10 ppm (M-24b); **Melting Range**—NLT 130° (p. 519); **Res. on Ignit.**—0.05% (p. 533)
IR	98.0% of $C_{11}H_{12}O_2$ (M-6)	3.0	1.539–1.543	1 ml in 5 ml 70% alc	1.047–1.051	
IR	98.0% of $C_9H_{10}O$ (M-12)			1 g in 1 ml 70% alc, remains in soln to 10 ml		**Aldehydes**—1.5% (M-16a); **Chlorinated Cmpds.**—passes test (p. 500) **Solidification Pt.**—NLT 31° (p. 538)
IR	96.0% of $C_{16}H_{15}NO_2$ (M-6)			1 g in 20 ml 95% alc gives clear soln		**Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27); **Solidification Pt.**—NLT 60° (p. 538)
IR	92.0% of $C_{10}H_{10}O_2$ (M-8a)	3.0	1.550–1.556	1 ml in 10 ml 70% alc, and in 2 ml 80% alc, gives clear solns	1.074–1.079	**Cinnamyl Alcohol**—8.0% (M-8a)
IR	95.0% of $C_{14}H_{18}O_2$ (M-6)	3.0	1.518–1.524	1 ml in 1 ml 90% alc	0.991–0.996	
IR	98.0% of $C_{12}H_{14}O_2$ (M-6)	3.0	1.532–1.537		1.029–1.033	
IR	96.0% of $C_{10}H_{16}O$ (M-3)		1.486–1.490	1 ml in 7 ml 70% alc	0.885–0.891	**Solubility in Bisulfite**—passes test (M-39)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Citronellal (3,7-Dimethyl-6-octen-1-al) [FEMA No. 2307]	154.25/$C_{10}H_{18}O$/ 	colorless to slightly yel liq/intense lemon-citronella-rose	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water	provided
Citronellol (3,7-Dimethyl-6-octen-1-ol) [FEMA No. 2309]	156.27/$C_{10}H_{20}O$/ 	colorless, oily liq/ roselike	*s*—most fixed oils, prop glycol; *ss*—water; *ins*—gly/225°	provided
Citronellyl Acetate (3,7-Dimethyl-6-octen-1-yl Acetate) [FEMA No. 2311]	198.30/$C_{12}H_{22}O_2$/ 	colorless liq/ fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/229°	provided
Citronellyl Butyrate (3,7-Dimethyl-6-octen-1-yl Butyrate) [FEMA No. 2312]	226.36/$C_{14}H_{26}O_2$/ 	colorless liq/strong, fruity-rosy	*m*—alc, ether, chloroform, most fixed oils; *ins*—water/245°	
Citronellyl Formate (3,7-Dimethyl-6-octen-1-yl Formate) [FEMA No. 2314]	184.28/$C_{11}H_{20}O_2$/ 	colorless liq/strong, fruity, floral	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/235°	provided
Citronellyl Isobutyrate (3,7-Dimethyl-6-octen-1-yl Isobutyrate) [FEMA No. 2313]	226.36/$C_{14}H_{26}O_2$/ 	colorless liq/ rosy-fruity	*m*—alc, chloroform, ether, most fixed oils; *ins*—water/249°	
Citronellyl Propionate [FEMA No. 2316]	212.33/$C_{13}H_{24}O_2$/ 	colorless liq/ fruity-rosy	*m*—alc, most fixed oils; *ins*—water/242°	provided
Cresyl Acetate (*p*-Tolyl Acetate) [FEMA No. 3073]	150.18/$C_9H_{10}O_2$/ 	colorless liq/strong, floral	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Cuminic Aldehyde (*p*-Cuminic Aldehyde; Cumaldehyde; *p*-Isopropylbenzaldehyde; Cuminal) [FEMA No. 2957]	148.21/$C_{10}H_{12}O$/ 	colorless to pale yel liq/strong, pungent odor of cumin oil	*s*—alc, ether; *ins*—water	provided

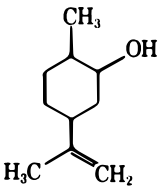
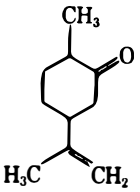
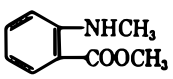
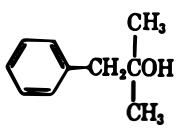
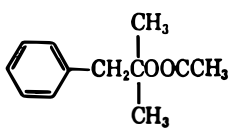
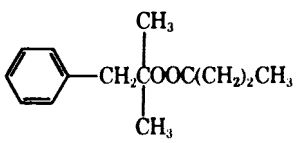
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	85.0% of aldehydes, as $C_{10}H_{18}O$ (M-3)	3.0	1.446–1.456	1 ml in 5 ml 70% alc, remains clear on dilution	0.850–0.860	**Angular Rotation**—between −1° and +11° (p. 530, 100-mm tube)
IR	90.0% of $C_{10}H_{20}O$ (M-12)		1.454–1.462	1 ml in 2 ml 70% alc, remains in soln to 10 ml	0.850–0.860	**Aldehydes**—1.0% (M-16a); **Angular Rotation**—between −1° and +5° (p. 530, 100-mm tube); **Esters**—1.0% (M-23)
IR	92.0% of total esters, as $C_{12}H_{22}O_2$ (M-6)	1.0	1.440–1.450	1 ml in 9 ml 70% alc	0.883–0.893	
IR	90.0% of $C_{14}H_{28}O_2$ (M-6)	1.0	1.444–1.448	1 ml in 6 ml 80% alc gives clear soln	0.873–0.883	
IR	86.0% of total esters, as $C_{11}H_{20}O_2$ (M-6)	3.0	1.443–1.449	1 ml in 3 ml 80% alc, remains in soln to 10 ml	0.890–0.903	
IR	92.0% of $C_{14}H_{26}O_2$ (M-6)	1.0	1.440–1.448	1 ml in 6 ml 80% alc gives clear soln	0.870–0.880	
IR	90.0% of $C_{13}H_{24}O_2$ (M-6)	1.0	1.443–1.449	1 ml in 4 ml 80% alc gives clear soln	0.877–0.886	
IR	98.0% of $C_9H_{10}O_2$ (M-7)	1.0 (phenol red TS)	1.499–1.502	1 ml in 2 ml 70% alc	1.044–1.050	**Free Cresol**—1.0% (M-33c)
IR	95.0% of $C_{10}H_{12}O$ (M-4)	5.0	1.529–1.534	1 ml in 4 ml 70% alc	0.976–0.980	**Chlorinated Cmpds.**—passes test (p. 500)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Cyclamen Aldehyde [2-Methyl-3-(*p*-isopropylphenyl)-propionaldehyde] [FEMA No. 2743]	190.28/$C_{13}H_{18}O$/ 	colorless to pale yel liq/strong, floral	*s*—most fixed oils; *ins*—prop glycol, gly	provided
***trans,trans*-2,4-Decadienal** [FEMA No. 3135]	152.23/$C_{10}H_{16}O$/ 	yel liq/powerful, oily, like chicken fat	*s*—alc, fixed oils; *ins*—water/104° (7 mm)	
Δ-Decalactone [FEMA No. 2361]	170.25/$C_{10}H_{18}O_2$/ 	colorless liq/ coconut-fruity, butterlike on dilution	*vs*—alc, prop glycol, veg oil; *ins*—water/281°	
Decanal (Aldehyde C-10; Capraldehyde) [FEMA No. 2362]	156.27/$C_{10}H_{20}O$/ $CH_3(CH_2)_8CHO$	colorless to light yel liq/fatty, floral-orange on dilution	*m*—alc, fixed oils, prop glycol (may be turbid); *ins*—gly, water/209°	provided
Decanoic Acid [see p. 94]				
1-Decanol, Natural (Decyl Alcohol; Alcohol C-10) [FEMA No. 2365]	158.28/$C_{10}H_{22}O$/ $CH_3(CH_2)_8CH_2OH$	colorless liq/ floral, waxy, fruity	*s*—alc, ether, min oil, prop glycol, most fixed oils; *ins*—gly, water/233°	provided
***trans*-2-Decen-1-al** [FEMA No. 2366]	154.24/$C_{10}H_{18}O$/ 	slightly yel liq/ orange, waxlike odor	*s*—alc, most fixed oils; *ins*—water	
***cis*-4-Decen-1-al** [FEMA No. 3264]	154.24/$C_{10}H_{18}O$/ 	colorless to slightly yel liq/orangelike, fatty	*s*—alc, most fixed oils; *ins*—water	
Diacetyl (2,3-Butanedione; Dimethyldiketone; Dimethylglyoxal) [FEMA No. 2370]	86.09/$C_4H_6O_2$/ 	yel to yel-green liq/powerful, buttery in very dilute soln	*m*—alc, most fixed oils, prop glycol; *s*—gly, water	
Diethyl Malonate (Ethyl Malonate; Malonic Ester) [FEMA No. 2375]	160.17/$C_7H_{12}O_4$/ 	colorless liq/ slight, fruitlike	*s*—most fixed oils, prop glycol; *ss*—alc, water; *ins*—gly, min oil/200°	

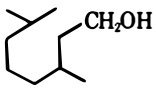
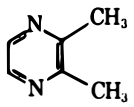
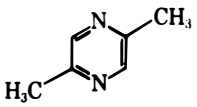
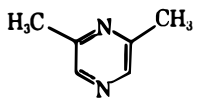
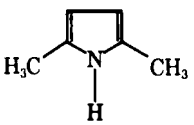
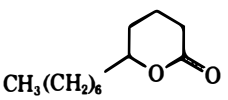
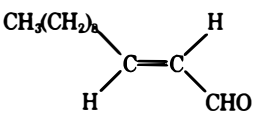
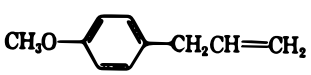
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	90.0% of $C_{13}H_{18}O$ (M-2a)	5.0	1.503–1.508	1 ml in 1 ml 80% alc	0.946–0.952	
IR	89.0% of $C_{10}H_{16}O$ (M-8a)		1.514–1.516		0.866–0.876	
IR	98.0% of $C_{10}H_{16}O_2$ (M-8c)	5.0 (Method II, p. 504)	1.456–1.459			Sap. Value—323 to 333 (p. 501, 1-g samp)
IR	92.0% of $C_{10}H_{20}O$ (M-2a)	10.0	1.426–1.430		0.823–0.832	
IR	98.0% of $C_{10}H_{22}O$ (M-12)	1.0	1.435–1.439	1 ml in 3 ml 60% alc	0.826–0.831	Solidification Pt.—NLT 5° (p. 538)
IR	99.0% of $C_{10}H_{18}O$ (M-8a)		1.454–1.458		0.836–0.846	
IR	95.0% of $C_{10}H_{18}O$ (M-8a)		1.442–1.444		0.847–0.848	
IR	95.0% of $C_4H_6O_2$ (M-3)		1.393–1.397		0.979–0.985	Solidification Pt.—between −2.0° and −4.0° (p. 538)
IR	98.0% of $C_7H_{12}O_4$ (M-6)	1.0	1.413–1.416	1 ml in 1.5 ml 60% alc	1.053–1.056	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Diethyl Sebacate (Ethyl Sebacate) [FEMA No. 2376]	258.36/$C_{14}H_{26}O_4$/ $C_2H_5OOC(CH_2)_8COOC_2H_5$	colorless to slightly yel liq/faint, winy, fruity	*m*—alc, ether, other org solvents, most fixed oils; *ins*—water/302°	
Diethyl Succinate [FEMA No. 2377]	174.20/$C_8H_{14}O_4$/ $C_2H_5OOCCH_2CH_2COOC_2H_5$	colorless, mobile liq/faint, pleasant	*m*—alc, ether, most fixed oils; 1 ml in 50 ml water/217°	provided
Dihydrocarveol [FEMA No. 2379]	154.24/$C_{10}H_{18}O$/ 	almost colorless, oily liq/like spearmint	*s*—alc, most fixed oils; *ins*—water	
***d*-Dihydrocarvone** [*d*-2-Methyl-5-(1-methylethenyl)-cyclohexanone] [FEMA No. 3565]	152.23/$C_{10}H_{16}O$/ 	almost colorless liq/herbaceous, spearmintlike	*s*—alc, most fixed oils; *ins*—water	
Dimethyl Anthranilate (Methyl *N*-Methyl Anthranilate) [FEMA No. 2718]	165.19/$C_9H_{11}NO_2$/ 	pale yel liq with bluish fluorescence/grapelike	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly, water	provided
Dimethyl Benzyl Carbinol (α,α-Dimethylphenethyl Alcohol) [FEMA No. 2393]	150.22/$C_{10}H_{14}O$/ 	white cryst solid that melts readily; may exist in supercooled form as colorless to pale yel liq/floral	*s*—most fixed oils, min oil, prop glycol; *ins*—gly	provided
Dimethyl Benzyl Carbinyl Acetate (α,α-Dimethylphenethyl Acetate) [FEMA No. 2392]	192.26/$C_{12}H_{16}O_2$/ 	colorless liq; solidifies at room temp/floral, fruity	*s*—most fixed oils; *ss*—prop glycol; *ins*—water	provided
Dimethyl Benzyl Carbinyl Butyrate (α,α-Dimethylphenethyl Butyrate) [FEMA No. 2394]	220.31/$C_{14}H_{20}O_2$/ 	almost colorless liq/prunelike	*s*—alc, most fixed oils; *ins*—water, prop glycol	

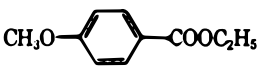
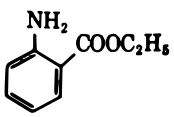
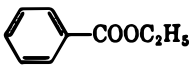
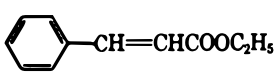
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{14}H_{26}O_4$ (M-6)	1.0	1.435–1.438		0.960–0.965	
IR	99.0% of $C_8H_{14}O_4$ (M-8c)					Acidity—0.02% (M-15a); Diethyl Maleate—0.03% (M-8c); Water—0.05% (p. 552, *KF*)
	96.0% of $C_{10}H_{18}O$ (mixed isomers) (M-8a)		1.477–1.481		0.921–0.926	
	92.0% of $C_{10}H_{16}O$ (*cis* + *trans*) (M-8a)		1.470–1.474		0.923–0.928	Angular Rotation—min. +14.0° (p. 530)
	98.0% to 101.3% of $C_9H_{11}NO_2$ (M-6)		1.578–1.581	1 ml in 3 ml 80% alc, remains in soln to 10 ml	1.126–1.132	Solidification Pt.—NLT 14° (p. 538)
IR	97.0% of $C_{10}H_{14}O$ (M-9b)	1.0	1.514–1.517 (20°, as supercooled liq)	1 ml in 3 ml 50% alc, remains in soln to 10 ml	0.972–0.977	Chlorinated Cmpds.—passes test (p. 500), Solidification Pt.—NLT 22° (p. 538)
IR	98.0% of $C_{12}H_{16}O_2$ (M-6)	1.0	1.490–1.495	1 ml in 4 ml 70% alc	0.995–1.002	Chlorinated Cmpds.—passes test (p. 500); Solidification Pt.—NLT 28° (p. 538)
IR	95.0% of $C_{14}H_{20}O_2$ (M-8a)		1.473–1.493 (25°)		0.960–0.981	Angular Rotation—between −1° and +2° (p. 530)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
3,7-Dimethyl-1-Octanol (Dimethyl Octanol; Tetrahydrogeraniol) [FEMA No. 2391]	158.28/$C_{10}H_{22}O$/ 	colorless liq/sweet, roselike	*s*—most fixed oils, prop glycol; *ins*—gly	provided
2,3-Dimethylpyrazine [FEMA No. 3271]	108.14/$C_6H_8N_2$/ 	colorless to slightly yel liq/nutty, cocoalike	*m*—water, organic solvents	
2,5-Dimethylpyrazine [FEMA No. 3272]	108.14/$C_6H_8N_2$/ 	colorless to slightly yel liq/earthy, potatolike	*m*—water, organic solvents	
2,6-Dimethylpyrazine [FEMA No. 3273]	108.14/$C_6H_8N_2$/ 	white to yel, lumpy crystals, m.p. 48°, sp. gr. 0.965 (50°)/ nutty, coffeelike	*s*—water, organic solvents/155°	
2,5-Dimethylpyrrole [FEMA No. 7071]	95.15/C_6H_9N/ 	colorless to yellowish, oily liq	*vs*—alc, ether; *vss*—water	
Δ-Dodecalactone [FEMA No. 2401]	198.31/$C_{12}H_{22}O_2$/ 	colorless to yel liq/ coconut-fruity, butterlike on dilution	*vs*—alc, prop glycol, veg oil; *ins*—water	
***trans*-2-Dodecen-1-al** [FEMA No. 2402]	182.31/$C_{12}H_{22}O$/ 	slightly yel liq/ fatty, citruslike	*s*—alc, most fixed oils; *ins*—water	
Estragole (*p*-Allylanisole) [FEMA No. 2411]	148.20/$C_{10}H_{12}O$/ 	colorless to light yel liq/aniselike	*s*—alc; *ins*—water	
Ethyl Acetate [FEMA No. 2414]	88.11/$C_4H_8O_2$/ $CH_3COOC_2H_5$	colorless liq; vol at low temp; flammable/fragrant, acetous, ethereal	*m*—alc, ether, gly, fixed oils, vol oils; 1 ml in 10 ml water/54°	

	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	90.0% of total alcohols, as $C_{10}H_{22}O$ (M-12)	1.0	1.435–1.445	1 ml in 3 ml 70% alc	0.826–0.842	
IR	95.0% of $C_6H_8N_2$ (M-8a)		1.506–1.509		1.000–1.022 (20°)	**Dist. Range**—152° to 157° (p. 478); **Heavy Metals**—10 ppm (M-24b); **Solidification Pt.**—11° to 13° (p. 538); **Tri- and Tetrapyrazines**—5% (by GLC assay); **Water**—0.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	99.0% of $C_6H_8N_2$ (M-8a)		1.497–1.501		0.980–1.000	**Heavy Metals**—10 ppm (M-24b); **Solidification Pt.**—12° to 17° (p. 538); **Water**—0.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_6H_8N_2$ (M-8a)					**Melting Range**—35° to 40° (p. 519); **Res. on Ignit.**—0.1% (p. 533); **Water**—0.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of C_6H_9N (M-8a)		1.503–1.506		0.935–0.945 (20°)	**Water**—0.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_{12}H_{22}O_2$ (M-8c)	8.0 (Method II, p. 504)	1.458–1.461			**Sap. Value**—278 to 286 (p. 501, 1-g samp)
IR	95.0% of $C_{12}H_{22}O$ (M-8a)		1.462–1.464		0.839–0.849	
IR			1.517–1.522	1 ml in 6 ml 80% alc gives clear soln	0.957–0.965	
IR	99.0% of $C_4H_8O_2$ (M-11c)				0.894–0.898	**Acidity**—passes test (M-15b); **Butylic and Amylic Derivs.**—passes test (M-21); **Dist. Range**—76° to 77.5° (p. 478); **Methyl Cmpds.**—passes test (M-29); **Readily Carb. Subs.**—passes test (M-34); **Res. on Evap.**—0.02% (p. 502, 10-g samp, 105°/1 h)

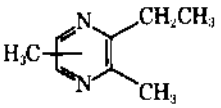
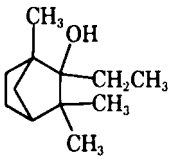
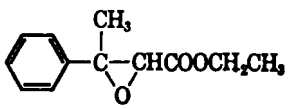
General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Ethyl Acetoacetate (Acetoacetic Ester; Ethyl 3-Oxybutanoate) [FEMA No. 2415]	130.14/$C_6H_{10}O_3$/ $CH_3COCH_2CO_2C_2H_5 \rightleftarrows CH_3C(OH)=CHCOOC_2H_5$	colorless to very light yel, mobile liq/agreeable odor	*m*—alc, ether, ethyl acetate; 1 ml in 12 ml water	
Ethyl Acrylate [FEMA No. 2418]	100.12/$C_5H_8O_2$/ $CH_2=CHCOOC_2H_5$	colorless, mobile liq; lachrymator/ intense, harsh, fruity	*m*—alc, ether; 1 ml in 50 ml water	
Ethyl *p*-Anisate (Ethyl *p*-Methoxybenzoate) [FEMA No. 2420]	180.20/$C_{10}H_{12}O_3$/ 	colorless to slightly yel liq/light, fruity, aniselike	*s*—alc, chloroform, ether; *ins*—water	
Ethyl Anthranilate (Ethyl *o*-Aminobenzoate) [FEMA No. 2421]	165.19/$C_9H_{11}NO_2$/ 	colorless to amber-colored liq/floral, orange blossom-like	*s*—alc, most fixed oils, prop glycol; 1 ml in 2 ml 70% alc	
Ethyl Benzoate [FEMA No. 2422]	150.18/$C_9H_{10}O_2$/ 	colorless liq/heavy, floral, fruity	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/212°	provided
2-Ethylbutyraldehyde [FEMA No. 2426]	100.16/$C_6H_{12}O$/ $CH_3CH_2CH(C_2H_5)CHO$	colorless, mobile liq/pungent	*m*—alc, ether; 1 ml in 50 ml water	
Ethyl Butyrate [FEMA No. 2427]	116.16/$C_6H_{12}O_2$/ $CH_3CH_2CH_2COOC_2H_5$	colorless liq/ banana-pineapple	*s*—fixed oils, prop glycol; *ins*—gly/121°	provided
2-Ethylbutyric Acid [FEMA No. 2429]	116.16/$C_6H_{12}O_2$/ $CH_3CH_2CH(C_2H_5)COOH$	colorless liq/mildly rancid odor	*m*—alc, ether; 1 ml in 65 ml water	
Ethyl Cinnamate (Ethyl 3-Phenylpropenate) [FEMA No. 2430]	176.21/$C_{11}H_{12}O_2$/ 	colorless, oily liq/ faint, cinnamonlike	*m*—alc, ether, most fixed oils; *ins*—gly, water/272°	
Ethyl Decanoate (Ethyl Caprate) [FEMA No. 2432]	200.32/$C_{12}H_{24}O_2$/ $CH_3(CH_2)_8COOC_2H_5$	colorless liq/ oily, brandylike odor	*s*—most fixed oils; *ins*—gly, prop glycol/243°	

Requirements

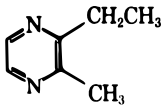
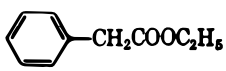
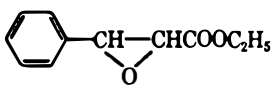
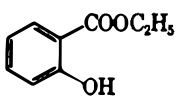
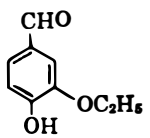
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.5% of $C_6H_{10}O_3$ (M-11d)		1.418–1.421		1.022–1.027	**Acidity**—0.2% (M-15b); **Ignition Residue**—0.01% (M-37)
IR	99.5% of $C_5H_8O_2$ (M-8c)				0.916–0.919	**Acidity**—0.005% (M-15b); **Antioxidants**—0.022% (M-17); **Water**—0.05% (p. 552, *KF*)
IR	97.0% of $C_{10}H_{12}O_3$ (M-6)	1.0	1.522–1.526	1 ml in 7 ml 60% alc gives clear soln	1.101–1.104	
IR	96.0% of $C_9H_{11}NO_2$ (M-6)	1.0	1.563–1.566		1.115–1.120	**Solidification Pt.**—NLT 13° (p. 538)
IR	98.0% of $C_9H_{10}O_2$ (M-6)	1.0	1.502–1.506	1 ml in 6 ml 60% alc	1.043–1.046	**Chlorinated Cmpds.**—passes test (p. 500)
IR	95.0% of $C_6H_{12}O$ (M-5b)				0.808–0.814	**Acidity**—2.0% (M-15a); **Dist. Range**—NLT 95% between 100° and 120° (p. 478)
IR	98.0% of $C_6H_{12}O_2$ (M-6)	1.0	1.391–1.394	1 ml in 3 ml 60% alc	0.870–0.877	
IR	98.0% of $C_6H_{12}O_2$ (M-11b)				0.917–0.922	**Dist. Range**—190° to 200° (p. 478); **Water**—0.2% (p. 552, *KF*)
IR	99.0% of $C_{11}H_{12}O_2$ (M-6)	1.0	1.558–1.560	1 ml in 5 ml 70% alc	1.045–1.051	
IR	98.0% of $C_{12}H_{24}O_2$ (M-6)	1.0	1.424–1.427	1 ml in 4 ml 80% alc	0.863–0.868	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
2-Ethyl-3,5(6)-dimethylpyrazine [FEMA No. 3149]	136.20/$C_8H_{12}N_2$/ 	colorless to slightly yel liq/roasted cocoa	*s*—water, organic solvents	
2-Ethyl Fenchol [FEMA No. 3491]	182.30/$C_{12}H_{22}O$/ 	pale yel liq/sharp, camphoraceous, earthy character	*s*—alc, prop glycol, most fixed oils; *ins*—water	
Ethyl Formate [FEMA No. 2434]	74.08/$C_3H_6O_2$/ $HCOOC_2H_5$	colorless, flammable liq/sharp, rumlike	*s*—most fixed oils, prop glycol, water (decomp.); *ss*—min oil; *ins*—gly/54°	
Ethyl Heptanoate (Ethyl Heptoate) [FEMA No. 2437]	158.24/$C_9H_{18}O_2$/ $CH_3(CH_2)_5COOC_2H_5$	colorless liq/ winy-brandy	*m*—alc, chloroform, most fixed oils; *ss*—prop glycol; *ins*—gly/189° (72% water azeotrope, 98.5°)	provided
Ethyl Hexanoate (Ethyl Caproate; Ethyl Capronate) [FEMA No. 2439]	144.21/$C_8H_{16}O_2$/ $CH_3(CH_2)_4COOC_2H_5$	colorless liq/ winy	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly/166°	provided
Ethyl Isovalerate (Ethyl 3-Methylbutyrate) [FEMA No. 2463]	130.19/$C_7H_{14}O_2$/ $(CH_3)_2CHCH_2COOC_2H_5$	colorless liq/strong, fruity, vinous, applelike on dilution	*m*—alc, most fixed oils; *s*—prop glycol; 1 ml in 350 ml water/135°	provided
Ethyl Lactate (Ethyl 2-Hydroxypropionate) [FEMA No. 2440]	118.13/$C_5H_{10}O_3$/ $CH_3CHOHCOOC_2H_5$	colorless liq/ characteristic odor	*vs*—alc, ether, chloroform, water	
Ethyl Laurate (Ethyl Dodecanoate) [FEMA No. 2441]	228.37/$C_{14}H_{28}O_2$/ $CH_3(CH_2)_{10}COOC_2H_5$	colorless, oily liq/fruity-floral	*m*—alc, chloroform, ether; *ins*—water/ 269°	
Ethyl 2-Methylbutyrate [FEMA No. 2443]	130.19/$C_7H_{14}O_2$/ $CH_3CH_2OOCCHCH_2CH_3$ (CH$_3$ on CH)	colorless liq/strong, green-fruity, applelike	*s*—alc, prop glycol; *m*—most fixed oils; *vss*—water	
Ethyl Methylphenylglycidate (Aldehyde C-16; Strawberry Aldehyde) [FEMA No. 2444]	206.24/$C_{12}H_{14}O_3$/ 	colorless to pale yel liq/strong, fruity, strawberrylike	*s*—most fixed oils, prop glycol; *ins*—gly	

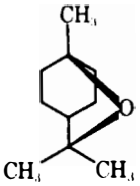
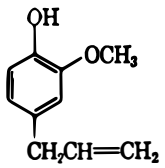
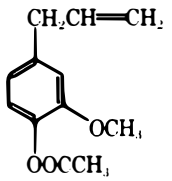
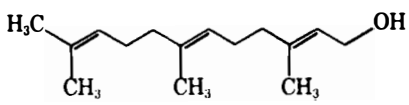
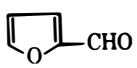
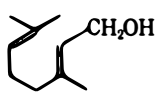
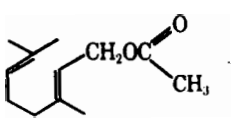
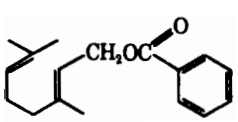
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_8H_{12}N_2$ (M-8a)		1.500–1.503		0.950–0.980 (20°)	Water—0.1% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_{12}H_{22}O$ (M-8a)		1.470–1.491 (26°)		0.946–0.967	
IR	95.0% of $C_3H_6O_2$ (M-6)		1.359–1.363	1 ml in 0.5 ml 50% alc	0.916–0.921	Acidity—0.1% (M-15c)
IR	98.0% of $C_9H_{18}O_2$ (M-6)	1.0	1.411–1.415	1 ml in 3 ml 70% alc	0.867–0.872	
IR	98.0% of $C_9H_{16}O_2$ (M-6)	1.0	1.406–1.409	1 ml in 2 ml 70% alc	0.867–0.871	
IR	98.0% of $C_7H_{14}O_2$ (M-6)	2.0	1.395–1.399		0.862–0.866	
IR	98.0% of $C_5H_{10}O_3$ (M-6)	1.0	1.410–1.420		1.029–1.032	
IR	98.0% of $C_{14}H_{28}O_2$ (M-6)	1.0	1.430–1.434	1 ml in 9 ml 80% alc gives clear soln	0.858–0.862	
IR	90.0% of $C_7H_{14}O_2$ (M-8a)	2.0	1.396–1.400		0.861–0.866	
IR	98.0% of $C_{12}H_{14}O_2$ (M-6)	2.0	1.504–1.513	1 ml in 3 ml 70% alc	1.086–1.112	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
2-Ethyl-3-methylpyrazine [FEMA No. 3155]	122.17/$C_7H_{10}N_2$/ 	colorless to slightly yel liq/strong, raw potato	*s*—water, organic solvents	
Ethyl Nonanoate (Ethyl Pelargonate) [FEMA No. 2447]	186.29/$C_{11}H_{22}O_2$/ $CH_3(CH_2)_7COOC_2H_5$	colorless liq/fatty, fruity, cognaclike	*m*—alc, prop glycol; *ins*—water; 1 ml in 10 ml 70% alc/229°	
Ethyl Octanoate (Ethyl Caprylate; Ethyl Octoate) [FEMA No. 2449]	172.27/$C_{10}H_{20}O_2$/ $CH_3(CH_2)_6COOC_2H_5$	colorless liq/ winy-brandy, fruity-floral	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly, water/209°	provided
Ethyl Oxyhydrate (Rum Ether, So-Called) [FEMA No. 2996]		colorless liq/ sharp rumlike	*m*—alc, gly, prop glycol	
Ethyl Phenylacetate [FEMA No. 2452]	164.20/$C_{10}H_{12}O_2$/ 	colorless or nearly colorless liq/sweet, honeylike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/228°	provided
Ethyl Phenylglycidate [FEMA No. 2454]	192.21/$C_{11}H_{12}O_3$/ 	colorless to slightly yel liq/strong, strawberrylike	*s*—alc, chloroform, ether; *ins*—water	provided
Ethyl Propionate [FEMA No. 2456]	102.13/$C_5H_{10}O_2$/ $CH_3CH_2COOC_2H_5$	colorless liq/ fruity, rumlike, ethereal	*m*—alc, prop glycol; *s*—most fixed oils; 1 ml in 42 ml water/ 99°	
Ethyl Salicylate [FEMA No. 2458]	166.18/$C_9H_{10}O_3$/ 	colorless liq/ wintergreenlike	*s*—alc, acetic acid, most fixed oils; *ss*—gly, water	provided
Ethyl Vanillin (3-Ethoxy-4-hydroxy-benzaldehyde) [FEMA No. 2464]	166.18/$C_9H_{10}O_3$/ 	fine, white or slightly yellowish crystals; affected by strong light; m.p. 78° strong, vanillalike	*s*—alc, chloroform, ether, prop glycol, solns of alkali hydroxides; 1 g in 100 ml water at 50°	

	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_7H_{10}N_2$ (M-8a)		1.502–1.505		0.980–0.999 (20°)	**Water**—0.1% (p. 552, *KF*; use freshly dist. pyridine as solvent)
IR	98.0% of $C_{11}H_{22}O_2$ (M-6)	3.0	1.420–1.424		0.863–0.867	
IR	98.0% of $C_{10}H_{20}O_2$ (M-6)	1.0	1.417–1.419	1 ml in 4 ml 70% alc	0.865–0.869	
						Alcohol Content—min. 14.0%, by vol, at 15.56° (M-14a); **Ester Value**—min. 25 (p. 501, 1- to 3-g samp); **Methanol-Formaldehyde**—1.5% (M-28)
IR	98.0% of $C_{10}H_{12}O_2$ (M-6)	1.0	1.496–1.500	1 ml in 3 ml 70% alc	1.027–1.032	**Chlorinated Cmpds.**—passes test (p. 500)
IR	98.0% of $C_{11}H_{12}O_3$ (M-6)		1.516–1.521	1 ml in 6 ml 70% alc, and in 1 ml 80% alc, gives clear solns	1.120–1.125	
IR	97.0% of $C_5H_{10}O_2$ (M-6)	2.0	1.383–1.385		0.886–0.889	
IR	99.0% of $C_9H_{10}O_3$ (M-6)	1.0 (phenol red TS)	1.520–1.523	1 ml in 4 ml 80% alc gives clear soln	1.127–1.129	
IR	98.0% to 101.0% of $C_9H_{10}O_3$ (M-11d)					**Arsenic**—3 ppm (M-18); **Heavy Metals**—10 ppm (M-24a); **Melting Range**—76° to 78° (p. 519, dry over P_2O_5/4 h); **Loss on Drying**—0.5% (p. 518, P_2O_5/4 h); **Res. on Ignit.**—0.05% (p. 533, 2-g samp)

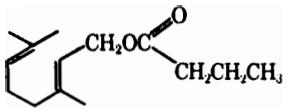
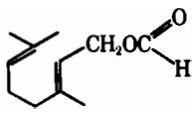
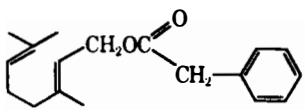
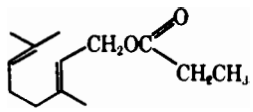
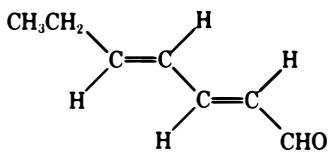
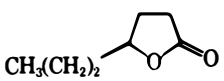
General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Eucalyptol (1,8-Cineol; Anhydride of Menthane 1:8 Diole; 1:8 Oxido-*p*-menthane; 1:8 Epoxy-*p*-menthane) [FEMA No. 2465]	154.25/$C_{10}H_{18}O$/ 	colorless liq/ characteristic odor; pungent, cooling taste	*s*—alc, most fixed oils, gly, prop glycol	provided
Eugenol (4-Allyl-2-methoxy-phenol; Eugenic Acid; 4-Allylguaiacol) [FEMA No. 2467]	164.20/$C_{10}H_{12}O_2$/ 	colorless to pale yel liq with a pungent spicy taste; darkens and thickens on exposure to air/ strong aromatic odor of clove	*m*—alc, chloroform, ether, fixed oils; *ss*—water	
Eugenyl Acetate (4-Allyl-2-methoxy-phenyl Acetate; Eugenol Acetate; Acetyl Eugenol; Aceteugenol) [FEMA No. 2469]	206.24/$C_{12}H_{14}O_3$/ 	fused solid, melts at warm room temp to a pale yel liq/ mild, clovelike	*s*—alc, ether; *ins*—water	provided
Farnesol (3,7,11-Trimethyl-2,6,10-dodecatrien-1-ol) [FEMA No. 2478]	222.36/$C_{15}H_{26}O$/ 	slightly yel liq/mild, oily odor	*ins*—water/263°	
Furfural (2-Furaldehyde; Pyromucic Aldehyde) [FEMA No. 2489]	96.09/$C_5H_4O_2$/ 	colorless to yel oily liq, turns reddish brown on long storage/ typical of cyclic aldehydes	*s*—water; *m*—alc	
Geraniol (trans-3,7-Dimethyl-2,6-octadien-1-ol) [FEMA No. 2507]	154.25/$C_{10}H_{18}O$/ 	colorless liq/ roselike	*s*—most fixed oils, prop glycol; *ss*—water; *ins*—gly/230°	provided
Geranyl Acetate (3,7-Dimethyl-2,6-octadien-1-yl Acetate) [FEMA No. 2509]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ floral	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/245°	provided
Geranyl Benzoate (3,7-Dimethyl-2,6-octadien-1-yl Benzoate) [FEMA No. 2511]	258.36/$C_{17}H_{22}O_2$/ 	slightly yellowish liq/floral, resembling ylang ylang oil	*m*—alc, chloroform *ins*—water/305°	

Requirements

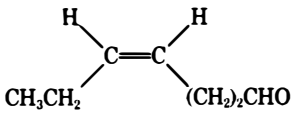
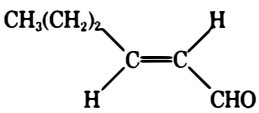
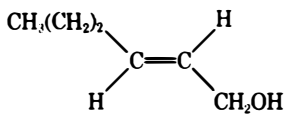
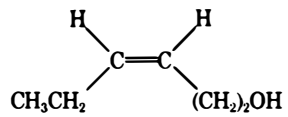
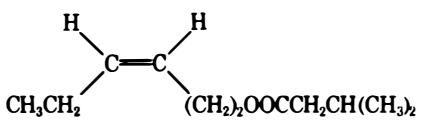
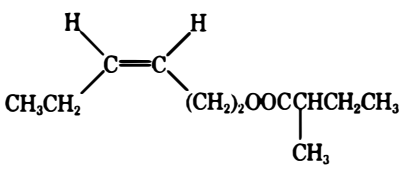
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR			1.455–1.460	1 ml in 5 ml 60% alc	0.921–0.924	**Angular Rotation**—between −0.5° and + 0.5° (p. 530, 100-mm tube); **Solidification Pt.**—NLT 0° (p. 538)
IR	100% of phenols by vol (M-10)		1.540–1.542	1 ml in 2 ml 70% alc	1.064–1.070	**Dist. Range**—NLT 95% between 250° and 255° (p. 478); **Hydrocarbons**—passes test (M-25)
IR	98.0% of $C_{12}H_{14}O_3$ (M-7)	1.0		1 ml in 5 ml 70% alc	1.077–1.082 (melted, supercooled)	**Solidification Pt.**—NLT 25° (p. 538)
IR	97.0% of $C_{15}H_{26}O$ (M-8a)		1.487–1.489		0.887–0.889 (20°)	
IR	96.0% of $C_5H_4O_2$ (M-2a)	1.0	1.522–1.528		1.154–1.158	
IR	88.0% of total alcohols, as $C_{10}H_{18}O$ (M-12)		1.469–1.478	1 ml in 3 ml 70% alc, remains in soln to 10 ml	0.870–0.885	**Aldehydes**—1.0% (M-16a); **Esters**—1.0% (M-23)
IR	90.0% of total esters, as $C_{12}H_{20}O_2$ (M-6)		1.458–1.464	1 ml in 8 ml 70% alc	0.900–0.914	
IR	95.0% of $C_{17}H_{22}O_2$ (M-6)	1.0	1.513–1.581	1 ml in 4 ml 90% alc gives clear soln	0.978–0.984	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Geranyl Butyrate (3,7-Dimethyl-2,6-octadien-1-yl Butyrate) [FEMA No. 2512]	224.34/$C_{14}H_{24}O_2$/ 	colorless to pale yel liq/fruity, roselike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/253°	provided
Geranyl Formate (3,7-Dimethyl-2,6-octadien-1-yl Formate) [FEMA No. 2514]	182.26/$C_{11}H_{18}O_2$/ 	colorless to pale yel liq/fresh, leafy, roselike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/216°	provided
Geranyl Phenylacetate (3,7-Dimethyl-2,6-octadien-1-yl Phenylacetate) [FEMA No. 2516]	272.39/$C_{18}H_{24}O_2$/ 	yel liq/honey-rose	*m*—alc, chloroform, ether; *ins*—water	provided
Geranyl Propionate (3,7-Dimethyl-2,6-octadien-1-yl Propionate) [FEMA No. 2517]	210.32/$C_{13}H_{22}O_2$/ 	colorless liq/ rosy, fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/253°	provided
***trans, trans*-2,4-Heptadienal** [FEMA No. 3164]	110.16/$C_7H_{10}O$/ 	slightly yel liq/ fatty, green odor	*s*—alc, most fixed oils; *ins*—water	
γ-Heptalactone [FEMA No. 2539]	128.17/$C_7H_{12}O_2$/ 	colorless, slightly oily liq/coconut, sweet, malty, caramel	*m*—alc, most fixed oils; *vss*—water	
Heptanal (Aldehyde C-7; Heptaldehyde) [FEMA No. 2540]	114.19/$C_7H_{14}O$/ $CH_3(CH_2)_5CHO$	colorless to slightly yel liq/penetrating, oily odor	*m*—alc, ether, fixed oils; *ss*—water/153°	provided
2-Heptanone (Methyl Amyl Ketone) [FEMA No. 2544]	114.19/$C_7H_{14}O$/ $CH_3CO(CH_2)_4CH_3$	colorless, mobile liq/ fruity, spicy	*m*—alc, ether; 1 ml in 250 ml water/151°	
3-Heptanone (Ethyl Butyl Ketone) [FEMA No. 2545]	114.19/$C_7H_{14}O$/ $CH_3(CH_2)_3COCH_2CH_3$	colorless, mobile liq/fruity, green, fatty odor	*m*—alc, ether; 1 ml in 70 ml water/149°	

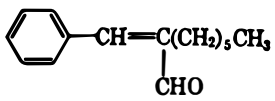
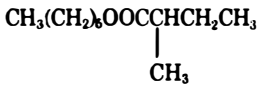
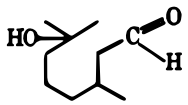
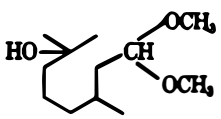
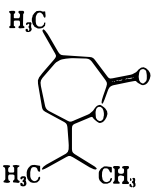
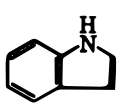
		Requirements				
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	92.0% of $C_{14}H_{24}O_2$ (M-6)	1.0	1.455–1.462	1 ml in 4 ml 80% alc	0.889–0.904	
IR	85.0% of $C_{11}H_{18}O_2$ (M-6)	3.0 (see M-15d)	1.457–1.466	1 ml in 3 ml 80% alc	0.906–0.920	
IR	97.0% of $C_{18}H_{24}O_2$ (M-6)	2.0	1.507–1.511	1 ml in 4 ml 90% alc gives clear soln	0.971–0.978	
IR	92.0% of $C_{13}H_{22}O_2$ (M-6)	1.0	1.456–1.464	1 ml in 4 ml 80% alc	0.896–0.913	
IR	92.0% of $C_7H_{10}O$ (M-8a)		1.478–1.480			
IR	98.0% of $C_7H_{12}O_2$ (M-8a)		1.439–1.445		0.997–1.004 (20°)	
IR	90.0% of $C_7H_{14}O$ (M-2a)	10.0	1.412–1.420	1 ml in 2 ml 70% alc gives clear soln	0.814–0.819	**Solubility in Bisulfite**—passes test (M-39)
IR	95.0% of $C_7H_{14}O$ (M-5b)				0.813–0.818	**Acidity**—0.05% (M-15a); **Dist. Range**—147° to 154° (p. 478); **Res. on Evap.**—5 mg/100 ml (p. 502 100-ml samp); **Water**—0.3% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	97.0% of $C_7H_{14}O$ (M-5b)				0.813–0.818	**Acidity**—0.02% (M-15a); **Dist. Range**—143° to 151° (p. 478); **Water**—0.3% (p. 552, *KF;* use freshly dist. pyridine as solvent)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
***cis*-4-Hepten-1-al** [FEMA No. 3289]	112.17/$C_7H_{12}O$/ 	slightly yel liq/ fatty, green odor	*s*—alc, most fixed oils; *ins*—water	
Heptyl Alcohol (Enanthic Alcohol) [FEMA No. 2548]	116.20/$C_7H_{16}O$/ $CH_3(CH_2)_5CH_2OH$	colorless liq/ citrus	*m*—alc, ether, most fixed oils; *ss*—water/175°	
Hexanal (Caproic Aldehyde; Hexaldehyde) [FEMA No. 2557]	100.16/$C_6H_{12}O$/ $CH_3(CH_2)_4CHO$	almost colorless liq/ fatty-green, grassy odor	*m*—alc, prop glycol, most fixed oils; *vss*—water	
Hexanoic Acid (Caproic Acid) [FEMA No. 2559]	116.16/$C_6H_{12}O_2$/ $CH_3(CH_2)_4COOH$	colorless to very pale yel, oily liq/ cheesy, sweatlike	*m*—alc, most fixed oils, ether; 1 ml in 250 ml water/223°	
***trans*-2-Hexen-1-al** [FEMA No. 2560]	98.15/$C_6H_{10}O$/ 	pale yel liq/strong, fruity-green, vegetablelike	*s*—alc, prop glycol, most fixed oils; *vss*—water	
***trans*-2-Hexen-1-ol** [FEMA No. 2562]	100.16/$C_6H_{12}O$/ 	almost colorless liq/ strong, fruity-green	*s*—alc, prop glycol, most fixed oils; *vss*—water	
***cis*-3-Hexen-1-ol** [FEMA No. 2563]	100.16/$C_6H_{12}O$/ 	colorless liq/ powerful, grassy-green	*s*—alc, prop glycol, most fixed oils; *vss*—water	
***cis*-3-Hexenyl Isovalerate** [FEMA No. 3498]	184.28/$C_{11}H_{20}O_2$/ 	colorless liq/ sweet, applelike	*s*—alc, prop glycol, most fixed oils; *ins*—water	
***cis*-3-Hexenyl 2-Methylbutyrate** [FEMA No. 3497]	184.28/$C_{11}H_{20}O_2$/ 	almost colorless liq/ powerful, fruity, like unripe apples	*s*—alc, most fixed oils; *ins*—water	

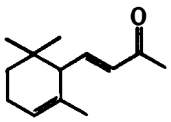
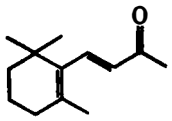
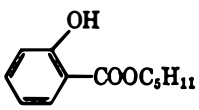
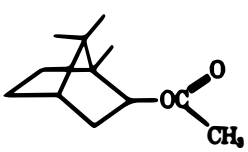
		Requirements				
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of $C_7H_{12}O_2$ (M-8a)		1.432–1.436			
IR	97.0% of $C_7H_{16}O$ (M-12)	1.0	1.423–1.427	1 ml in 2 ml 60% alc gives clear soln	0.820–0.824	**Aldehydes**—1.0% (M-16b)
	97.0% of $C_6H_{12}O$ (M-8a)	10.0	1.403–1.407		0.808–0.812	
IR	98.0% of $C_6H_{12}O_2$ (M-11a)		1.415–1.418		0.923–0.928	**Solidification Pt.**—NLT −4.5° (p. 538)
	92.0% of $C_6H_{10}O$ (M-8a)		1.445–1.449		0.841–0.848	
IR	95.0% of $C_6H_{12}O$ (M-8a)		1.437–1.442		0.836–0.841	
IR	98.0% of $C_6H_{12}O$ (M-8a)		1.439–1.441		0.846–0.850	
	95.0% of $C_{11}H_{20}O_2$ (M-8a)	2.0	1.429–1.435		0.869–0.874	
	90.0% of $C_{11}H_{20}O_2$ (M-8a)	2.0	1.430–1.434		0.876–0.880	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Hexyl Alcohol, Natural (1-Hexanol; Alcohol C-6) [FEMA No. 2567]	102.18/$C_6H_{14}O$/ $CH_3(CH_2)_4CH_2OH$	colorless, mobile liq/ mild, sweet, green	*m*—alc, ether; 1 ml in 175 ml water/157°	
Hexyl-2-butenoate [FEMA No. 3354]	170.24/$C_{10}H_{18}O_2$/ $CH_3(CH_2)_5OOCCH{=}CHCH_3$	colorless liq/fruity	*s*—alc, most fixed oils; *ins*—water, prop glycol	
α-Hexylcinnamaldehyde [FEMA No. 2569]	216.32/$C_{15}H_{20}O$/ 	pale yel liq/ jasminelike	*s*—most fixed oils; *ins*—gly, prop glycol	provided
Hexyl Isovalerate [FEMA No. 3500]	186.30/$C_{11}H_{22}O_2$/ $(CH_3)_2CHCH_2COOCH_2(CH_2)_4CH_3$	colorless liq/ pungent, fruity	*s*—alc, most fixed oils; *ins*—water	
Hexyl 2-Methylbutyrate [FEMA No. 3499]	186.30/$C_{11}H_{22}O_2$/ 	colorless liq/ strong, fresh-green, fruity	*s*—alc, most fixed oils; *ins*—water	
Hydroxycitronellal (7-Hydroxy-3,7-dimethyl Octanal) [FEMA No. 2583]	172.27/$C_{10}H_{20}O_2$/ 	colorless liq/sweet, floral, lilylike	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Hydroxycitronellal Dimethyl Acetal (7-Hydroxy-3,7-dimethyl Octanal: Acetal) [FEMA No. 2585]	218.34/$C_{12}H_{26}O_3$/ 	colorless liq/floral	*s*—most fixed oils, prop glycol; *ins*—gly	provided
6-Hydroxy-3,7-Dimethyl-octanoic Acid Lactone [FEMA No. 3355]	170.25/$C_{10}H_{18}O_2$/ 	colorless, low-melting solid/odor like maple syrup or brown sugar	*s*—alc; *vss*—water	
Indole [FEMA No. 2593]	117.15/C_8H_7N/ 	white, lustrous, flaky, cryst solid/unpleasant odor in high conc, but free from fecal quality; odor becomes floral in higher dilutions	*s*—alc, most fixed oils, prop glycol; *ins*—gly	provided

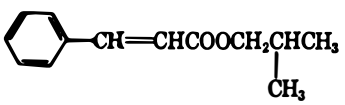
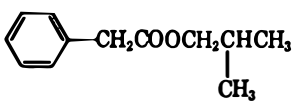
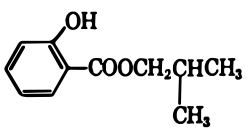
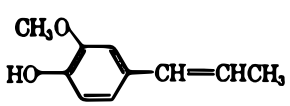
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	96.5% of $C_6H_{14}O$ (M-11e)				0.816–0.821	Acidity—0.01% (M-15a)
IR	95.0% of $C_{10}H_{18}O_2$ (M-8a)		1.428–1.449		0.880–0.900	
IR	95.0% of $C_{15}H_{20}O$ (M-3)	5.0	1.548–1.552	1 ml in 1 ml 90% alc	0.953–0.959	Chlorinated Cmpds.—passes test (p. 500)
	95.0% of $C_{11}H_{22}O_2$ (M-8a)	2.0	1.417–1.421		0.853–0.857	
	95.0% of $C_{11}H_{22}O_2$ (M-8a)	2.0	1.416–1.421		0.854–0.859	
IR	95.0% of $C_{10}H_{20}O_2$ (M-2a)	5.0	1.447–1.450	1 ml in 1 ml 50% alc	0.918–0.923	Solubility in Bisulfite—passes test (M-39)
IR	95.0% of $C_{12}H_{26}O_2$ (M-1b)	1.0	1.441–1.444	1 ml in 2 ml 50% alc	0.925–0.930	Free Aldehydes—3.0% (M-16a)
	90.0% of $C_{10}H_{18}O_2$ (M-8a)		1.457–1.461		0.966–0.973	
IR				1 g in 3 ml 70% alc		Solidification Pt.—NLT 51° (p. 538, dry over H_2SO_4)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
α-Ionone [4(2,6,6-Trimethyl-2-cyclohexenyl)-3-butene-2-one] [FEMA No. 2594]	192.30/$C_{13}H_{20}O$/ 	colorless to pale yel liq/warm, woody, violet-floral	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/237°	provided
β-Ionone [4(2,6,6-Trimethyl-1-cyclohexenyl)-3-butene-2-one] [FEMA No. 2595]	192.30/$C_{13}H_{20}O$/ 	colorless to pale straw-colored liq/ warm, woody, dry	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/239°	provided
Isoamyl Acetate (Amyl Acetate; β-Methyl Butyl Acetate) [FEMA No. 2055]	130.19/$C_7H_{14}O_2$/ $CH_3COOC_5H_{11}$	colorless liq/ fruity, pearlike, bananalike	*m*—alc, ether, ethyl acetate, most fixed oils; *ss*—water; *ins*—gly, prop glycol/145°	provided
Isoamyl Butyrate (Amyl Butyrate) [FEMA No. 2060]	158.24/$C_9H_{18}O_2$/ $CH_3(CH_2)_2COOC_5H_{11}$	colorless liq/ fruity	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/179°	provided
Isoamyl Formate (Amyl Formate) [FEMA No. 2069]	116.16/$C_6H_{12}O_2$/ $HCOOC_5H_{11}$	colorless liq/ plumlike	*s*—alc, most fixed oils, prop glycol; *ss*—water; *ins*—gly/124°	provided
Isoamyl Hexanoate (Amyl Hexanoate; Isoamyl Caproate; Pentyl Hexanoate) [FEMA No. 2075]	186.29/$C_{11}H_{22}O_2$/ $CH_3(CH_2)_4COOC_5H_{11}$	colorless liq/ fruity	*s*—alc, fixed oils; *ins*—gly, prop glycol, water/222°	provided
Isoamyl Isovalerate (Amyl Valerate; Amyl Isovalerate) [FEMA No. 2085]	172.27/$C_{10}H_{20}O_2$/ $(CH_3)_2CHCH_2COOC_5H_{11}$	colorless liq/ fruity, applelike	*m*—alc, most fixed oils; *ss*—prop glycol; *ins*—water; 1 ml in 6 ml 70% alc/192°	
Isoamyl Salicylate (Amyl Salicylate) [FEMA No. 2084]	208.26/$C_{12}H_{16}O_3$/ 	colorless liq/ pleasant odor	*m*—alc, chloroform, ether, most fixed oils; *ins*—gly, prop glycol, water	provided
Isobornyl Acetate [FEMA No. 2160]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq when fresh, develops very pale straw shade on storage/ camphoraceous, piney, balsamic	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly, water/227°	provided

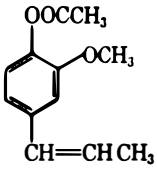
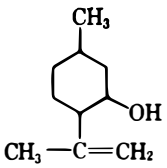
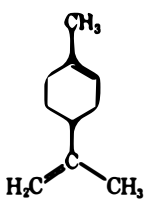
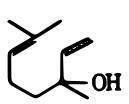
		Requirements				
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	99.0% of $C_{13}H_{20}O$ (M-3)		1.497–1.502	1 mli n 10 ml 60% alc	0.927–0.933	
IR	90.0% of $C_{13}H_{20}O$ (M-3)		1.517–1.522		0.940–0.947	
IR	95.0% of $C_7H_{14}O_2$ (M-6)	1.0	1.400–1.404	1 ml in 3 ml 60% alc gives clear soln	0.868–0.878	
IR	98.0% of $C_9H_{18}O_2$ (M-6)	1.0	1.409–1.414	1 ml in 4 ml 70% alc	0.860–0.864	
IR	92.0% of $C_8H_{12}O_2$ (M-6)	1.0	1.396—1.400	1 ml in 4 ml 60% alc, remains in soln to 10 ml	0.878–0.885	
IR	98.0% of $C_{11}H_{22}O_2$ (M-6)	1.0	1.418–1.422	1 ml in 3 ml 80% alc gives clear soln	0.858–0.863	
	98.0% of $C_{10}H_{20}O_2$ (M-6)	2.0	1.411–1.414		0.851–0.857	
IR	98.0% of $C_{12}H_{16}O_3$ (M-6)	1.0	1.505–1.509	1 ml in 3 ml 90% alc, remains in soln on dilution	1.047–1.053	
IR	97.0% of $C_{12}H_{20}O_2$ (M-6)	1.0	1.462–1.465	1 ml in 3 ml 70% alc	0.980–0.984	**Angular Rotation**—between −1° and +1° (p. 530, 100-mm tube)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Isobutyl Acetate [FEMA No. 2175]	116.16/$C_6H_{12}O_2$/ $CH_3COOCH_2CH(CH_3)_2$	colorless liq/fruity, bananalike on dilution	*s*—alc, most fixed oils, prop glycol; 1 ml in 180 ml water/116°	provided
Isobutyl Alcohol [FEMA No. 2179]	74.12/$C_4H_{10}O$/ $(CH_3)_2CHCH_2OH$	colorless, mobile liq/penetrating, winy	*m*—alc, ether; 1 ml in 140 ml water/108°	
Isobutyl-2-butenoate [FEMA No. 3432]	142.19/$C_8H_{14}O_2$/ $(CH_3)_2CHCH_2OOCCH{=}CHCH_3$	colorless liq/ powerful, fruity	*s*—alc, prop glycol, most fixed oils; *ss*—water	
Isobutyl Butyrate (2-Methyl Propanyl Butyrate) [FEMA No. 2187]	144.22/$C_8H_{16}O_2$/ $C_3H_7COOCH_2CH(CH_3)_2$	colorless liq/ sweet, fruity, applelike, pineapplelike	*s*—alc, most fixed oils; *ss*—water; *ins*—gly	provided
Isobutyl Cinnamate [FEMA No. 2193]	204.27/$C_{13}H_{16}O_2$/ 	colorless liq/ sweet, fruity, balsamic	*m*—alc, chloroform, ether, most fixed oils; *ins*—water	
Isobutyl Phenylacetate [FEMA No. 2210]	192.23/$C_{12}H_{16}O_2$/ 	colorless liq/rose, honeylike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water	provided
Isobutyl Salicylate [FEMA No. 2213]	194.23/$C_{11}H_{14}O_3$/ 	colorless liq/ orchidlike	*s*—most fixed oils; *ins*—gly, prop glycol	provided
Isobutyraldehyde [FEMA No. 2220]	72.11/C_4H_8O/ $(CH_3)_2CHCHO$	colorless, mobile liq/sharp, pungent	*m*—alc, ether; 1 ml in 125 ml water	
Isobutyric Acid (2-Methyl Propanoic Acid; Isophenyl-formic Acid) [FEMA No. 2222]	88.11/$C_4H_8O_2$/ $(CH_3)_2CHCOOH$	colorless liq/strong, penetrating odor of rancid butter	*m*—alc, most fixed oils, gly, prop glycol; *ins*—water	
Isoeugenol (2-Methoxy-4-propenylphenol) [FEMA No. 2468]	164.20/$C_{10}H_{12}O_2$/ 	pale yel, viscous liq/floral, carnationlike	*s*—most fixed oils, prop glycol; *ins*—gly	provided

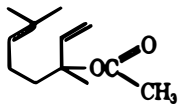
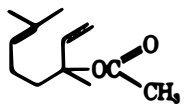
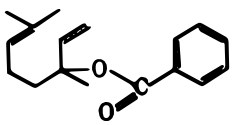
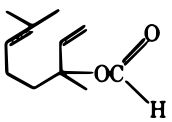
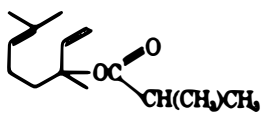
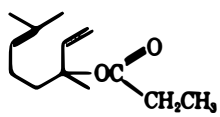
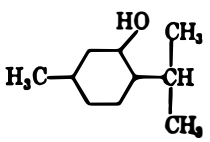
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	90.0% of $C_6H_{12}O_2$ (M-6)	1.0	1.389–1.392		0.862–0.871	
IR					0.799–0.801	
IR	95.0% of $C_8H_{14}O_2$ (M-8a)		1.426–1.430		0.880–0.900	
IR	98.0% of $C_8H_{16}O_2$ (M-6)	1.0	1.402–1.405	1 ml in 8 ml 60% alc	0.858–0.863	
IR	98.0% of $C_{13}H_{16}O_2$ (M-6)	1.0	1.539–1.541	1 ml in 3 ml 80% alc gives clear soln	1.001–1.004	
IR	98.0% of $C_{12}H_{16}O_2$ (M-6)	1.0	1.486–1.488	1 ml in 2 ml 80% alc, remains in soln to 10 ml	0.984–0.988	
IR	98.0% of $C_{11}H_{14}O_3$ (M-6)	1.0 (phenol red TS)	1.507–1.510	1 ml in 9 ml 80% alc, remains in soln to 10 ml	1.062–1.066	
IR	98.0% of C_4H_8O (M-5a)	0.3			0.783–0.788	**Acidity**—0.3% (M-15b)
IR	99.0% to 101.1% of $C_4H_8O_2$ (M-11a)		1.392–1.395		0.944–0.948	**Reducing Subs.**—passes test (M-36)
IR	99.0% of phenols by vol (M-10)		1.572–1.577	1 ml in 5 ml 50% alc	1.079–1.085	**Solidification Pt.**—NLT 12° (p. 538)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Isoeugenyl Acetate (2-Methoxy-4-propenyl Phenyl Acetate) [FEMA No. 2470]	206.24/$C_{12}H_{14}O_3$/ 	white crystals/ spicy, clovelike	*s*—alc, chloroform, ether; *ins*—water	
Isopropyl Acetate [FEMA No. 2926]	102.13/$C_5H_{10}O_2$/ $CH_3COOCH(CH_3)_2$	colorless, mobile liq/ characteristic odor	*m*—alc, ether, fixed oils; 1 ml in 35 ml water/88°	
Isopulegol (*p*-Menth-4-en-3-ol) [FEMA No. 2962]	154.25/$C_{10}H_{18}O$/ 	colorless liq/harsh, camphoraceous, mintlike, with rose leaf and geranium background		provided
Isovaleric Acid (Isopropylacetic Acid) [FEMA No. 3102]	102.13/$C_5H_{10}O_2$/ $(CH_3)_2CHCH_2COOH$	colorless liq/ disagreeable, rancid, cheeselike	*s*—alc, chloroform, ether, water	
Lauric Acid [see p. 165]				
Lauryl Alcohol, Natural (1-Dodecanol; Alcohol C-12) [FEMA No. 2617]	186.34/$C_{12}H_{26}O$/ $CH_3(CH_2)_{10}CH_2OH$	colorless liq above 21°/fatty odor	*s*—most fixed oils, prop glycol; *ins*—gly, water/259°	provided
Lauryl Aldehyde (Aldehyde C-12; Dodecanal) [FEMA No. 2615]	184.32/$C_{12}H_{24}O$/ $CH_3(CH_2)_{10}CHO$	colorless to light yel liq/fatty odor	*s*—alc, most fixed oils, prop glycol (may be turbid); *ins*—gly, water/249°	provided
***d*-Limonene** (*d*-*p*-Mentha-1,8-diene; Cinene) [FEMA No. 2633]	136.25/$C_{10}H_{16}$/ 	colorless liq/mildly citrus odor, free from camphoraceous and terpenelike notes	*m*—alc, most fixed oils; *ss*—gly; *ins*—prop glycol, water	provided
***l*-Limonene** (*l*-*p*-Mentha-1,8-diene)	136.25/$C_{10}H_{16}$/	colorless liq/ refreshing, light, clean odor	*m*—alc, most fixed oils; *ins*—water	
Linalool (3,7-Dimethyl-1,6-octadien-3-ol) [FEMA No. 2635]	154.25/$C_{10}H_{18}O$/ 	colorless liq/ pleasant, floral odor	*s*—fixed oils, prop glycol; *ins*—gly	provided

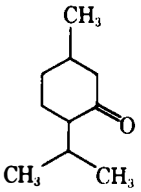
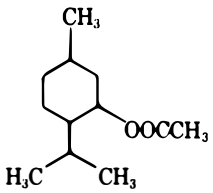
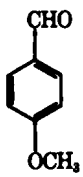
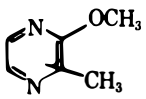
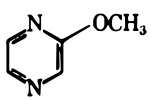
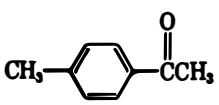
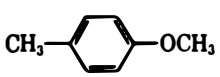
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{12}H_{14}O_2$ (M-6)	2.0 (phenol red TS)		1 g in 27 ml 95% alc gives clear soln		**Solidification Pt.**—NLT 76° (p. 538)
IR	99.0% of $C_5H_{10}O_2$ (M-11c)				0.866–0.869	
IR	95.0% of $C_{10}H_{18}O$ (M-12)	1.0	1.470–1.475	1 ml in 4 ml 60% alc gives clear soln	0.904–0.913	**Aldehydes**—1.0% (M-16a); **Angular Rotation**—between 0° and −7° (p. 530, 100-mm tube)
IR	99.0% of $C_5H_{10}O_2$ (M-11a)		1.403–1.405		0.928–0.931	
IR	97.0% of $C_{12}H_{26}O$ (M-12)	1.0	1.440–1.444	1 ml in 3 ml 70% alc, remains clear to 10 ml	0.830—0.836	**Solidification Pt.**—NLT 21° (p. 538)
IR	92.0% of $C_{12}H_{24}O$ (M-2a)	10.0	1.433–1.439		0.826–0.836	
IR	93.0% of $C_{10}H_{16}$ (M-8a)		1.471–1.474		0.838–0.843	**Angular Rotation**—between +96° and +104° (p. 530, 100-mm tube); **Peroxide Value**—5.0 (M-32)
	95.0% of $C_{10}H_{16}$ (M-8a)		1.469–1.473		0.837–0.841	**Angular Rotation**—min. −90° (p. 530, 100-mm tube); **Peroxide Value**—5.0 (M-32)
IR	92.0% of $C_{10}H_{18}O$ (M-9a)		1.461–1.465	1 ml in 4 ml 60% alc	0.858–0.867	**Angular Rotation**—between −2° and +2° (p. 530, 100-mm tube); **Esters**—0.5% (M-23)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Linalyl Acetate (3,7-Dimethyl-1,6-octadien-3-yl Acetate) [FEMA No. 2636]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ floral, fruity	*m*—alc; *s*—fixed oils; *ss*—prop glycol; *ins*—gly; water/220°	provided
Linalyl Acetate, Synthetic (3,7-Dimethyl-1,6-octadien-3-yl Acetate) [FEMA No. 2636]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/ floral, fruity	*m*—alc; *s*—fixed oils; *ss*—prop glycol; *ins*—gly; water/220°	provided
Linalyl Benzoate (3,7-Dimethyl-1,6-octadien-3-yl Benzoate) [FEMA No. 2638]	258.36/$C_{17}H_{22}O_2$/ 	yellowish to brownish yel liq/tuberoselike	*s*—alc, chloroform, ether; *ins*—water/263°	
Linalyl Formate (3,7-Dimethyl-1,6-octadien-3-yl Formate) [FEMA No. 2642]	182.26/$C_{11}H_{18}O_2$/ 	colorless liq/fresh, citrus, green, herbaceous, bergamotlike	*s*—alc, most fixed oils; *ss*—prop glycol, water; *ins*—gly/202°	provided
Linalyl Isobutyrate (3,7-Dimethyl-2,6-octadien-3-yl Isobutyrate) [FEMA No. 2640]	224.34/$C_{14}H_{24}O_2$/ 	colorless to slightly yel liq/sweet, fresh, rosy	*m*—alc, chloroform, ether; *ins*—water/ 230°	provided
Linalyl Propionate (3,7-Dimethyl-2,6-octadien-3-yl Propionate) [FEMA No. 2645]	210.32/$C_{13}H_{22}O_2$/ 	colorless or almost colorless liq/fresh, floral, sweet, fruity, pearlike	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—gly/226°	provided
Menthol (3-*p*-Menthanol) [NOTE: *l*-Menthol is obtained from natural sources or by synthetic processes; *dl*-menthol is produced synthetically.] [FEMA No. 2665]	156.27/$C_{10}H_{20}O$/ 	colorless, hexagonal crystals, usually needlelike; fused masses or cryst powder; m.p. 43°/ pleasant, peppermintlike	*vs*—alc, fixed and vol oils; *ss*—water	

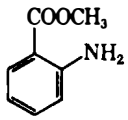
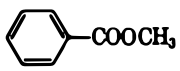
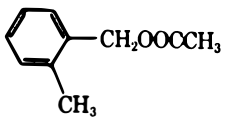
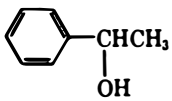
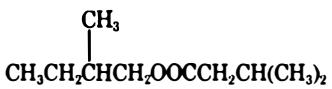
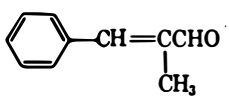
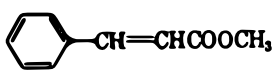
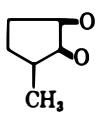
	Requirements					
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	90.0% of total esters, as $C_{12}H_{20}O_2$ (M-6)	1.0	1.449–1.457	1 ml in 5 ml 70% alc	0.895–0.914	**Angular Rotation**—between −1° and +1° (p. 530, 100-mm tube)
IR	97.0% of $C_{12}H_{20}O_2$ (M-6)	1.0	1.449–1.452		0.895–0.908	
IR	75.0% of $C_{17}H_{22}O_2$ (M-6)	5.0	1.505–1.520	1 ml in 1 ml 90% alc gives clear soln	0.980–0.999	
IR	90.0% of $C_{11}H_{18}O_2$ (M-6)	3.0	1.453–1.458	1 ml in 6 ml 70% alc	0.910–0.918	
IR	95.0% of $C_{14}H_{24}O_2$ (M-7)	1.0	1.446–1.451	1 ml in 3 ml 80% alc gives clear soln	0.882–0.888	
IR	92.0% of $C_{13}H_{22}O_2$ (M-6)	1.0	1.450–1.455	1 ml in 7 ml 70% alc	0.895–0.902	**Angular Rotation**—between −1° and +1° (p. 530, 100-mm tube)
IR						**Arsenic**—3 ppm (M-18); **Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27); **Melting Range** (*l*-menthol)—41° to 43° (p. 519); **Nonvol. Res.**—0.05% (M-30); **Readily Ox. Subs.** (*dl*-menthol)—passes test (M-35); **Solidification Pt.** (*dl*-menthol)—27° to 28° (M-14b); **Specific Rotation** (*l*-menthol)—between −45° and −51°; **Specific Rotation** (*dl*-menthol)—between −2° and +2° (p. 530)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
***l*-Menthone** (*l-p*-Menthan-3-one) [FEMA No. 2667]	154.25/$C_{10}H_{18}O$/ 	almost colorless liq/ mintlike	*s*—alc, most fixed oils; *vss*—water	
***dl*-Menthyl Acetate** (*dl-p*-Menthan-3-yl Acetate) [FEMA No. 2668]	198.31/$C_{12}H_{22}O_2$/ 	colorless liq/mild, minty	*s*—alc, prop glycol, most fixed oils; *ss*—water, gly	
***l*-Menthyl Acetate** (*l-p*-Menthan-3-yl Acetate) [FEMA No. 2668]	198.31/$C_{12}H_{22}O_2$/	colorless liq/mild, minty	*s*—alc, prop glycol, most fixed oils; *ss*—water, gly	
***p*-Methoxybenzaldehyde** (Anisic Aldehyde; *p*-Anisaldehyde) [FEMA No. 2670]	136.15/C_8H_8O/ 	colorless to slightly yel liq/ hawthornlike	*m*—alc, ether, most fixed oils; *s*—prop glycol; *ins*—gly, water	provided
2-Methoxy-3(5)-methylpyrazine [FEMA No. 3183]	124.14/$C_6H_8N_2O$/ 	colorless liq/roasted, like hazlenut	*s*—water, organic solvents	
2-Methoxypyrazine [FEMA No. 3302]	110.12/$C_5H_6N_2O$/ 	colorless to yellowish liq/nutty, cocoalike	*s*—alc; *ins*—water/ 61° (29 mm)	
4'-Methyl Acetophenone (Methyl *p*-Tolyl Ketone) [FEMA No. 2677]	134.18/$C_9H_{10}O$/ 	colorless or nearly colorless liq/fruity floral odor resembling acetophenone	*s*—most fixed oils, prop glycol; *ins*—gly	provided
***p*-Methyl Anisole** (*p*-Cresyl Methyl Ether; Methyl *p*-Cresol) [FEMA No. 2681]	122.17/$C_8H_{10}O$/ 	colorless liq/ ylang-ylang	*s*—most fixed oils; *ins*—gly, prop glycol	provided

		Requirements				
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
	96.0% of $C_{10}H_{18}O$ (M-8a)		1.448–1.453		0.888–0.895	**Angular Rotation**—min. −20° (p. 530, 100-mm tube)
	97.0% of $C_{12}H_{22}O_2$ (M-8a)	2.0	1.443–1.447		0.919–0.924	
	98.0% of $C_{12}H_{22}O_2$ (M-8a)	2.0	1.443–1.447		0.919–0.924	**Angular Rotation**—min. −69.0° (p. 530, 100-mm tube)
IR	97.5% of C_8H_8O (M-2a)	6.0	1.571–1.574	1 ml in 7 ml 50% alc gives clear soln	1.119–1.123	**Chlorinated Cmpds.**—passes test (p. 500)
IR	99.0% of $C_8H_8N_2O$ (M-8b)		1.506–1.510		1.060–1.090 (20°)	
IR	99.0% of $C_5H_6N_2O$ (M-8b)		1.508–1.511		1.110–1.140 (20°)	**Dist. Range**—145° to 150° (p. 478)
IR	98.0% of $C_9H_{10}O$ (M-3)		1.532–1.535	1 ml in 10 ml 50% alc	1.001–1.004	**Chlorinated Cmpds.**—passes test (p. 500)
IR			1.510–1.513	1 ml in 3 ml 80% alc, remains in soln on dilution	0.966–0.970	**Cresol**—0.5% (M-33a)

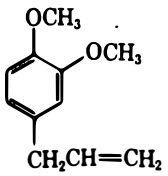
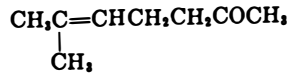
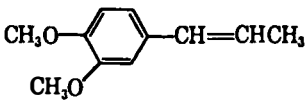
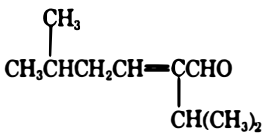
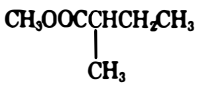
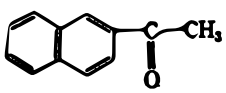
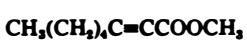
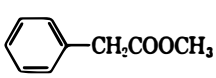
General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Methyl Anthranilate [FEMA No. 2682]	151.16/$C_8H_9NO_2$/ 	colorless to pale yel liq with bluish fluorescence/ grapelike	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Methyl Benzoate [FEMA No. 2683]	136.15/$C_8H_8O_2$/ 	colorless liq/deep, pungent, floral	*s*—alc, most fixed oils, prop glycol; *ins*—gly	provided
Methylbenzyl Acetate[a] (Tolyl Acetate So-Called)	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/ sweet, nutty	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly	provided
α-Methylbenzyl Alcohol (Methyl Phenylcarbinol; α-Phenethyl Alcohol) [FEMA No. 2685]	122.17/$C_8H_{10}O$/ 	colorless liq above room temp/mild, hyacinthlike	*s*—most fixed oils, prop glycol; *vs*—gly	provided
2-Methylbutyl Isovalerate (2-Methylbutyl-3-methylbutanoate) [FEMA No. 2753]	172.27/$C_{10}H_{20}O_2$/ 	colorless liq/ herbaceous, fruity	*s*—alc, most fixed oils; *ins*—water	
α-Methylcinnamaldehyde [FEMA No. 2697]	146.19/$C_{10}H_{10}O$/ 	yel liq/cinnamonlike	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Methyl Cinnamate [FEMA No. 2698]	162.19/$C_{10}H_{10}O_2$/ 	white to slightly yel cryst mass/ fruity, balsamic	*s*—alc, most fixed oils, gly, prop glycol; *ins*—water	provided
Methyl Cyclopentenolone (3-Methylcyclopentane-1,2-dione) [FEMA No. 2700]	112.13/$C_6H_8O_2$/ 	white, cryst powder/nutty odor, maple-licorice aroma in dilute soln	*s*—alc, prop glycol; *ss*—most fixed oils; 1 g in 72 ml water	

[a] The specifications for *Methylbenzyl Acetate* are based on EOA No. 228. The substance previously known as *α-Methylbenzyl Acetate* (see *FCC II*, p. 514) appears in this table under the new title *Methyl Phenylcarbinyl Acetate*.

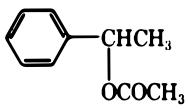
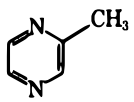
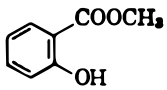
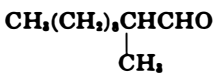
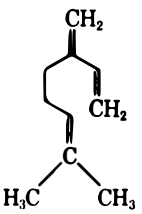
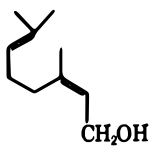
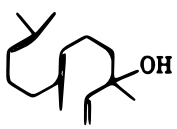
			Requirements			
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_8H_9NO_2$ (M-6)		1.582–1.584 (as super-cooled liq)	1 ml in 5 ml 60% alc, remains in soln to 10 ml	1.161–1.169	**Solidification Pt.**—NLT 23.8° (p. 538)
IR	98.0% of $C_8H_8O_2$ (M-6)	1.0	1.514–1.518	1 ml in 4 ml 60% alc	1.082–1.088	**Chlorinated Cmpds.**—passes test (p. 500)
IR	98.0% of $C_{10}H_{12}O_2$ (M-6)	1.0	1.501–1.504	1 ml in 2 ml 70% alc, remains clear on dilution	1.030–1.035	**Chlorinated Cmpds.**—passes test (p. 500)
IR	99.0% of $C_8H_{10}O$ (M-12)		1.525–1.529	1 ml in 3 ml 50% alc	1.009–1.014	**Ketones**—1.0% (M-16a); **Solidification Pt.**—NLT 19° (p. 538)
	94.0% of $C_{16}H_{20}O_2$ (M-8a)	2.0	1.413–1.416		0.852–0.857	
IR	97.0% of $C_{16}H_{10}O$ (M-2a)	5.0	1.602–1.607	1 ml in 2 ml 70% alc, remains clear on dilution	1.035–1.039	
IR	98.0% of $C_{16}H_{10}O_2$ (M-6)	2.0		1 ml in 4 ml 80% alc		**Arsenic**—3 ppm (M-18); **Chlorinated Cmpds.**—passes test (p. 500); **Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27)
				1 g in 5 ml 90% alc		**Heavy Metals**—0.004% (M-24a); **Melting Range**—104° to 108° (p. 519)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Methyl Eugenol (Eugenyl Methyl Ether;1,2-Dimethoxy-4-allylbenzene) [FEMA No. 2475]	178.23/$C_{11}H_{14}O_2$/ 	colorless to pale yel liq/delicate, clove-carnation	*s*—most fixed oils; *ins*—gly, prop glycol	provided
6-Methyl-5-hepten-2-one (Methyl Heptenone) [FEMA No. 2707]	126.20/$C_8H_{14}O$/ 	slightly yel liq/ sharp, citrus-lemongrass	*m*—alc, chloroform, ether; *ins*—water	provided
Methyl Isoeugenol (4-Allyl-1,2-dimethoxy Benzene; Isoeugenyl Methyl Ether; 4-Propenyl Veratrole [FEMA No. 2476]	178.23/$C_{11}H_{14}O_2$/ 	colorless to pale yel liq/delicate, clove-carnation	*s*—most fixed oils; *ins*—gly, prop glycol	provided
5-Methyl-2-isopropyl-2-hexenal [FEMA No. 3406]	154.25/$C_{10}H_{18}O$/ 	slightly yel liq/ herbaceous, woody, fruity, chocolate	*s*—alc, most fixed oils; *ins*—water, prop glycol	
Methyl 2-Methylbutyrate (Methyl 2-Methyl-butanoate) [FEMA No. 2719]	116.16/$C_6H_{12}O_2$/ 	almost colorless liq/ sweet, fruity, applelike	*s*—alc, most fixed oils; *ins*—water	
Methyl β-Naphthyl Ketone (2-Acetonaphthone) [FEMA No. 2723]	170.21/$C_{12}H_{10}O$/ 	white or nearly white cryst solid/orange blossom	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly	provided
Methyl 2-Octynoate (Methyl Heptine Carbonate) [FEMA No. 2729]	154.21/$C_9H_{14}O_2$/ 	colorless to slightly yel liq/powerful, unpleasant, violetlike when diluted	*s*—most fixed oils; *ss*—prop glycol; *ins*—gly	provided
4-Methyl-2-pentanone (Methyl Isobutyl Ketone) [FEMA No. 2731]	100.16/$C_6H_{12}O$/ 	colorless, mobile liq/fruity, ethereal	*m*—alc, ether; 1 ml in 50 ml water	
Methyl Phenylacetate [FEMA No. 2733]	150.18/$C_9H_{10}O_2$/ 	colorless or nearly colorless liq/ honeylike, jasminelike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/215°	provided

			Requirements			
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR			1.532–1.536	1 ml in 2 ml 70% alc, remains clear to 10 ml	1.032–1.036	**Eugenol**—1.0% (M-33a)
IR	95.0% of $C_9H_{14}O$ (M-3)		1.438–1.442	1 ml in 2 ml 70% alc gives clear soln	0.846–0.851	
IR			1.566–1.569	1 ml in 2 ml 70% alc, remains in soln to 10 ml	1.047–1.053	**Isoeugenol**—1.0% (M-33a)
IR	90.0% of $C_{10}H_{18}O$ (M-8a)		1.448–1.453		0.845–0.860	
	92.0% of $C_9H_{12}O_2$ (M-8a)	2.0	1.393–1.397		0.879–0.883	
IR	99.0% of $C_{10}H_{16}O$ (M-3)			1 g in 5 ml 95% alc		**Solidification Pt.**—NLT 53° (p. 538)
IR	96.0% of $C_9H_{14}O_2$ (M-6)	1.0	1.446–1.449	1 ml in 5 ml 70% alc	0.919–0.924	**Chlorinated Cmpds.**—passes test (p. 500)
	99.0% of $C_9H_{12}O$ (M-5a)				0.796–0.799	**Acidity**—0.01% (M-15a); **Dist. Range**—114° to 117° (p. 478); **Water**—0.1% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_9H_{10}O_2$ (M-6)	1.0	1.503–1.509	1 ml in 6 ml 60% alc	1.061–1.067	**Chlorinated Cmpds.**—passes test (p. 500)

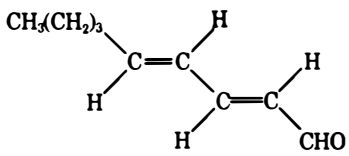
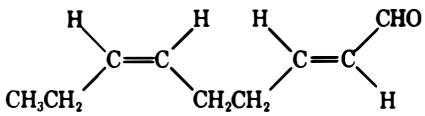
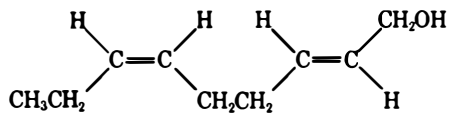
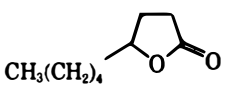
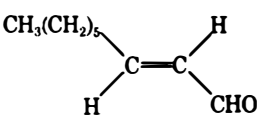
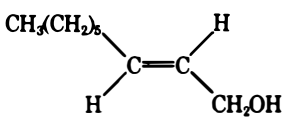
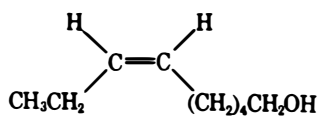
General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Methyl Phenylcarbinyl Acetate[b] (α-Phenyl Ethyl Acetate) [FEMA No. 2684]	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/ gardenialike	*s*—most fixed oils, gly; *ins*—water	
2-Methylpyrazine [FEMA No. 3309]	94.12/$C_5H_6N_2$/ 	colorless to slightly yel liq/nutty, cocoalike	*m*—water, alc, acetone, most fixed oils/137°	
Methyl Salicylate [FEMA No. 2745]	152.15/$C_8H_8O_3$/ 	colorless, yellowish, or reddish liq; optically inactive/ odor and taste of wintergreen	*s*—alc, glacial acetic acid; *ss*—water/222° (decomp)	
2-Methylundecanal (Aldehyde C-12 MNA; Methyl *n*-Nonyl Acetaldehyde) [FEMA No. 2749]	184.32/$C_{12}H_{24}O$/ 	colorless to slightly yel liq/fatty odor	*s*—fixed oils, alc, prop glycol (may be turbid); *ins*—gly	provided
Myrcene (7-Methyl-3-methylene-1,6-octadiene) [FEMA No. 2762]	136.24/$C_{10}H_{16}$/ 	colorless to pale yel liq/sweet, balsamic	*s*—alc, most fixed oils; *ins*—water	
Myristic Acid [see p. 204]				
Nerol (*cis*-3,7-Dimethyl-2,6-octadien-1-ol) [FEMA No. 2770]	154.25/$C_{10}H_{18}O$/ 	colorless liq/fresh, sweet, roselike	*m*—alc, chloroform, ether; *ins*—water/227°	
Nerolidol (3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol) [FEMA No. 2772]	222.37/$C_{15}H_{26}O$/ 	colorless to straw-colored liq/faint, floral, roselike, applelike	*s*—most fixed oils, prop glycol; *ins*—gly	provided

[b] The specifications for *Methyl Phenylcarbinyl Acetate*, previously known in *FCC II* (p. 514) as *α-Methylbenzyl Acetate*, are based on EOA No. 46.

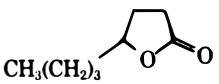
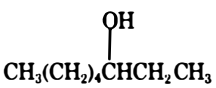
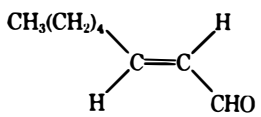
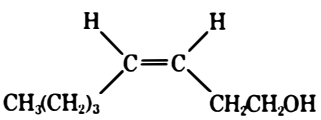
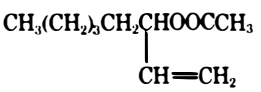
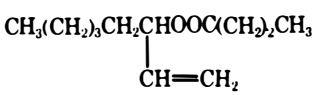
		Requirements				
I.D. Test[4]	Assay Min (%)[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_{10}H_{12}O_2$ (M-6)	2.0	1.493–1.497	1 ml in 7 ml 60% alc	1.023–1.026	**Chlorinated Cmpds.**—passes test (p. 500)
IR	99.0% of $C_5H_6N_2$ (M-8a)		1.504–1.506		1.010–1.030 (20°)	**Water**—0.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_9H_8O_3$ (M-6)	1.0	1.535–1.538	1 ml in 7 ml 70% alc (may be slightly cloudy)	1.180–1.185	
IR	94.0% of $C_{12}H_{24}O$ (M-2a)	10.0	1.431–1.436		0.822–0.830	
	90.0% of $C_{10}H_{16}$ (M-8a)		1.466–1.471		0.789–0.793	**Peroxide Value**—50.0 (M-32)
IR	95.0% of total alcohols, as $C_{10}H_{18}O$ (M-12)		1.467–1.478	1 ml in 9 ml 50% alc gives clear soln	0.875–0.880	
IR	90.0% of total alcohols, as $C_{15}H_{26}O$ (M-9b)		1.478–1.483	1 ml in 4 ml 70% alc	0.870–0.880	**Angular Rotation** *Natural*—between +11° and +14°; *synthetic*—inactive (p. 530, 100-mm tube); **Esters**—0.5% (M-23)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
***trans,trans*-2,4-Nonadienal [FEMA No. 3212]**	138.21/$C_9H_{14}O$/ 	slightly yellow liq/ strong, fatty, floral	*s*—alc, most fixed oils; *ins*—water	
***trans,cis*-2,6-Nonadienal [FEMA No. 3377]**	138.21/$C_9H_{14}O$/ 	slightly yellow liq/ powerful, violet, cucumber	*s*—alc, most fixed oils; *ins*—water	
***trans,cis*-2,6-Nonadienol [FEMA No. 2780]**	140.22/$C_9H_{16}O$/ 	white to yellowish liq/powerful, green, vegetable	*ins*—water	
γ-Nonalactone (Aldehyde C-18, So-Called) [FEMA No. 2781]	156.23/$C_9H_{16}O_2$/ 	colorless to slightly yel liq/coconutlike	*s*—alc, most fixed oils, prop glycol; *ins*—water	provided
Nonanal (Aldehyde C-9; Pelargonic Aldehyde) [FEMA No. 2782]	142.24/$C_9H_{18}O$/ $CH_3(CH_2)_7CHO$	colorless to light yel liq/fatty odor, citrus-rose on dilution	*s*—alc, most fixed oils, prop glycol; *ins*—gly	provided
***trans*-2-Nonenal [FEMA No. 3213]**	140.22/$C_9H_{16}O$/ 	white to slightly yellowish liq/ fatty, violet	*s*—alc, most fixed oils; *ins*—water	
***trans*-2-Nonen-1-ol [FEMA No. 3379]**	142.23/$C_9H_{18}O$/ 	white liq/fatty, violet	*ins*—water	
***cis*-6-Nonen-1-ol [FEMA No. 3465]**	142.23/$C_9H_{18}O$/ 	white to slightly yel liq/powerful, melonlike	*ins*—water	
Nonyl Acetate [FEMA No. 2788]	186.29/$C_{11}H_{22}O_2$/ $CH_3COO(CH_2)_8CH_3$	colorless liq/floral, fruity	*s*—alc, ether; *ins*—water	

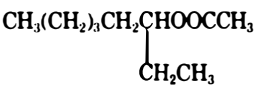
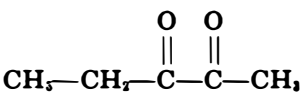
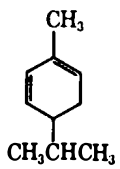
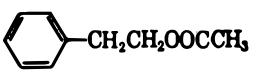
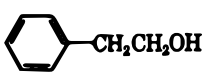
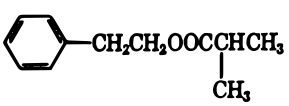
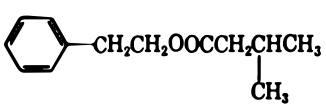
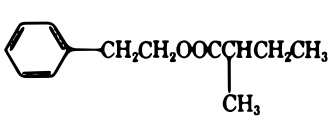
		Requirements				
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	89.0% of $C_9H_{14}O$ (M-8a)		1.522–1.525		0.850–0.870	
IR	92.0% of $C_9H_{14}O$ (M-8a)		1.470–1.475		0.850–0.870	
IR	92.0% of $C_9H_{16}O$ (M-8a)		1.463–1.465		0.860–0.880	
IR	98.0% of $C_9H_{16}O_2$ (M-6)	2.0	1.446–1.450	1 ml in 5 ml 60% alc	0.958–0.966	
IR	92.0% of $C_9H_{18}O$ (M-2a)	10.0	1.422–1.429		0.820–0.830	
IR	92.0% of $C_9H_{16}O$ (M-8a)		1.457–1.460		0.850–0.870	
IR	98.0% of $C_9H_{18}O$ (M-8a)		1.444–1.448		0.830–0.850	
IR	95.0% of $C_9H_{18}O$ (M-8a)		1.448–1.450		0.850–0.870	
IR	97.0% of $C_{11}H_{22}O_2$ (M-6)	1.0	1.422–1.426	1 ml in 6 ml 70% alc gives clear soln	0.864–0.868	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Nonyl Alcohol (1-Nonanol; Alcohol C-9) [FEMA No. 2789]	144.26/$C_9H_{20}O$/ $CH_3(CH_2)_7CH_2OH$	colorless liq/ rose-citrus	*m*—alc, chloroform, ether; *ins*—water/213°	
γ-Octalactone [FEMA No. 2796]	142.20/$C_8H_{14}O_2$/ 	colorless to slightly yel liq/sweet, coconut, fruity	*s*—alc; *ss*—water	
Octanal (Aldehyde C-8; Caprylic Aldehyde) [FEMA No. 2797]	128.21/$C_8H_{16}O$/ $CH_3(CH_2)_6CHO$	colorless to light yel liq/fatty-orange odor	*s*—alc, most fixed oils, prop glycol; *ins*—gly	provided
Octanoic Acid [see p. 207]				
1-Octanol, Natural (Alcohol C-8; Octyl Alcohol; Capryl Alcohol) [FEMA No. 2800]	130.23/$C_8H_{18}O$/ $CH_3(CH_2)_6CH_2OH$	colorless liq/ sharp fatty-citrus	*s*—most fixed oils, prop glycol; *ins*—gly/195°	provided
3-Octanol [FEMA No. 3581]	130.23/$C_8H_{18}O$/ 	colorless liq/ strong, oily-nutty, herbaceous	*s*—alc, most fixed oils; *ins*—water	
***trans*-2-Octen-1-al** [FEMA No. 3215]	126.20/$C_8H_{14}O$/ 	slightly yel liq/ fatty, green odor	*s*—alc, most fixed oils; *ss*—water	
***cis*-3-Octen-1-ol** [FEMA No. 3467]	128.22/$C_8H_{16}O$/ 	white to slightly yellowish liq/musty, mushroomlike	*ins*—water	
1-Octen-3-yl Acetate [FEMA No. 3582]	170.24/$C_{10}H_{18}O_2$/ 	almost colorless liq/ metallic, mushroomlike	*s*—alc, most fixed oils; *ins*—water, prop glycol	
1-Octen-3-yl Butyrate [FEMA No. 3612]	198.31/$C_{12}H_{22}O_2$/ 	almost colorless liq/ metallic, mushroomlike	*s*—alc, most fixed oils; *ss*—prop glycol; *ins*—water	
Octyl Acetate [FEMA No. 2806]	172.27/$C_{10}H_{20}O_2$/ $CH_3COO(CH_2)_7CH_3$	colorless liq/ fruity, orangelike, jasminelike	*m*—alc, most fixed oils, other org solvents; *ins*—water	

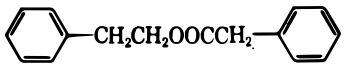
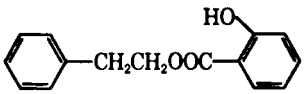
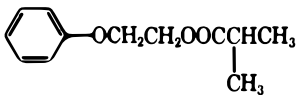
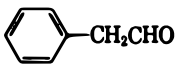
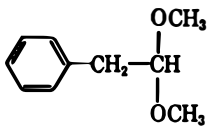
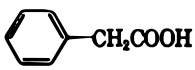
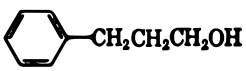
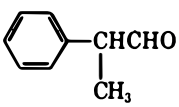
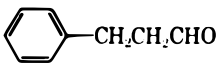
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	97.0% of $C_9H_{20}O$ (M-12)	1.0	1.431–1.435	1 ml in 3 ml 60% alc gives clear soln	0.824–0.830	
IR	95.0% of $C_8H_{14}O_2$ (M-8a)	8.0	1.443–1.447		0.970–0.980	
IR	92.0% of $C_8H_{16}O$ (M-2a)	10.0	1.417–1.425		0.810–0.830	
IR	98.0% of $C_8H_{18}O$ (M-12)	1.0	1.428–1.431	1 ml in 5 ml 50% alc	0.822–0.830	
	97.0% of $C_8H_{18}O$ (M-8a)		1.425–1.429		0.816–0.821	
IR	92.0% of $C_8H_{14}O$ as *trans* isomer (M-8a)		1.421–1.424		0.830–0.850	
IR	95.0% of $C_8H_{16}O$ as *cis* isomer (M-8a)		1.440–1.446		0.830–0.850	
IR	95.0% of $C_{10}H_{18}O_2$ (M-8a)		1.414–1.434 (25°)		0.865–0.886	
IR	95.0% of $C_{12}H_{22}O_2$ (M-8a)		1.416–1.437 (25°)		0.859–0.880	
IR	98.0% of $C_{10}H_{20}O_2$ (M-6)	1.0	1.418–1.421	1 ml in 4 ml 70% alc gives clear soln	0.865–0.868	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
3-Octyl Acetate [FEMA No. 3583]	172.27/$C_{10}H_{20}O_2$/ 	colorless liq/ rosy-minty	*s*—alc, prop glycol, most fixed oils; *ss*—water	
Octyl Formate [FEMA No. 2809]	158.24/$C_9H_{18}O_2$/ $HCOO(CH_2)_7CH_3$	colorless liq/fruity	*s*—most fixed oils, min oil, prop glycol; *ins*—gly	provided
2,3-Pentanedione (Acetyl Propionyl) [FEMA No. 2841]	100.12/$C_5H_8O_2$/ 	yel to yel green liq/penetrating, buttery on dilution	*m*—alc, prop glycol, fixed oils; *ins*—gly, water	
2-Pentanone (Methyl Propyl Ketone) [FEMA No. 2842]	86.13/$C_5H_{10}O$/ $CH_3COCH_2CH_2CH_3$	colorless, mobile liq/fruity, ethereal	*m*—alc, ether; 1 ml in 25 ml water/102°	
α-Phellandrene (*p*-Mentha-1,5-diene) [FEMA No. 2856]	136.24/$C_{10}H_{16}$/ 	colorless to slightly yel liq/herbaceous odor, mintlike background	*s*—alc; *ins*—water	
Phenethyl Acetate (2-Phenethyl Acetate) [FEMA No. 2857]	164.20/$C_{10}H_{12}O_2$/ 	colorless liq/sweet, rosy, honeylike	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/232°	provided
Phenethyl Alcohol (2-Phenylethyl Alcohol) [FEMA No. 2858]	122.17/$C_8H_{10}O$/ 	colorless liq/ roselike	*s*—most fixed oils, gly, prop glycol	provided
Phenethyl Isobutyrate [FEMA No. 2862]	192.26/$C_{12}H_{16}O_2$/ 	colorless to slightly yel liq/fruity, rosy	*s*—alc, most fixed oils; *ins*—water/230°	
Phenethyl Isovalerate [FEMA No. 2871]	206.28/$C_{13}H_{18}O_2$/ 	colorless to slightly yel liq/fruity, rosy	*s*—alc, most fixed oils; *ins*—water/263°	
2-Phenethyl 2-Methylbutyrate [FEMA No. 3632]	206.28/$C_{13}H_{18}O_2$/ 	colorless liq/ floral-fruity	*s*—alc, most fixed oils; *ins*—water	

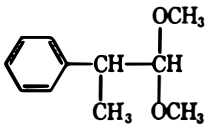
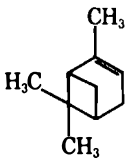
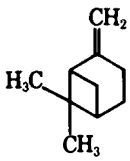
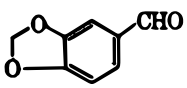
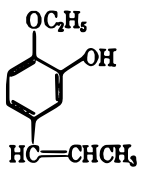
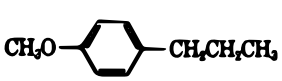
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
	98.0% of $C_{10}H_{20}O_2$ (M-8a)	2.0	1.414–1.419		0.856–0.860	
IR	96.0% of $C_8H_{16}O_2$ (M-6)	1.0	1.418–1.420	1 ml in 5 ml 70% alc, remains in soln to 10 ml	0.869–0.872	
IR	93.0% of $C_5H_8O_2$ (M-3)		1.402–1.406	1 ml in 3 ml 50% alc	0.952–0.962	
IR	95.0% of $C_5H_{10}O$ (M-5b)				0.801–0.806	
IR			1.471–1.477	1 ml in 1 ml 95% alc gives clear soln	0.835–0.865	Angular Rotation—between −80° and −120° (p. 530, 100-mm tube)
IR	98.0% of $C_{10}H_{12}O_2$ (M-6)	1.0	1.497–1.501	1 ml in 2 ml 70% alc, remains clear to 10 ml	1.030–1.034	
IR			1.531–1.534	1 ml in 2 ml 50% alc, remains clear to 10 ml	1.017–1.020	Chlorinated Cmpds.—passes test (p. 500); Odor—passes test (M-31)
IR	98.0% of $C_{12}H_{16}O_2$ (M-6)	1.0	1.486–1.490	1 ml in 3 ml 80% alc gives clear soln	0.987–0.990	
IR	98.0% of $C_{13}H_{18}O_2$ (M-6)	1.0	1.484–1.486	1 ml in 11 ml 70% alc gives clear soln	0.973–0.976	
	95.0% of $C_{13}H_{18}O_2$ (M-8a)	2.0	1.484–1.488		0.973–0.977	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Phenethyl Phenylacetate [FEMA No. 2866]	240.30/$C_{16}H_{16}O_2$/ 	colorless to slightly yel liq above 26°/ rosy, hyacinthlike	*s*—alc; *ins*—water	
Phenethyl Salicylate [FEMA No. 2868]	242.27/$C_{15}H_{14}O_3$/ 	white crystals/ balsamic	*s*—alc; *ins*—water	
Phenoxyethyl Isobutyrate [FEMA No. 2873]	208.26/$C_{12}H_{16}O_3$/ 	colorless liq/ honey, roselike	*m*—alc, chloroform, ether; *ins*—water	
Phenylacetaldehyde (α-Toluic Aldehyde) [FEMA No. 2874]	120.15/C_8H_8O/ 	colorless, slightly yel, oily liq; becomes more viscous on aging/ harsh odor, hyacinthlike on dilution	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Phenylacetaldehyde Dimethyl Acetal [FEMA No. 2876]	166.22/$C_{10}H_{14}O_2$/ 	colorless liq/ strong odor	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Phenylacetic Acid (α-Toluic Acid) [FEMA No. 2878]	136.15/$C_8H_8O_2$/ 	glistening white cryst solid/ persistent, disagreeable, suggestive of geranium leaf and rose when diluted	*s*—most fixed oils, gly; *ss*—water	
3-Phenyl-1-propanol (Phenylpropyl Alcohol; Hydrocinnamyl Alcohol) [FEMA No. 2885]	136.19/$C_9H_{12}O$/ 	colorless, slightly viscous liq/sweet, hyacinth-mignonette	*s*—most fixed oils, prop glycol; *ins*—gly	provided
2-Phenylpropionaldehyde (Hydratropic Aldehyde; α-Methyl Phenylacetaldehyde) [FEMA No. 2886]	134.18/$C_9H_{10}O$/ 	water-white to pale yel liq/floral	*s*—most fixed oils; *ins*—gly; *ss*—prop glycol	provided
3-Phenylpropionaldehyde (Hydrocinnamaldehyde; Phenylpropyl Aldehyde) [FEMA No. 2887]	134.18/$C_9H_{10}O$/ 	colorless to slightly yel liq/strong, pungent, floral, hyacinthlike	*m*—alc, ether; *ins*—water	provided

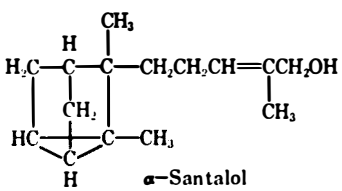
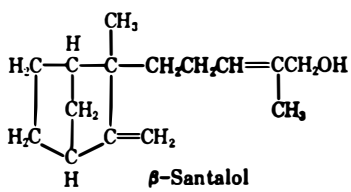
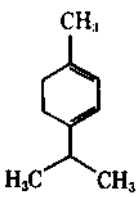
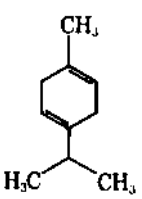
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of $C_{16}H_{16}O_2$ (M-6)	1.0		1 ml in 4 ml 90% alc gives clear soln	1.079–1.082	**Solidification Pt.**—NLT 26° (p. 538)
IR	98.0% of $C_{15}H_{14}O_3$ (M-6)	1.0 (phenol red TS)		1 g in 20 ml 95% alc gives clear soln		**Solidification Pt.**—NLT 41° (p. 538)
IR	97.0% of $C_{12}H_{16}O_3$ (M-6)	1.0	1.492–1.495	1 ml in 2 ml 70% alc gives clear soln	1.044–1.048	
IR	90.0% of C_8H_8O (M-2a)	5.0	1.524–1.532	1 ml in 2 ml 80% alc	1.025–1.035	
IR	95.0% of $C_{10}H_{14}O_2$ (M-1a)	1.0	1.493–1.496	1 ml in 2 ml 70% alc, remains clear to 10 ml	1.000–1.006	**Chlorinated Cmpds.**—passes test (p. 500); **Free Aldehydes**—1.0% (M-16b)
IR	99.0% of $C_8H_8O_2$ after drying (M-11b)					**Arsenic**—3 ppm (M-18); **Heavy Metals**—0.004% (M-24a); **Melting Range**—76° to 78° (p. 519, *Class Ia*); **Lead**—10 ppm (M-27)
IR	98.0% of $C_9H_{12}O$ (M-12)		1.524–1.528	1 ml in 1 ml 70% alc	0.998–1.002	**Aldehydes**—0.5% (M-16b)
IR	95.0% of $C_9H_{10}O$ (M-2a)	5.0	1.515–1.520		0.998–1.006	
IR	90.0% of aldehydes by vol (M-2b)	10.0	1.520–1.532	1 ml in 7 ml 60% alc, remains clear on dilution	1.010–1.020	**Chlorinated Cmpds.**—passes test (p. 500)

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
2-Phenylpropionaldehyde Dimethyl Acetal (Hydratropic Aldehyde Dimethyl Acetal) [FEMA No. 2888]	180.25/$C_{11}H_{16}O_2$/ 	colorless to slightly yel liq/ mushroomlike	*s*—alc, ether; *ins*—water	
3-Phenylpropyl Acetate [FEMA No. 2890]	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/ spicy, floral	*s*—alc; *ins*—water	
α-Pinene (2,6,6-Trimethyl-bicyclo[3.1.1]hept-2-ene; 2-Pinene) [FEMA No. 2902]	136.24/$C_{10}H_{16}$/ 	colorless liq/ fresh, piney	*s*—alc, most fixed oils; *ins*—water	
β-Pinene (6,6-Dimethyl-2-methylene-bicyclo-[3.1.1]heptane) [FEMA No. 2903]	136.24/$C_{10}H_{16}$/ 	colorless liq/ resinous-piney	*s*—most fixed oils; *ins*—water, prop glycol, gly	
Piperonal (3,4-Methylenedioxy-benzaldehyde; Heliotropine; Piperonyl Aldehyde) [FEMA No. 2911]	150.13/$C_8H_6O_3$/ 	white cryst substance; m.p. 37°/ floral odor, like heliotropine, free from safrole by-odor	*vs*—alc; *s*—most fixed oils, prop glycol; *ins*—gly, water	provided
Propenylguaethol (1-Ethoxy-2-hydroxy-4-propenylbenzene) [FEMA No. 2922]	178.23/$C_{11}H_{14}O_2$/ 	white cryst powder/ vanillalike	*s*—most veg oils, *ins*—water; 1 g in 20 ml 95% alc	
Propionaldehyde [FEMA No. 2923]	58.08/C_3H_6O/ CH_3CH_2CHO	colorless, mobile liq/sharp, pungent	*m*—alc, ether, water/ 49°	
Propionic Acid [see p. 254]				
***p*-Propyl Anisole** (Dihydroanethole) [FEMA No. 2930]	150.22/$C_{10}H_{14}O$/ 	colorless to pale yel liq/anise-type odor, with sassafras background	*s*—most fixed oils; *ins*—gly, prop glycol	provided

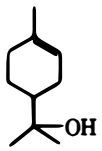
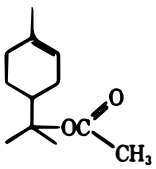
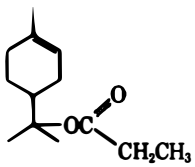
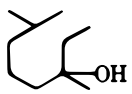
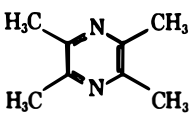
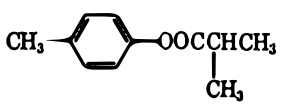
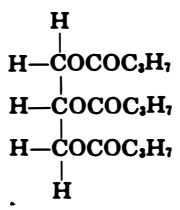
	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	95.0% of $C_{11}H_{16}O_2$ (M-1a)		1.492–1.497	1 ml in 7 ml 60% alc, and in 3 ml 70% alc, gives clear solns	0.989–0.994	**Free Aldehydes**—3.0% (M-16a)
IR	98.0% of $C_{11}H_{14}O_2$ (M-6)	1.0	1.494–1.497	1 ml in 3 ml 70% alc gives clear soln	1.012–1.015	
	97.0% of $C_{10}H_{16}$ (M-8a)		1.464–1.468		0.851–0.855	**Angular Rotation**—levorotatory activity NLT −40° (p. 530)
	97.0% of $C_{10}H_{16}$ (M-8a)		1.477–1.481		0.864–0.868	**Angular Rotation**—levorotatory activity NLT −20° (p. 530)
IR	99.0% of $C_9H_6O_3$ (M-2a)			1 g in 4 ml 70% alc		**Arsenic**—3 ppm (M-18); **Heavy Metals**—0.004% (M-24a); **Lead**—10 ppm (M-27); **Solidification Pt.**—NLT 35° (p. 538)
IR						**Heavy Metals**—10 ppm (M-24a); **Melting Range**—85° to 88° (p. 519); **Res. on Ignit.**—0.1% (p. 533, 2-g samp)
IR	97.0% of C_9H_6O (M-5a)				0.800–0.805	**Acidity**—0.1% (M-15b); **Dist. Range**—46° to 50° (first 97%, p. 478); **Water**—2.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR			1.502–1.506	1 ml in 5 ml 80% alc, remains in soln on dilution	0.940–0.943	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Pyrrole [FEMA No. 3386]	67.09/C_4H_5N/ 	colorless to yellowish liq, darkens on aging/ nutty, sweet, warm, ethereal	*s*—alc, most fixed oils; *ss*—water/ 130° (decomp)	
Rhodinol [FEMA No. 2980]	[see *Citronellol*, *Geraniol*, and *Nerol*]	colorless liq/ pronounced, roselike	*s*—most fixed oils, prop glycol; *ins*—gly	provided
Rhodinyl Acetate [FEMA No. 2981]	[see *Citronellyl Acetate* and *Geranyl Acetate*]	colorless to slightly yel liq/light, fresh, roselike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/237°	provided
Rhodinyl Formate [FEMA No. 2984]	[see *Citronellyl Formate*]	colorless to slightly yel liq/leafy, roselike	*s*—alc, most fixed oils; *ins*—gly, prop glycol, water/220°	
Santalol [Mixture of α- and β-isomers] [FEMA No. 3006]	220.35/$C_{15}H_{24}O$/  α-Santalol  β-Santalol	colorless to slightly yel, viscous liq/ sandalwoodlike	*vs*—alc, fixed oils, prop glycol; *ins*—gly, water	
Santalyl Acetate [FEMA No. 3007]	[mixture of α- and β-isomers from acetylation of *Santalol*]	colorless to slightly yel liq/ sandalwoodlike	*s*—alc; *ins*—water	
α-Terpinene [1-Methyl-4-(1-methylethyl)-1,3-cyclohexadiene] [FEMA No. 3558]	136.24/$C_{10}H_{16}$/ 	colorless liq/ lemonlike	*s*—alc, most fixed oils; *ins*—water	
γ-Terpinene [1-Methyl-4-(1-methylethyl)-1,4-cyclohexadiene] [FEMA No. 3559]	136.24/$C_{10}H_{16}$/ 	colorless liq/ herbaceous, citrusy	*s*—alc, most fixed oils; *ins*—water	

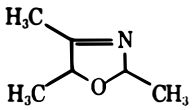
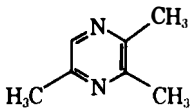
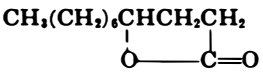
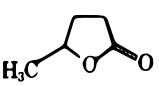
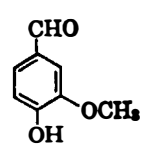
	Requirements					
I.D. Test[4]	Assay Min., %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	98.0% of C_4H_5N (M-8a)		1.507–1.510		0.950–0.980 (20°)	**Dist. Range**—125° to 130° (p. 478); **Water**—0.5% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	82.0% of total alcohols, as $C_{10}H_{20}O$ (M-12)		1.463–1.473	1 ml in 1.2 ml 70% alc	0.860–0.880	**Angular Rotation**—between −4° and −9° (p. 530, 100-mm tube); **Esters**—1.0% (M-23)
IR	87.0% of total esters, as $C_{12}H_{22}O_2$ (M-6)	1.0	1.453–1.458	1 ml in 2 ml 80% alc, remains in soln to 10 ml	0.895–0.908	**Angular Rotation**—between −2° and −6° (p. 530, 100-mm tube)
IR	85.0% of total esters, as $C_{11}H_{20}O_2$ (M-6)	2.0	1.453–1.458	1 ml in 2 ml 80% alc gives clear soln	0.901–0.908	
IR	95.0% of total alcohols, as $C_{15}H_{24}O$ (M-12)		1.505–1.509	1 ml in 4 ml 70% alc gives clear soln	0.965–0.975	**Angular Rotation**—between −11° and −19° (p. 530, 100-mm tube)
IR	95.0% of total esters, as $C_{17}H_{22}O_2$ (M-6)	1.0	1.488–1.491	1 ml in 9 ml 80% alc gives clear soln	0.980–0.986	
	89.0% of $C_{10}H_{16}$ (M-8a)		1.475–1.480		0.833–0.838	
	95.0% of $C_{10}H_{16}$ (M-8a)		1.473–1.477		0.841–0.845	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
Terpineol (Menthen-1-ol-8) [FEMA No. 3045]	154.25/$C_{10}H_{18}O$/ 	colorless, viscous liq/lilaclike	*ss*—gly, water	provided
Terpinyl Acetate (Menthen-1-yl-8 Acetate) [FEMA No. 3047]	196.29/$C_{12}H_{20}O_2$/ 	colorless liq/sweet, refreshing, herbaceous	*s*—alc, most fixed oils, min oil, prop glycol; *ss*—gly; *ins*—water/220°	provided
Terpinyl Propionate (Menthen-1-yl-8 Propionate) [FEMA No. 3053]	210.32/$C_{13}H_{22}O_2$/ 	colorless to slightly yel liq/sweet, floral, herbaceous, lavenderlike	*m*—alc, chloroform, ether, most fixed oils; *ss*—prop glycol; *s*—gly; *ins*—water/240°	
Tetrahydrolinalool (3,7-Dimethyl-3-octanol) [FEMA No. 3060]	158.29/$C_{10}H_{22}O$/ 	colorless liq/ distinct floral odor	*s*—alc, most fixed oils; *ins*—water	
2,3,5,6-Tetramethylpyrazine [FEMA No. 3237]	136.20/$C_8H_{12}N_2$/ 	white crystals or powder/fermented soybeans	*s*—alc, prop glycol, most fixed oils; *ss*—water	
***p*-Tolyl Isobutyrate** (*p*-Cresyl Isobutyrate) [FEMA No. 3075]	178.23/$C_{11}H_{14}O_2$/ 	colorless liq/ characteristic odor	*s*—alc; *ins*—water	provided
Tributyrin (Glyceryl Tributyrate; Butyrin) [FEMA No. 2223]	302.37/$C_{15}H_{26}O_6$/ 	colorless, somewhat oily liq/ characteristic odor	*s*—alc, chloroform, ether; *ins*—water/308°	
2-Tridecenal [FEMA No. 3082]	196.33/$C_{13}H_{24}O$/ $CH_3(CH_2)_9CH{=}CHCHO$	white or slightly yellowish liq/ oily, citrus odor	*s*—alc, most fixed oils; *ins*—water	

	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	96.0% of $C_{10}H_{18}O$ as mixed isomers, principally alpha (M-8a)		1.482–1.485	1 ml in 2 ml 70% alc, 4 ml 60% alc, 8 ml 50% alc	0.930–0.936	**Dist. Range**—NLT 90% within a 5° range between 214° and 224° (p. 478); **Solidification Pt.**—NLT 2° (p. 538)
IR	97.0% of $C_{12}H_{20}O_2$ (M-6)		1.464–1.467	1 ml in 5 ml 70% alc, remains in soln to 10 ml	0.953–0.962	
IR	95.0% of $C_{13}H_{22}O_2$ (M-7)	1.0	1.461–1.466	1 ml in 2 ml 80% alc gives clear soln	0.944–0.949	
	95.0% of $C_{12}H_{22}O$ (M-8a)		1.431–1.435		0.923–0.927	
IR	95.0% of $C_6H_{12}N_2$ (M-8a)					**Melting Range**—85° to 90° (p. 519); **Water**—0.2% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	95.0% of $C_{11}H_{14}O_2$ (M-7)	1.0	1.485–1.489	1 ml in 7 ml 70% alc gives clear soln	0.990–0.996	
IR	99.0% of $C_{15}H_{26}O_6$ (M-6)				1.034–1.037	**Acidity**—0.04% (M-15b)
IR	92.0% of $C_{13}H_{24}O$ (M-8a)		1.457–1.460		0.842–0.862	

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility[1]/B.P.[2]	GLC Profile[3]
2,4,5-Trimethyl Δ-3-Oxazoline [FEMA No. 3525]	113.16/$C_6H_{11}NO$/ 	yellow orange liq/ powerful, musty, slight green, wood, nutlike	*s*—alc, prop glycol, water; *ins*—most fixed oils	
2,3,5-Trimethylpyrazine [FEMA No. 3244]	122.17/$C_7H_{10}N_2$/ 	colorless to slightly yel liq/baked potato, peanut	*s*—water, organic solvents	
γ-Undecalactone (Aldehyde C-14 Pure, So-Called; Peach Aldehyde) [FEMA No. 3091]	184.28/$C_{11}H_{20}O_2$/ 	colorless to slightly yel liq/fruity, peachlike	*s*—alc, most fixed oils, prop glycol; *ins*—gly, water/297°	provided
Undecanal (Aldehyde C-11 Undecyclic; *n*-Undecyl Aldehyde) [FEMA No. 3092]	170.30/$C_{11}H_{22}O$/ $CH_3(CH_2)_9CHO$	colorless to slightly yel liq/sweet, fatty, floral odor	*s*—most fixed oils, prop glycol; *ins*—gly, water/223°	provided
10-Undecenal (Aldehyde C-11 Undecylenic; Undecen-10-al) [FEMA No. 3095]	168.28/$C_{11}H_{20}O$/ $CH_2=CH(CH_2)_8CHO$	colorless to light yel liq/fatty; roselike odor on dilution	*s*—most fixed oils, prop glycol; *ins*—gly, water/235°	provided
2-Undecenol	170.30/$C_{11}H_{22}O$/ $CH_3(CH_2)_7CH=CHCH_2OH$	white to slightly yel liq/oily, sweet, floral	*ins*—water	
Undecyl Alcohol (Alcohol C-11) [FEMA No. 3097]	172.31/$C_{11}H_{24}O$/ $CH_3(CH_2)_9CH_2OH$	colorless liq/ fatty-floral	*s*—most fixed oils; *ins*—water	
Valeric Acid (Pentanoic Acid) [FEMA No. 3101]	102.13/$C_5H_{10}O_2$/. $CH_3(CH_2)_3COOH$	colorless, mobile liq/ unpleasant, penetrating, rancid odor	*m*—alc, ether; 1 ml in 40 ml water/186°	
γ-Valerolactone [FEMA No. 3103]	100.12/$C_5H_8O_2$/ 	colorless to slightly yel liq/warm, sweet, herbaceous	*m*—alc, most fixed oils, water	
Vanillin (4-Hydroxy-3-methoxybenzaldehyde) [FEMA No. 3107]	152.15/$C_8H_8O_3$/ 	fine, white to slightly yel crystals, usually needlelike; affected by light/odor and taste of vanilla	*s*—alc, chloroform, ether; 1 g in 100 ml water at 25°, in 20 ml gly, in 20 ml water at 80°	

	Requirements					
I.D. Test[4]	Assay Min, %[5]	A.V. Max[6]	Ref. Index[7]	Solubility in Alcohol[8]	Sp. Gr.[9]	Other Requirements[10]
IR	94.0% of $C_9H_{11}NO$ (M-8a)		1.414–1.435		0.911–0.932	
IR	99.0% of $C_7H_{10}N_2$ (M-8a)		1.503–1.507		0.960–0.990 (20°)	Water—0.2% (p. 552, *KF;* use freshly dist. pyridine as solvent)
IR	98.0% of $C_{11}H_{20}O_2$ (M-6)	5.0	1.450–1.454	1 ml in 5 ml 60% alc	0.942–0.945	Solubility in Alkali—passes test (M-38)
IR	92.0% of $C_{11}H_{22}O$ (M-2a)	10.0	1.430–1.435		0.825–0.832	
IR	90.0% of $C_{11}H_{20}O$ (M-2a)	6.0	1.441–1.447		0.840–0.850	
IR	92.0% of $C_{11}H_{22}O$ (M-8a)		1.450–1.452 (22°)		0.847–0.848	
IR	97.0% of $C_{11}H_{24}O$ (M-8a)		1.437–1.443		0.820–0.840	
IR	99.0% of $C_2H_{10}O_2$ (M-11b)		1.405–1.414 (25°)		0.935–0.940	
IR	95.0% of $C_5H_8O_2$ (M-8a)		1.431–1.434		1.045–1.050	
IR	97.0% to 103.0% of $C_9H_8O_3$, on dried basis (M-13)					Arsenic—3 ppm (M-18); Heavy Metals—10 ppm (M-24a); Loss on Drying—0.5% (p. 518, silica gel/4 h); Melting Range—81° to 83° (p. 519); Res. on Ignit.—0.05% (p. 533, 2-g samp)

4 / *Test Methods for Flavor Aromatic Chemicals and Isolates*

This section provides the test methods by which certain flavoring agents listed in *Section 3* are to be analyzed (see *Notes 5* through *10* on page 353, *Section 3*).

M-1 Assay by Determination of Acetals

M-1a GENERAL METHOD

Proceed as directed under *Acetals*, page 499, using the sample weight and equivalence factor (*f*), respectively, as specified for each substance below.

Phenylacetaldehyde Dimethyl Acetal: 1 g/83.11
2-Phenylpropionaldehyde Dimethyl Acetal: 2 g/90.13

M-1b MODIFIED GENERAL METHOD

For hydroxycitronellal dimethyl acetal, weigh accurately about 1.5 g of the sample, and proceed as directed under *Acetals*, page 499, but reflux between 5 and 10 min. Record the number of ml of 0.5 *N* alcoholic potassium hydroxide consumed per g of sample as *A*. Weigh accurately a separate 5-g portion of the sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, recording the number of ml of 0.5 *N* hydrochloric acid liberated per g of sample as *B*. Calculate the percentage of $C_{12}H_{26}O_3$ by the formula $10.92(A - B)$.

M-2 Assay by Determination of Aldehydes

M-2a GENERAL METHOD

Proceed as directed under *Aldehydes*, page 499, using the sample weight and equivalence factor (*e*), respectively, as specified for each substance below.

Benzaldehyde: 1 g/53.06
Cinnamaldehyde: 1.5 g/66.08
Cyclamen Aldehyde: 1.5 g/95.15
Decanal: 1.5 g/78.14
Furfural: 1.5 g/48.05
Heptanal: 700 mg/57.10
Hydroxycitronellal: 1.3 g/86.13
Lauryl Aldehyde: 1.2 g/92.16
p-Methoxybenzaldehyde: 1.2 g/68.08 (NOTE: Allow the sample and blank to stand at room temperature for 15 min.)
α-Methylcinnamaldehyde: 1 g/73.10
2-Methylundecanal: 1.2 g/92.16
Nonanal: 1.5 g/71.12
Octanal: 1.5 g/64.11
Phenylacetaldehyde: 1 g/60.08
2-Phenylpropionaldehyde: 1 g/67.09
Piperonal: 1.5 g/75.06
Undecanal: 1 g/85.15
10-Undecenal: 1 g/84.14

M-2b METABISULFITE-CASSIA FLASK METHOD

For 3-phenylpropionaldehyde, pipet 5 ml of the sample into a 100-ml cassia flask, and add 70 ml of a 1 in 10, weight in weight, solution of sodium metabisulfite. Warm the mixture on a water bath to 50°–60°, and shake the flask vigorously for 15 min. When the liquids have separated completely, add sufficient sodium metabisulfite solution to raise the lower level of the oily layer within the graduated portion of the neck of the flask. Not more than 0.5 ml of oil separates.

M-3 Assay by Determination of Aldehydes and Ketones—Hydroxylamine Method

Proceed as directed under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, using the sample weight and equivalence factor (*e*), respectively, as specified for each substance below.

Acetanisole: 1.2 g/75.09
Acetophenone: 1 g/60.08
Allyl α-Ionone: 1.5 g/116.18
α-Amylcinnamaldehyde: 1.5 g/101.2
d-Carvone: 1 g/75.11
l-Carvone: 1 g/75.11
Citral: 1 g/76.12
Citronellal: 1.1 g/77.13 (NOTE: Allow to stand for 1 h before titrating.)
Diacetyl: 500 mg/21.52
α-Hexylcinnamaldehyde: 1.5 g/108.2 (NOTE: Allow to stand for 30 min before titrating.)
α-Ionone: 1.3 g/96.15
β-Ionone: 1.3 g/96.15
4-Methyl Acetophenone: 1 g/67.09
6-Methyl-5-hepten-2-one: 700 mg/63.10
Methyl β-Naphthyl Ketone: 1.5 g/85.10
2,3-Pentanedione: 400 mg/25.03

M-4 Assay by Determination of Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method

For cuminic aldehyde, weigh accurately about 1 g of the sample, and proceed as directed under *Aldehydes and Ketones—Hydroxylamine/Tert-Butyl Alcohol Method*, page 500, allowing the mixture to stand at room temperature for 1 h before titrating. Use 74.11 as the equivalence factor (*e*) in the calculation.

M-5 Assay for Certain Aldehydes and Ketones by Special Procedures

M-5a PROCEDURES REQUIRING USE OF SEALED GLASS AMPULES

Transfer 65 ml of 0.5 *N* hydroxylamine hydrochloride and 50.0 ml of 0.5 *N* triethanolamine into a suitable heat-resistant pressure bottle provided with a tight closure that can be fastened securely. Replace the air in the bottle by passing a gentle stream of nitrogen for 2 min through a glass tube positioned so that the end is just above the surface of the liquid. To the mixture in the pressure bottle add the amount of sample, as specified below, contained in a sealed glass ampule and accurately weighed. Introduce several pieces of 8-mm glass rod, cap the bottle, and shake vigorously to break the ampule. Allow the bottle to stand at room temperature for the time specified, swirling occasionally. Cool, if necessary, and uncap the bottle cautiously to prevent any loss of the contents. Titrate with 0.5 *N* sulfuric acid to a greenish blue endpoint. Perform a residual blank titration (see page 2). Each ml of 0.5 *N* sulfuric acid is equivalent to the amount of substance as specified below.

Substance	Sample Weight (mg)	Reaction Time (min)	1 ml 0.5 *N* H_2SO_4 Equivalent to
Acetaldehyde	600	30	22.03 mg of C_2H_4O
Butyraldehyde	900	60	36.06 mg of C_4H_8O
Isobutyraldehyde	900	60	36.06 mg of C_4H_8O
4-Methyl-2-pentanone	1200	60	50.08 mg of $C_6H_{12}O$
Propionaldehyde	750	30	29.04 mg of C_3H_6O

M-5b PROCEDURES REQUIRING USE OF WEIGHING PIPETS

Transfer 65 ml of 0.5 *N* hydroxylamine hydrochloride and 50.0 ml of 0.5 *N* triethanolamine into a suitable heat-resistant pressure bottle provided with a tight closure that can be securely fastened. Replace the air in the bottle by passing a gentle stream of nitrogen for 2 min through a glass tube positioned so that the end is just above the surface of the liquid. To the mixture in the pressure bottle add the amount of sample, as specified below, accurately weighed by means of a suitable weighing pipet. Cap the bottle, and allow it to stand at room temperature (except for 2-ethylbutyraldehyde, which should be wrapped securely in a canvas bag and placed in a hot water bath maintained at 98° ± 2°) for the time specified, shaking occasionally. Cool, if necessary, and uncap the bottle to prevent any loss of its contents. Titrate with 0.5 *N* sulfuric acid to a greenish blue endpoint. Perform a residual blank titration (see page 2). Each ml of 0.5 *N* sulfuric acid is equivalent to the amount of substance as specified below.

Substance	Sample Weight (g)	Reaction Time (min)	1 ml 0.5 *N* H_2SO_4 Equivalent to
2-Ethylbutyraldehyde	1	60	50.08 mg of $C_6H_{12}O$
2-Heptanone	1.2	60	57.10 mg of $C_7H_{14}O$
3-Heptanone	1	60	57.10 mg of $C_7H_{14}O$
2-Pentanone	1	15	43.06 mg of $C_5H_{10}O$

M-6 Assay by Determination of Esters

Proceed as directed under *Ester Determination*, page 500, using the sample weight and equivalence factor (*e*), respectively, as specified for each substance below.

Allyl Cyclohexanepropionate: 1.2 g/98.15
Allyl Hexanoate: 1 g/78.12
Amyl Cinnamate: 1.3 g/109.1
Amyl Octanoate: 1.3 g/107.2
Amyl Propionate: 1 g/72.10
Anisyl Acetate: 1.5 g/90.11
Benzyl Acetate: 900 mg/75.10
Benzyl Benzoate: 1 g/106.1
Benzyl Butyrate: 1 g/89.10
Benzyl Cinnamate: 1.6 g/119.1
Benzyl Isobutyrate: 1.1 g/89.10
Benzyl Isovalerate: 1.1 g/96.13
Benzyl Phenylacetate: 1.5 g/113.1
Benzyl Propionate: 1 g/82.10
Benzyl Salicylate: 1.4 g/114.1 (NOTE: Reflux for 2 h, and use phenol red TS as indicator.)
Bornyl Acetate: 1 g/98.14
Butyl Butyrate: 1 g/72.10
Butyl Butyryllactate: 1 g/54.07
Butyl Isobutyrate: 900 mg/72.10
Cinnamyl Acetate: 1.2 g/88.11
Cinnamyl Anthranilate: 1.5 g/126.7
Cinnamyl Isovalerate: 1.5 g/109.2
Cinnamyl Propionate: 1.2 g/95.12
Citronellyl Acetate: 1.4 g/99.15
Citronellyl Butyrate: 1.5 g/113.2
Citronellyl Formate: 1 g/92.14
Citronellyl Isobutyrate: 1.5 g/113.2
Citronellyl Propionate: 1.2 g/95.12
Diethyl Malonate: 1 g/40.04
Diethyl Sebacate: 1 g/64.59
Dimethyl Anthranilate: 1.1 g/82.60
Dimethyl Benzyl Carbinyl Acetate: 1.3 g/96.13
Ethyl *p*-Anisate: 1.2 g/90.10
Ethyl Anthranilate: 1.5 g/82.60
Ethyl Benzoate: 900 mg/75.09
Ethyl Butyrate: 1 g/58.08
Ethyl Cinnamate: 1.2 g/88.11
Ethyl Decanoate: 1.3 g/100.2
Ethyl Formate: 500 mg/37.04 (NOTE: Allow to stand at room temperature for 15 min, instead of heating on a steam bath for 1 h.)
Ethyl Heptanoate: 1 g/79.12
Ethyl Hexanoate: 1 g/72.10
Ethyl Isovalerate: 1.5 g/65.10
Ethyl Lactate: 700 mg/59.07
Ethyl Laurate: 1.5 g/114.2
Ethyl Methyl Phenylglycidate: 1 g/103.1
Ethyl Nonanoate: 1.5 g/93.15
Ethyl Octanoate: 1.1 g/86.13
Ethyl Phenylacetate: 1 g/82.10
Ethyl Phenylglycidate: 1.2 g/96.11
Ethyl Propionate: 1 g/51.07
Ethyl Salicylate: 1 g/83.09 (NOTE: Reflux for 2 h, and use phenol red TS as the indicator.)
Geranyl Acetate: 1 g/98.15
Geranyl Benzoate: 1.5 g/129.2
Geranyl Butyrate: 1 g/112.2
Geranyl Formate: 1 g/91.13
Geranyl Phenylacetate: 1.6 g/136.2
Geranyl Propionate: 1.2 g/105.2
Isoamyl Acetate: 800 mg/65.10
Isoamyl Butyrate: 1 g/79.12
Isoamyl Formate: 1 g/58.08
Isoamyl Hexanoate: 1.2 g/93.15
Isoamyl Isovalerate: 1.5 g/86.14
Isoamyl Salicylate: 1.3 g/86.14
Isobornyl Acetate: 1 g/98.15
Isobutyl Acetate: 1 g/58.08
Isobutyl Butyrate: 900 mg/72.11
Isobutyl Cinnamate: 1.4 g/102.1
Isobutyl Phenylacetate: 1.2 g/96.13
Isobutyl Salicylate: 1.3 g/97.12 (NOTE: Reflux for 2 h, and use phenol red TS as the indicator.)
Isoeugenyl Acetate: 1.4 g/103.1 (NOTE: Reflux for 4 h, and use phenol red TS as the indicator.)
Linalyl Acetate: 1 g/98.15 (NOTE: The volume of 0.5 *N* potassium hydroxide consumed by the sample should be corrected for the acid value.)
Linalyl Acetate, Synthetic: 1 g/98.15
Linalyl Benzoate: 1.5 g/129.2 (NOTE: Reflux for 4 h before titrating.)
Linalyl Formate: 1.1 g/91.10
Linalyl Propionate: 1 g/105.2
Methyl Anthranilate: 1 g/75.59
Methyl Benzoate: 900 mg/68.07
Methylbenzyl Acetate: 1 g/82.10
Methyl Cinnamate: 1 g/81.10
Methyl 2-Octynoate: 1 g/77.11
Methyl Phenylacetate: 1 g/75.09
Methyl Phenylcarbinyl Acetate: 1 g/82.10
Methyl Salicylate: 2 g/76.08 (NOTE: Use 50.0 ml of 0.5 *N* alcoholic potassium hydroxide, and reflux for 2 h.)
γ-Nonalactone: 1 g/78.12 (NOTE: The volume of 0.5 *N* alcoholic potassium hydroxide consumed by the sample should be corrected for the acid value.)
Nonyl Acetate: 1.2 g/93.15
Octyl Acetate: 1.1 g/86.14
Octyl Formate: 1 g/79.12
Phenethyl Acetate: 1 g/82.10
Phenethyl Isobutyrate: 1.2 g/96.13
Phenethyl Isovalerate: 1.5 g/103.2
Phenethyl Phenylacetate: 1.5 g/120.2
Phenethyl Salicylate: 1.5 g/121.2 (NOTE: Use phenol red TS as the indicator.)
Phenoxyethyl Isobutyrate: 1.4 g/104.1
3-Phenylpropyl Acetate: 1.2 g/89.12
Rhodinyl Acetate: 1.3 g/99.15
Rhodinyl Formate: 1.2 g/92.14
Santalyl Acetate: 1.6 g/131.2

Terpinyl Acetate: 1 g/98.15 (NOTE: Reflux for 4 h.)
Tributyrin: 1.5 g/50.40
γ-Undecalactone: 1 g/92.14

M-7 Assay by Determination of Esters, High-Boiling Method

Proceed as directed under *Ester Determination (High-Boiling Solvent)*, page 501, using the sample weight and equivalence factor (*e*), respectively, as specified for each substance below.

Cresyl Acetate: 1.2 g/75.09
Eugenyl Acetate: 1.7 g/103.1
Linalyl Isobutyrate: 1.5 g/112.2
Terpinyl Propionate: 1.5 g/105.2
p-Tolyl Isobutyrate: 1.2 g/89.12

M-8 Assay by Gas-Liquid Chromatography

M-8a EOA GENERAL METHOD, POLAR COLUMN

Proceed as directed in *Section 5, GLC Analysis of Flavor Aromatic Chemicals and Isolates*. The composition of the polar column and the conditions of analysis may be varied at the discretion of the analyst, provided that such changes would result in equal or improved separations and/or quantification as would be obtained by use of the particular column material and test conditions specified therein.

M-8b EOA GENERAL METHOD, NONPOLAR COLUMN

Proceed as directed in *Section 5, GLC Analysis of Flavor Aromatic Chemicals and Isolates*. The composition of the nonpolar column and the conditions of analysis may be varied at the discretion of the analyst, provided that such changes would result in equal or improved separations and/or quantification as would be obtained by use of the particular column material and test conditions specified therein.

M-8c MISCELLANEOUS PROCEDURES

Use the procedure as specified for each substance below.

Acetoin Determine the percentage of acetoin by gas-liquid chromatography (see page 475), using an instrument containing a thermal conductivity detector. Prepare a 1.5-m × 6.35-mm column consisting of 20% Carbowax 20M on acid-washed, 60/80-mesh Chromosorb W, or other components capable of separating diacetyl, water, and acetoin. Observe the following operating conditions during the determination: *sample*, 2 µl; *injector*, about 195°; *column*, about 130°; *detector*, about 230°; and *helium flow rate*, about 35 ml per min. The approximate retention time for diacetyl is 2-1/4 min, for water, 3 min, and for acetoin, 12 min. The area of the acetoin peak is not less than 96.0% of the total area of all peaks.

2-Butanone Determine the purity of 2-butanone by gas-liquid chromatography (see page 475), using an instrument containing a thermal conductivity detector. Prepare a 4-m × 6-mm column consisting of a blend of equal quantities of 20% Carbowax 20M on acid-washed, 60/80-mesh Chromosorb W and 20% tetrahydroxyethyl ethylenediamine on 30/60-mesh Chromosorb P, or use other suitable column materials capable of separating 2-butanone and the impurities whose retention times are listed below. Observe the following operating conditions during the determination: *sample*, 10 µl; *column temperature*, about 80°; *helium flow rate*, 30 to 32 ml per min; and *detector voltage*, 8.0. The approximate retention times, in min, are as follows: acetone, 7; ethyl acetate, 9; 2-butanone, 11; *t*-butanol, 14; methanol, 15; ethanol, 19; 2-butanol, 30; and propanol, 36.

Measure the area under each peak of the chromatogram so obtained, calculate the area percent of each impurity, and record the sum of the impurities as *A*. (NOTE: In this procedure, area percent and weight percent may be assumed to be identical.) Calculate the percentage of C_4H_8O in the sample by the formula

$$100.0 - (A + B + C),$$

in which *B* is the percentage of water determined by the *Karl Fischer Titrimetric Method*, page 552, and *C* is the acidity (as acetic acid) determined under M-15a.

Δ-Decalactone The purity of Δ-decalactone is determined by gas-liquid chromatography (see page 475), using an instrument containing a thermal conductivity detector and helium as the carrier gas. The operating conditions of the apparatus may vary, depending upon the particular instrument used, but a suitable chromatogram is obtained with a glass, stainless steel, aluminum, or copper column, 2.74-m × 4.8-mm od, packed with 25% polyester (polydiethylene glycol glutarate, stabilized with 2% phosphoric acid, is satisfactory) on 60- to 80-mesh Chromosorb P or W, and operated at a constant temperature between 190° and 210°. If the detector is separately thermostated, it should be maintained at the column temperature or up to 25° hotter. The recorder should be equipped with an attenuator switch and should be operated in the 0- to 1-mV range, with 1-s full-scale deflection at a chart speed of 1/2 in. per second. A constant gas flow should be established and maintained throughout the determination. The inlet gas pressure, which will vary between columns and instruments, should not exceed 40 psi.

With helium gas flowing through the apparatus, adjust the column and detector to the operating temperature and record a baseline. Inject a sample of 0.4 to 4 µl into the apparatus, adjusting the sample size, if necessary, so that the major peak is not attenuated more than 8 times, preferably less, and obtain the chromatogram. Under the conditions described, and using a helium flow rate of about 15 ml per min, the Δ-decalactone is eluted in about 15 min and an area of about 600 cm^2 is

generated at attenuation ×8. The area of the Δ-decalactone peak is not less than 98.0% of the total area of all peaks.

Diethyl Succinate Determine the purity of diethyl succinate by gas-liquid chromatography (see page 475), using an instrument containing a thermal conductivity detector and helium as the carrier gas. The operating conditions of the apparatus may vary, depending upon the particular instrument used, but a suitable chromatogram is obtained with an aluminum column, approximately 2 m × 6 mm, packed with 30%, by weight, Carbowax 20M on 60/80-mesh Chromosorb W, and operated at a constant temperature of about 200°. The injection port temperature should be about 250°, and the detector should be thermostated at about 260°. The recorder should be operated in the 0- to 1-mV range, with the detector current set at 200 mA. The gas flow rate should be adjusted (about 55 ml per min) so that, after the injection of a 20-μl sample, the retention time for diethyl succinate will be about 8.5 min, resulting in a retention time for diethyl maleate of about 10.8 min under the conditions described. After the chromatogram is obtained, measure the area under all peaks and calculate the concentrations of diethyl succinate and diethyl maleate, in weight percent, by applying an appropriate correction factor. (NOTE: In the calculation of diethyl succinate content, the area percent and weight percent may be assumed to be identical.) Determine the calibration factor for diethyl maleate by analyzing a sample of known composition to which measured amounts of diethyl maleate have been added, following the procedure described above.

Δ-Dodecalactone Determine as directed for *Δ-Decalactone* above. The Δ-dodecalactone is eluted in about 25 min, and an area of about 600 cm^2 is generated at attenuation ×8. The area of the Δ-dodecalactone peak is not less than 98.0% of the total area of all peaks.

Ethyl Acrylate Determine the percentage of ethyl acrylate by gas-liquid chromatography (see page 475), using an instrument containing a thermal conductivity detector. Prepare a 2.5-m × 6-mm column packed with 25%, by weight, of Carbowax 20M on 42/60 GC-22. Observe the following operating conditions during the determination: *sample*, 10 μl; *injector temperature*, about 300°; *column temperature*, 50° to 225°, programmed at a rate of 5.6° per min; *detector temperature*, about 300°; *detector current*, 200 mA; and *helium flow*, about 100 ml per min. Under the conditions described, the retention time for ethyl acrylate is 9 min. The area of the ethyl acrylate peak is not less than 99.5% of the total area of all peaks.

M-9 Assay by Determination of Linalool Content

M-9a GENERAL METHOD

For linalool, proceed as directed under *Linalool Determination*, page 501, using about 1.2 g of acetylated oil, accurately weighed.

M-9b MODIFICATION OF GENERAL METHOD

Dimethyl Benzyl Carbinol Acetylate a 10-ml sample as directed under *Linalool Determination*, page 501. Weigh accurately about 1.5 g of the acetylated alcohol, and proceed as directed under *Ester Determination*, page 500. Calculate the percentage of dimethyl benzyl carbinol ($C_{10}H_{14}O$) in the original sample by the formula given for linalool, page 501, substituting 7.511 for 7.707 as the equivalence factor.

Nerolidol Proceed as directed under *Linalool Determination*, page 501, using 1.5 g of the dried acetylated oil, and reflux with 0.5 *N* alcoholic potassium hydroxide for 6 h. Calculate the total alcohols by the formula

$$\frac{100(b-S) \times 111.19}{W-0.042(b-S)},$$

in which b is the number of ml of 0.5 *N* hydrochloric acid consumed in the residual titration, S is the number of ml of 0.5 *N* hydrochloric acid consumed in the titration of the sample, and W is the weight, in mg, of the acetylated oil sample.

M-10 Assay by Determination of Phenols

Proceed as directed for *Phenols*, page 502, for the following substances:

Carvacrol
Eugenol
Isoeugenol

M-11 Assay by Titrimetric Procedures

M-11a DIRECT AQUEOUS ACID-BASE TITRATIONS

Transfer an accurately weighed amount of the sample, as specified below, to a 250-ml Erlenmeyer flask containing 75 to 100 ml of water, add phenolphthalein TS, and titrate with 0.5 *N* sodium hydroxide to the first pink color that persists for 15 s. Each ml of 0.5 *N* sodium hydroxide is equivalent to the amount of substance as specified below.

Substance	Sample Weight (g)	1 ml 0.5 *N* NaOH Equivalent to
Butyric Acid	1.5	44.06 mg of $C_4H_8O_2$
Hexanoic Acid	2.0	58.08 mg of $C_6H_{12}O_2$
Isobutyric Acid	1.5	44.06 mg of $C_4H_8O_2$
Isovaleric Acid	1.5	51.07 mg of $C_5H_{10}O_2$

Conditions for Direct Aqueous-Alcoholic Acid-Base Titrations

Substance	Sample Weight (mg)	Solvent	*N* NaOH	Equivalent to
Cinnamic Acid (dried in desiccator 3 h over silica gel)	500	25 ml 50% ethanol	0.1	14.82 mg of $C_9H_8O_2$
2-Ethylbutyric Acid	2200	100 ml 50% isopropanol	0.5	58.08 mg of $C_6H_{12}O_2$
Phenylacetic Acid (dried 3 h over H_2SO_4)	500	25 ml 50% ethanol	0.1	13.62 mg of $C_8H_8O_2$
Valeric Acid	1500	100 ml 50% isopropanol	0.5	51.07 mg of $C_5H_{10}O_2$

M-11b DIRECT AQUEOUS-ALCOHOLIC ACID-BASE TITRATIONS

Dissolve an accurately weighed amount of the sample, as specified in the table *Conditions for Direct Aqueous-Alcoholic Acid-Base Titrations* at the top of this page, in the specified solvent, which has been previously neutralized to phenolphthalein TS with 0.1 *N* sodium hydroxide. Titrate with the specified normality of sodium hydroxide to a pink color. Each ml of titrant is equivalent to the amount of substance specified.

M-11c ACID-BASE RESIDUAL BLANK TITRATIONS

Butyl Acetate Transfer about 1.5 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, add 25.0 ml of 1 *N* potassium hydroxide and 25 ml of anhydrous isopropanol, swirl to effect complete solution, and allow to stand at room temperature for 30 min. Add about 1 ml of phenolphthalein TS to the mixture, and titrate with 0.5 *N* sulfuric acid to the disappearance of the pink color. Perform a residual blank determination (see page 2). Each ml of 0.5 *N* sulfuric acid is equivalent to 58.08 mg of $C_6H_{12}O_2$.

Ethyl Acetate Transfer about 1.5 g, accurately weighed in a tared, stoppered weighing bottle, to a suitable flask, add 50.0 ml of 0.5 *N* sodium hydroxide, and heat on a steam bath under a reflux condenser for 1 h. Allow it to cool, add phenolphthalein TS, and titrate the excess sodium hydroxide with 0.5 *N* hydrochloric acid. Perform a blank determination (see page 2) and make any necessary correction. Each ml of 0.5 *N* sodium hydroxide is equivalent to 44.06 mg of $C_4H_8O_2$.

Isopropyl Acetate Transfer 25.0 ml of 1 *N* potassium hydroxide into a suitable heat-resistant pressure bottle provided with a tight closure that can be securely fastened, and then add 10 ml of isopropanol and a few pieces of glass rod. To the mixture in the pressure bottle add 1.3 g of the sample contained in a sealed glass ampule and accurately weighed. Cap the bottle, shake it vigorously to break the ampule, and allow it to stand at room temperature for 30 min. Uncap the bottle, add phenolphthalein TS, and titrate with 0.5 *N* sulfuric acid to the disappearance of the pink color. Perform a residual blank titration (see page 2). Each ml of 0.5 *N* sulfuric acid is equivalent to 51.07 mg of $C_5H_{10}O_2$.

M-11d NONAQUEOUS TITRATIONS

Ethyl Acetoacetate Introduce 50 ml of freshly distilled pyridine into a 250-ml Erlenmeyer flask, and add about 450 mg of the sample, accurately weighed. Stopper the flask, and swirl the mixture to effect complete solution. Add a few drops of thymolphthalein TS, and titrate with 0.1 *N* sodium methoxide in pyridine to the first appearance of a blue endpoint. Perform a blank determination (see page 2). During the titration direct a gentle stream of nitrogen into the flask through a short piece of 6-mm glass tubing attached near the tip of the buret. Each ml of 0.1 *N* sodium methoxide in pyridine is equivalent to 13.01 mg of $C_6H_{10}O_3$.

Ethyl Vanillin Transfer about 300 mg of the sample, previously dried over phosphorus pentoxide for 4 h and accurately weighed, into a 125-ml Erlenmeyer flask, and dissolve in 50 ml of dimethylformamide. Add 3 drops of thymol blue TS, and titrate with 0.1 *N* sodium methoxide, using a magnetic stirrer and taking precautions against absorption of atmospheric carbon dioxide. Perform a blank determination (see page 2). Each ml of 0.1 *N* sodium methoxide is equivalent to 16.62 mg of $C_9H_{10}O_3$.

M-11e MISCELLANEOUS TITRIMETRIC PROCEDURES

Allyl Isothiocyanate Transfer about 4 ml, accurately weighed, into a 100-ml volumetric flask, and add sufficient alcohol to make 100.0 ml. Pipet 5 ml of this solution into a 100-ml volumetric flask, and add 50.0 ml of 0.1 *N* silver nitrate and 5 ml of ammonia TS. Connect the flask to a reflux condenser, heat it on a water bath for 1 h, and allow the liquid to cool to room temperature. Disconnect the flask from the condenser, add sufficient water to make the mixture measure 100.0 ml, mix well, and filter through a dry filter, rejecting the first 10 ml of filtrate. To 50 ml of the subsequent filtrate, accurately measured, add about 5 ml of nitric acid and 2 ml of ferric

ammonium sulfate TS, and titrate the excess silver nitrate with 0.1 *N* ammonium thiocyanate. Perform a blank determination, using 5 ml of alcohol in place of the sample solution, and make any necessary corrections. Each ml of 0.1 *N* silver nitrate is equivalent to 4.958 mg of C_3H_5NCS.

Hexyl Alcohol Add about 115 g of phthalic anhydride to 700 ml of freshly distilled pyridine contained in a 1000-ml glass-stoppered, amber-colored bottle, and shake vigorously until complete solution is effected. Transfer 25.0 ml of this solution into a suitable heat-resistant pressure bottle provided with a tight closure that can be securely fastened. Introduce about 1 g of the sample, accurately weighed, into the pressure bottle, using a suitable weighing pipet. Cap the bottle, enclose it securely in a canvas bag, and heat for 3 h in a water bath maintained at a temperature between 98° and 100°, keeping the water level in the bath at about the same height as the liquid in the bottle. Remove the bottle from the bath, allow it to cool to room temperature, open it cautiously to prevent loss of the contents, and transfer 50.0 ml of 0.5 *N* sodium hydroxide into the bottle. (NOTE: This 50.0 ml of 0.5 *N* sodium hydroxide is not to be considered in the final calculation.) Add 5 drops of a 1 in 100 solution of phenolphthalein in pyridine to the mixture, and titrate with 0.5 *N* sodium hydroxide to a pink endpoint that persists for at least 15 s. Perform a residual blank titration (see page 2). Each ml of 0.5 *N* sodium hydroxide is equivalent to 51.09 mg of $C_6H_{14}O$.

M-12 Assay by Determination of Total Alcohols

Proceed as directed under *Total Alcohols*, page 499, using the weight of acetylated alcohol for saponification and the equivalence factor (*f*), respectively, as specified for each substance below.

Anisyl Alcohol: 1.5 g/69.09
Cinnamyl Alcohol, Synthetic: 1 g/67.09
Citronellol: 1.2 g/78.13
1-Decanol, Natural: 1.2 g/79.15
3,7-Dimethyl-1-octanol: 1.2 g/79.15
Geraniol: 1.2 g/77.13
Heptyl Alcohol: 1 g/58.10
Isopulegol: 1.2 g/77.12 (NOTE: Reflux for 2 h.)
Lauryl Alcohol, Natural: 1.5 g/93.17
α-Methylbenzyl Alcohol: 1 g/61.08
Nerol: 1.2 g/77.13
Nonyl Alcohol: 1.2 g/72.13
1-Octanol, Natural: 1.4 g/65.12
3-Phenyl-1-propanol: 1 g/68.10
Rhodinol: 1.2 g/78.14
Santalol: 1.6 g/110.2

M-13 Assay by Ultraviolet Absorption Spectrophotometry

Vanillin

Standard Solution Transfer about 100 mg, accurately weighed, of USP Vanillin Reference Standard into a 250-ml volumetric flask, add methanol to volume, and mix. Transfer 2.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with methanol, and mix.

Assay Solution Transfer about 100 mg, accurately weighed, of the sample into a 250-ml volumetric flask, add methanol to volume, and mix. Transfer 2.0 ml of this solution into a 100-ml volumetric flask, dilute to volume with methanol, and mix.

Procedure Determine the absorbance of each solution in a 1-cm quartz cell at the wavelength of maximum absorption at about 308 nm, with a suitable spectrophotometer, using methanol as the blank. Calculate the quantity, in mg, of $C_8H_8O_3$ in the sample taken by the formula $12.5C(A_U/A_S)$, in which C is the concentration, in μg per ml, of USP Vanillin Reference Standard in the *Standard Solution*, A_U is the absorbance of the *Assay Solution*, and A_S is the absorbance of the *Standard Solution*.

M-14 Quantitative Requirements Other Than Assays

M-14a ALCOHOL CONTENT OF ETHYL OXYHYDRATE

Mix 25.0 ml of the sample with an equal volume of water in a separator, saturate with sodium chloride, and extract with three 25-ml portions of solvent hexane. Extract the combined solvent hexane extracts with three 10-ml portions of a saturated solution of sodium chloride, and then discard the solvent hexane solutions. Combine the saline solutions in a suitable distillation flask, and distil, collecting 25 ml of distillate. The specific gravity of the distillate is not greater than 0.9814, indicating an alcohol content of not less than 14.0% by volume.

M-14b SOLIDIFICATION POINT OF *dl*-MENTHOL

Determine as directed in the general method, page 538, using a sample previously dried in a desiccator over silica gel for 24 h and adjusting the temperature of the cooling bath to a temperature between 23° and 25°. *dl*-Menthol solidifies between 27° and 28°. Continue the stirring. After a few min the temperature quickly rises to 30.5° to 32°.

Conditions for Acidity Limit Test

Substance	Sample Weight (g)	Acidity Expressed as	*N* Alc KOH	1 ml of Titrant Equivalent to
2-Butanone	60	acetic acid	0.02	1.2 mg of $C_2H_4O_2$
Butyl Acetate	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
Butyl Alcohol	60	acetic acid	0.02	1.2 mg of $C_2H_4O_2$
Diethyl Succinate	59	succinic acid	0.1	5.904 mg of $C_4H_6O_4$
2-Ethylbutyraldehyde	29	2-ethylbutyric acid	0.5	58.08 mg of $C_6H_{12}O_2$
2-Heptanone	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
3-Heptanone	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
Hexyl Alcohol	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
Isobutyl Alcohol	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
Isopropyl Acetate	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
4-Methyl-2-pentanone	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$
2-Pentanone	60	acetic acid	0.1	6.01 mg of $C_2H_4O_2$

M-15 Limit Test for Acidity

M-15a NO ADDITIONAL SOLVENT

Transfer an accurately weighed amount of the sample, as specified in the table *Conditions for Acidity Limit Test* at the top of this page, to a 250-ml Erlenmeyer flask, add phenolphthalein TS, and titrate to the first pink color that persists for 15 s with the normality of alcoholic potassium hydroxide specified. Calculate the acidity, in terms of the acid specified, using the equivalence factor shown for the appropriate normality of titrant.

M-15b VARIATIONS ON METHOD 15a

Acetaldehyde (Acidity as Acetic Acid) Mix a 3.9-ml (3-g) sample with 10 ml of water, previously cooled to about 5°, add phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium hydroxide to a pink color that persists for 15 s. Not more than 0.5 ml is required.

Butyraldehyde (Acidity as Butyric Acid) Transfer 54 ml (44 g) into a 250-ml Erlenmeyer flask through which a gentle stream of nitrogen has been passed for 2 min. Add phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium hydroxide in an atmosphere of nitrogen to a pink endpoint that persists for at least 15 s. Each ml of 0.1 *N* alcoholic potassium hydroxide is equivalent to 8.81 mg of $C_4H_8O_2$.

Ethyl Acetate A solution of 2 ml in 10 ml of neutralized alcohol requires not more than 0.1 ml of 0.1 *N* sodium hydroxide for neutralization using 2 drops of phenolphthalein TS as the indicator.

Ethyl Acetoacetate (Acidity as Acetic Acid) Transfer 10 ml of water, recently boiled and then cooled to about 5°, into a 250-ml Erlenmeyer flask containing 50 ml of alcohol, add about 0.5 ml of bromocresol purple TS, and neutralize the mixture with 0.1 *N* sodium hydroxide to the appearance of a blue endpoint. Introduce 25.0 ml of the sample, previously cooled to about 5°, into the flask, and titrate with 0.1 *N* sodium hydroxide to a blue endpoint that persists for at least 30 s. Not more than 8.7 ml is required.

Ethyl Acrylate (Acidity as Acrylic Acid) Transfer 50 ml (46 g) of the sample into a 250-ml Erlenmeyer flask, and add 50 ml of methanol previously neutralized to bromothymol blue TS with 0.1 *N* alcoholic potassium hydroxide. Add an additional 5 or 6 drops of the bromothymol blue TS, and titrate with 0.1 *N* alcoholic potassium hydroxide to a bluish green endpoint. Each ml of 0.1 *N* alcoholic potassium hydroxide is equivalent to 7.21 mg of $C_3H_4O_2$.

Isobutyraldehyde (Acidity as Butyric Acid) Transfer 56 ml (44 g) into a 250-ml Erlenmeyer flask through which a gentle stream of nitrogen has been passed for 2 min. Add phenolphthalein TS, and titrate with 0.1 *N* alcoholic potassium hydroxide in an atmosphere of nitrogen to a pink endpoint that persists for at least 15 s. Each ml of 0.1 *N* alcoholic potassium hydroxide is equivalent to 8.81 mg of $C_4H_8O_2$.

Propionaldehyde (Acidity as Propionic Acid) Transfer 50 ml of methanol into a 250-ml Erlenmeyer flask through which a gentle stream of nitrogen has previously been passed for 2 min. Add phenolphthalein TS to the methanol, and neutralize it with 0.02 *N* alcoholic potassium hydroxide. Introduce 20 ml (16 g) of the sample using a suitable transfer pipet. Add 3 or 4 additional

drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a pink endpoint that persists for at least 15 s. During the titration direct a gentle stream of nitrogen into the flask through a short piece of 6-mm glass tubing fastened near the tip of the buret. Each ml of 0.1 *N* sodium hydroxide is equivalent to 7.41 mg of $C_3H_6O_2$.

Tributyrin (Acidity as Acetic Acid) To 75 ml of methanol, contained in a 250-ml wide-mouth Erlenmeyer flask, add 1 ml of bromothymol blue TS, and titrate with 0.05 *N* sodium hydroxide to a blue endpoint. Transfer a 20.0-ml sample into the flask, calculate its weight from its specific gravity, and titrate back to the same blue endpoint. Each ml of 0.05 *N* sodium hydroxide is equivalent to 3 mg of acetic acid ($C_2H_4O_2$).

M-15c ACIDITY DETERMINATION BY IODOMETRIC METHOD

Ethyl Formate (Acidity as Formic Acid) Transfer about 5 g, accurately weighed, into a glass-stoppered flask containing a solution of 500 mg of potassium iodate and 2 g of potassium iodide in 50 ml of water. Titrate the liberated iodine with 0.1 *N* sodium thiosulfate, using starch TS as the indicator. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 4.603 mg of CH_2O_2.

M-16 Limit Tests for Aldehydes and Ketones

M-16a DETERMINATION BY HYDROXYLAMINE METHOD

Proceed as directed under *Aldehydes and Ketones—Hydroxylamine Method*, page 500, using the sample weight and equivalence factor (*e*) as specified below.

Substance	Sample Weight (g)	Aldehyde/Ketone Expressed as	*e*
Anisyl Alcohol	5[a]	anisic aldehyde	68.07
Cinnamyl Alcohol	5	cinnamaldehyde	66.08
Citronellol	5	citronellal	77.13
Geraniol	5	citronellal	77.13
Hydroxycitronellyl Dimethyl Acetal	5	hydroxycitronellal	86.13
Isopulegol	10	citronellal	77.13
α-Methylbenzyl Alcohol	10	acetophenone	60.07
2-Phenylpropionaldehyde Dimethyl Acetal	10	2-phenylpropionaldehyde	67.09

[a] Allow the mixture to stand at room temperature 30 min before titrating.

M-16b DETERMINATION BY SPECIFIC ALDEHYDES METHOD

Proceed as directed under *Aldehydes*, page 499, using the sample weight and equivalence factor (*e*) as specified below.

Substance	Sample Weight (g)	Aldehyde Expressed as	*e*
Benzyl Alcohol	5[a]	benzaldehyde	53.06
Heptyl Alcohol	10	heptanal	57.10
Phenylacetaldehyde Dimethyl Acetal	5	phenylacetaldehyde	60.07
3-Phenyl-1-propanol	5	phenyl propyl aldehyde	67.09

[a] Modify the procedure by using a 250-ml Erlenmeyer flask and 75 ml of *Hydroxylamine Hydrochloride Solution*.

M-16c DETERMINATION BY SEMIQUANTITATIVE PROCEDURES

Anethole (Test for Aldehydes and Ketones) Shake 10 ml with 50 ml of a saturated solution of sodium bisulfite in a glass-stoppered, graduated cylinder, and allow the mixture to stand for 6 h. The volume of the sample does not diminish appreciably, and no crystalline deposit separates.

Butyl Alcohol (Test for Aldehydes) Transfer 10 ml of ammoniacal silver nitrate TS into a 20- × 150-mm test tube, add 10 ml of the sample, mix thoroughly, and allow the mixture to stand in a dark place for 30 min. No color is produced, but a slight precipitate may form at the interface of the two layers.

M-17 Limit Test for Antioxidants in Ethyl Acrylate

Preliminary Examination of the Sample Wash a 25-ml portion of the sample with 25 ml of sodium hydroxide solution (1 in 10). Any yellow or brown coloration in the extract indicates the presence of hydroquinone, in which case both of the procedures below (*A* and *B*) must be followed to determine the antioxidant content. If the sodium hydroxide extract remains colorless, the first procedure (*A*) need not be run and the antioxidant content is determined by the second procedure (*B*) alone.

A. Determination of Hydroquinone

Carbonyl-Free Methanol To 500 ml of anhydrous methanol add 5 g of 2,4-dinitrophenylhydrazine, heat the mixture under a reflux condenser for 2 h, and then recover the methanol by distillation. Store the carbonyl-free methanol in tight containers.

2,4-Dinitrophenylhydrazine Solution Dissolve 100 mg of 2,4-dinitrophenylhydrazine in 50 ml of *Carbonyl-Free*

Methanol, add 4 ml of hydrochloric acid, and dilute to 100 ml with water.

Sodium Carbonate Solution Dissolve 530 mg of sodium carbonate in sufficient water to make 100 ml.

Pyridine-Diethanolamine Solution Mix 5 ml of diethanolamine with 500 ml of freshly distilled pyridine.

Calibration Curve Transfer 25 mg of hydroquinone, accurately weighed, into a 100-ml volumetric flask, add sufficient butyl acetate to volume, and mix thoroughly (250 µg per ml). Prepare a series of standards by transferring 1.0-, 2.0-, 3.0-, 4.0-, and 6.0-ml portions of this solution into separate 50-ml volumetric flasks, and dilute each aliquot to 50.0 ml with butyl acetate. One ml of each of these standards contains 5, 10, 15, 20, and 30 µg, respectively, of hydroquinone. Transfer 1.0 ml of each solution into separate 25-ml glass-stoppered graduates, and continue as directed in the *Procedure*, beginning with "add 2.0 ml of water. . . . " Plot a calibration curve of absorbance versus µg of hydroquinone. Fifteen µg of hydroquinone should be equivalent to approximately 0.30 units of absorbance, and the curve should intersect the origin.

Procedure Using a hypodermic syringe, transfer 0.2 ml of the sample, accurately weighed, into a 25-ml glass-stoppered graduate, add 2.0 ml of water, stopper the graduate, and mix the contents well without allowing contact between the liquid and the stopper. Add to the mixture 0.5 ml of the *Sodium Carbonate Solution*, and immediately shake gently for 5 s, avoiding contact between the solution and the stopper. Immediately add 1.0 ml of a 15%, volume in volume, solution of sulfuric acid, shake as previously directed, and add 1-ml of the *Dinitrophenylhydrazine Solution*. Stopper the graduate and place it in a water bath, maintained at a temperature between 70° and 72°, for 1 h. Shake samples three times during the heating period. Cool the graduate to room temperature, dilute the contents to 15 ml with water, add 5.8 ml of benzene, stopper, shake vigorously, and then allow the phases to separate. Transfer 2.0 ml of the benzene layer, using a suitable pipet, into a test tube, add 10.0 ml of *Pyridine-Diethanolamine Solution*, and mix. Transfer a portion of this solution into a 2-cm cell, and determine the absorbance at 620 nm with a suitable spectrophotometer, using as a blank 1.0 ml of butyl acetate treated in the same manner as the sample except that 5.0 ml of benzene is used for the extraction instead of 5.8 ml. From the previously prepared *Calibration Curve* read the µg of hydroquinone and/or benzoquinone corresponding to the absorbance of the solution from the sample, and record this value as w. Calculate the ppm of hydroquinone (ppm HQ) in the sample by the formula $1000w/W$, in which W is the weight of the sample taken, in mg.

B. Determination of Hydroquinone Monomethyl Ether

Antioxidant-Free Ethyl Acrylate Wash a suitable volume of the sample with three separate similar-sized volumes of sodium hydroxide solution (1 in 10). After the last washing add a small amount of sodium chloride, if necessary, to remove any turbidity that may be present.

Calibration Curve Transfer 25.0 mg of hydroquinone monomethyl ether, accurately weighed, into a 100-ml volumetric flask, add *Antioxidant-Free Ethyl Acrylate* to volume, and shake to effect complete solution (250 µg per ml). Prepare a series of standards by transferring 1.0-, 5.0-, 10.0-, and 20.0-ml portions of this solution into separate 25-ml volumetric flasks, dilute to volume with *Antioxidant-Free Ethyl Acrylate*, and mix. One ml of each of the standards contains 10, 50, 100, and 200 µg, respectively, of hydroquinone monomethyl ether. Transfer 5.0 ml of each solution into separate 50-ml volumetric flasks, dilute each to volume with isooctane, and mix. Determine the absorbance of each solution in a 1-cm silica cell at 292 nm with a suitable spectrophotometer, using a 1 in 10 dilution of *Antioxidant-Free Ethyl Acrylate* as the blank. Plot a calibration curve of absorbance versus µg of hydroquinone monomethyl ether. The curve should be linear and should intersect the origin.

Procedure Transfer 5.0 ml of the sample, accurately weighed, into a 50-ml volumetric flask, dilute to volume with isooctane, and mix. Determine the absorbance of this solution in a 1-cm silica cell at 292 nm with a suitable spectrophotometer, using a 1 in 10 dilution of *Antioxidant-Free Ethyl Acrylate* in isooctane as the blank. From the previously prepared *Calibration Curve* read the µg of hydroquinone monomethyl ether corresponding to the absorbance of the sample solution, and record this value as w. Calculate the ppm of hydroquinone monomethyl ether (ppm HMME) in the sample by the formula w/W, in which W is the weight of the sample taken, in g. [NOTE: If the first sodium hydroxide extract obtained under *Preliminary Examination of the Sample* (or under *Antioxidant-Free Ethyl Acrylate*) showed a yellow coloration, the true ppm HMME is obtained by subtracting the ppm HQ, obtained under section *A*, from the apparent ppm HMME.]

M-18 Limit Test for Arsenic

A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.

M-19 Limit Test for Butyl Ether in Butyl Alcohol

Determine the percentage of butyl ether in the sample by gas-liquid chromatography (see page 475), using an instrument containing a thermal conductivity detector. Prepare a 2-m × 6-mm column consisting of 25%, by weight, β,β′-thiodipropionitrile on 30/40-mesh Chromosorb P and operated at a constant temperature of about 85°. Establish a helium flow rate of about 75 ml per min, adjust the inlet pressure to about 30 psi, and inject a sample of about 10 µl. The recorder should be operated in the 0- to 1-mV range, with the detector voltage set at about 8, depending upon the particular instrument used. Under the conditions described, the butyl ether is eluted in about 6 min and the *n*-butyl alcohol in about 25 min, so that the relative retention of the butyl ether in butyl alcohol is about 0.24. A known mixture of about 1% butyl ether in butyl alcohol should

be chromatographed to verify the retention time of the ether. The area of the butyl ether peak is not more than 0.2% of the total area of all peaks.

M-20 Limit Test for *para*-Butyraldehyde in Butyraldehyde

Transfer about 30 ml of a freshly prepared 1 in 5 solution of sodium bisulfite into a Babcock bottle having a neck graduated into 8 units of 0.2 ml and each of which is further subdivided into 10 parts, representing a volume of 0.02 ml each. To the sodium bisulfite solution add 5.0 ml of the sample, stopper the bottle, immerse in an ice water bath, and shake vigorously until heat is no longer evolved. Remove the bottle from the bath, shake it at room temperature for about 5 min, then fill to the highest graduate with sodium bisulfite solution, mix, and centrifuge for 5 min. Read the volume of the oil layer in ml, and calculate the percentage of *para*-butyraldehyde by the formula

$$(a + 0.05) \times 92.0/(5 \times b),$$

where a is the volume of the oil layer, b is the specific gravity of the butyraldehyde, 0.05 is a solubility factor, and 92.0 relates to the specific gravity of *para*-butyraldehyde.

M-21 Limit Test for Butylic and Amylic Derivatives in Ethyl Acetate

Allow 10 ml of the sample to evaporate spontaneously from clean, odorless blotting paper. The final odor does not resemble that of pineapple or banana.

M-22 Quantitative Test for Chlorinated Compounds in Cinnamic Acid

Mix 1 g with 500 mg of sodium carbonate, add 15 ml of water, evaporate the solution on a steam bath, and then ignite the residue at the lowest possible temperature until it is thoroughly charred. Extract the charred mass with a mixture of 20 ml of water and 5.5 ml of nitric acid, filter, and wash the residue with sufficient water to make 50 ml. Dilute 10 ml of the filtrate to 25 ml with water, and add 1 ml of silver nitrate TS. Any turbidity does not exceed that shown in a control of equal volume containing 100 mg of sodium carbonate, 1.1 ml of nitric acid, and 10 µg of chloride ion (Cl) (see page 471).

M-23 Limit Test for Esters in Alcohols

Proceed as directed under *Ester Determination*, page 500, using the sample weight and equivalence factor (*e*) as specified below.

Substance	Sample Weight (g)	Ester Expressed as	*e*
Citronellol	5	citronellyl acetate	99.15
Geraniol	5	geranyl acetate	98.15
Linalool	10	linalyl acetate	98.15
Nerolidol	10	nerolidyl acetate	132.7
Rhodinol	5	rhodinyl acetate	99.15

M-24 Limit Test for Heavy Metals

M-24a UNMODIFIED GENERAL METHOD (page 512)

Prepare and test, as directed in *Method II* under the *Heavy Metals Test*, page 513, an accurately weighed sample corresponding to the following.

Heavy Metals Limit	Sample Weight (mg)
0.004%	500
0.002%	1000
10 ppm	2000
5 ppm	4000

Use 20 µg of lead ion (Pb) in the control (*Solution A*).

M-24b SPECIAL SAMPLE PREPARATION FOR CINNAMIC ACID

Volatilize 2 g over a low flame. To the residue add 2 ml of nitric acid and about 10 mg of sodium carbonate, and evaporate to dryness on a water bath. Dissolve the residue in a mixture of 1 ml of diluted acetic acid TS and 24 ml of water. This solution meets the requirements of the *Heavy Metals Test*, page 512, using 20 µg of lead ion (Pb) in the control (*Solution A*).

M-25 Limit Test for Hydrocarbons in Eugenol

Dissolve 1 ml of the sample in 20 ml of 0.5 *N* sodium hydroxide in a stoppered 50-ml tube, add 18 ml of water, and mix. A clear mixture results immediately, but it may become turbid when exposed to air.

M-26 Limit Test for Hydrocyanic Acid in Benzaldehyde

Shake 0.5 ml with 5 ml of water, add 0.5 ml of sodium hydroxide TS and 0.1 ml of ferrous sulfate TS, and warm the mixture gently. Upon the addition of a slight excess of hydrochloric acid, no greenish blue color or blue precipitate is produced within 15 min.

M-27 Limit Test for Lead

A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Lead Limit Test*, page 518, using 10 µg of lead ion (Pb) in the control.

M-28 Limit Test for Methanol-Formaldehyde in Ethyl Oxyhydrate

Chromotropic Acid Solution Dissolve 5 g of chromotropic acid sodium salt in sufficient water to make 100 ml, and filter, if necessary, to obtain a clear solution.

Potassium Permanganate Solution Dissolve 3.0 g of potassium permanganate and 15.0 ml of phosphoric acid in sufficient water to make 100.0 ml. Discard after 30 days.

Standard Preparation Prepare a solution containing 0.025%, by volume, of methanol in 5.5% ethanol.

Test Preparation Dilute an accurately measured volume of the sample to provide a total alcohol concentration of between 5% and 6%. Dilute an accurately measured volume of this solution with 5.5% ethanol to provide a methanol concentration of about 0.05%.

Procedure Transfer 1.0 ml each of the *Standard Preparation*, of the *Test Preparation*, and of 5.5% ethanol into separate 50-ml volumetric flasks containing 2.0 ml of *Potassium Permanganate Solution* previously chilled in an ice bath. Allow to stand for 30 min in the ice bath, then add sufficient dry sodium bisulfite to decolorize the solution, add 1.0 ml of *Chromotropic Acid Solution*, and mix. Slowly add, with swirling, 15 ml of sulfuric acid, and heat in a water bath at 60° to 75° for 15 min. Cool, dilute to volume with water, and mix. Determine the absorbance of each solution in a 1-cm cell at 575 nm, with a suitable spectrophotometer, using the blank to set the instrument. Calculate the percentage, by volume, of methanol-formaldehyde in the sample by the formula $0.025F(A_U/A_S)$, in which F is the dilution factor for the sample, A_U is the absorbance of the solution from the *Test Preparation*, and A_S is the absorbance of the solution from the *Standard Preparation*.

M-29 Limit Test for Methyl Compounds in Ethyl Acetate

Place 20 ml in a 500-ml separator, add a solution of 20 g of sodium hydroxide in 50 ml of water, stopper the separator, and wrap it securely in a towel for protection against the heat of the reaction. Shake the mixture vigorously for about 5 min, cautiously opening the stopcock from time to time to permit the escape of air. Continue the shaking vigorously until a homogeneous liquid results, then distil and collect about 25 ml of the distillate. To 1 drop of the distillate add 1 drop of dilute phosphoric acid (1 in 20) and 1 drop of potassium permanganate solution (1 in 20). Mix, allow to stand for 1 min, and add sodium bisulfite solution (1 in 20) dropwise until the color is discharged. If a brown color remains, add 1 drop of the dilute phosphoric acid. To the colorless solution add 5 ml of a freshly prepared solution of chromotropic acid (1 in 2000) in 75% sulfuric acid, and heat on a steam bath for 10 min at 60°. No violet color appears.

M-30 Limit Test for Nonvolatile Residue in Menthol

Heat 2 g of the sample, accurately weighed, in a tared porcelain dish on a steam bath until volatilized. Dry the residue at 105° for 1 h. The residue weighs no more than 1 mg.

M-31 Limit Test for Odor in Phenethyl Alcohol

Mix thoroughly 2 ml of the sample with 20 ml of ice cold, odorless water. No off-odor should be discernible in the mixture.

M-32 Limit Test for Peroxide Value

To 50 ml of a mixture of 3 volumes of glacial acetic acid and 2 volumes of chloroform add 10 ml of the sample. To this solution add 1 ml of a saturated solution of potassium iodide,

allow to stand for exactly 1 min with gentle shaking, and then introduce 100 ml of water and a few drops of starch TS. Titrate immediately with 0.1 *N* sodium thiosulfate. Each ml of 0.1 *N* sodium thiosulfate, multiplied by 5, equals the peroxide value, expressed in millimoles of peroxide per liter of the sample.

M-33 Limit Test for Phenolic Impurities

M-33a TEST FOR FREE PHENOLS

Proceed as directed under *Free Phenols*, page 502, using the sample weight and equivalence factor (*f*) as specified below.

Substance	Impurity Expressed as	*f*
p-Methyl Anisole	cresol	54.06
Methyl Eugenol	eugenol	82.10
Methyl Isoeugenol	isoeugenol	82.10

M-33b TEST FOR PHENOLS USING CASSIA FLASK METHOD

β-Caryophyllene: Proceed as directed under *Phenols*, page 502.

M-33c QUALITATIVE TEST FOR PHENOLS USING FERRIC CHLORIDE

Allyl Isothiocyanate Dilute 1 ml of the sample with 5 ml of alcohol, and add 1 drop of ferric chloride TS. A blue color is not produced immediately.

Anethole Shake 1 ml with 20 ml of water, and allow the liquids to separate. Filter the water layer through a filter paper previously moistened with water, and to 10 ml of the filtrate add 3 drops of ferric chloride TS. No purplish color is produced.

Anisole Shake 1 ml with about 20 ml of water, allow the layers to separate, collect the water layer in a test tube, and add to it a few drops of ferric chloride TS. No greenish, bluish, or purplish color is produced.

Cresyl Acetate (Test for Free Cresol)

Ferric Chloride Solution Add 1.5 g of anhydrous ferric chloride to 850 ml of chloroform in a 2-l beaker. Add 100 ml of ethylene glycol monobutyl ether. When the ferric chloride has dissolved, add 50 ml of pyridine, mix, and filter through a Buchner funnel.

Procedure Transfer a 5-ml sample to a 15-mm test tube, and add 10 ml of the *Ferric Chloride Solution*. The color of the solution is no darker green than a solution of 5 ml of a 1% solution of cresol in cresol-free methyl *p*-cresol mixed with 10 ml of the *Ferric Chloride Solution*.

M-34 Limit Test for Readily Carbonizable Substances in Ethyl Acetate

Pour 2 ml of the sample carefully upon 10 ml of sulfuric acid TS so as to form separate layers. No discoloration is developed within 15 min.

M-35 Limit Test for Readily Oxidizable Substances in *dl*-Menthol

Transfer 500 mg of *dl*-menthol into a clean, dry test tube, and add 10 ml of potassium permanganate solution prepared by diluting 3 ml of 0.1 *N* potassium permanganate with water to 100 ml. Place the test tube in a beaker of water maintained between 45° and 50°. At 30-s intervals, quickly remove the test tube from the bath and shake. The color of potassium permanganate is still apparent after 5 min.

M-36 Limit Test for Reducing Substances

Dilute 2 ml of the sample in a glass-stoppered flask with 50 ml of water and 5 ml of sulfuric acid, shaking the flask during the addition. While the solution is still warm, titrate with 0.1 *N* potassium permanganate. Not more than 1 ml is required to produce a pink color that persists for 30 min.

M-37 Limit Test for Ignition Residue in Ethyl Acetoacetate

Transfer a 50-g sample into a tared 125-ml platinum dish, heat until the vapors are ignited, withdraw the flame, protect the combustion from drafts, and allow the vapors to continue to burn spontaneously. Transfer the dish into a muffle furnace maintained at about 900°, heat until all carbonaceous material has been removed, then cool in a desiccator, and weigh.

M-38 Limit Test for Solubility in Alkali of γ-Undecalactone

Transfer 5.0 ml into a 100-ml cassia flask, add 70 ml of 1 *N* potassium hydroxide, warm the mixture in a water bath at 50°

to 60°, and shake for 15 min. Add sufficient 1 *N* potassium hydroxide to raise the level of the liquid into the graduated portion of the neck of the flask, and cool to room temperature. No oil layer forms, and the solution is not more than slightly cloudy.

M-39 Limit Test for Solubility in Bisulfite of Aldehydes

Benzaldehyde Transfer 5 ml to a 100-ml cassia flask. Add 70 ml of a 10%, by weight, solution of sodium metabisulfite. Shake the mixture vigorously for 15 min at room temperature. Add sufficient bisulfite solution to bring the liquid into the graduated neck of the flask. No oil should separate, and the solution should be clear or not more than slightly cloudy.

Cinnamaldehyde Measure 10 ml from a pipet into a 100-ml cassia flask, and add 75 ml of a freshly prepared solution of sodium bisulfite (12 in 100) previously heated to a temperature of 85°. Shake the flask vigorously until solution is complete, then add sufficient sodium bisulfite solution to raise the meniscus within the graduated portion of the neck. No oil separates.

Citral Pipet 10.0 ml into a 150-ml cassia flask, and add 10 ml of a freshly prepared solution containing 30%, by weight, of anhydrous sodium bisulfite ($NaHSO_3$). Shake the flask in a boiling water bath for 2 min. If the mixture has not solidified, continue shaking the flask in the boiling water bath for an additional 2 min. Add an additional 50 ml of the sodium bisulfite solution, return the flask to the water bath, and shake until the material is apparently clear. Add enough hot sodium bisulfite solution to the flask to raise the meniscus to the top of the graduated neck of the flask. Immerse the flask in the boiling water bath for 10 min. Remove the flask. Not more than 0.2 ml of oil separates when the flask and contents are cooled to 25°.

Heptanal Pipet 5 ml of the sample into a 100-ml cassia flask, and add 70 ml of a 1 in 10, weight in weight, solution of sodium metabisulfite. Warm the mixture on a water bath to 50°–60°, and shake the flask vigorously for 15 min. When the liquids have separated completely, add sufficient sodium metabisulfite solution to raise the lower level of the oily layer within the graduated portion of the neck of the flask. Not more than 0.25 ml of oil separates.

Hydroxycitronellal Transfer 5 ml to a 100-ml cassia flask. Add 70 ml of a 10%, by weight, solution of sodium metabisulfite. Shake the mixture vigorously for 15 min. Add the sodium metabisulfite solution to raise the liquid into the graduated neck of the flask. No oil separates, and the solution is no more than slightly cloudy.

5 / GLC Analysis of Flavor Aromatic Chemicals and Isolates

The functions of this section are twofold: (1) to provide "GLC Profile" data on a number of substances for the information of users of this Codex (see *Note 3* on page 353 of *Section 3*), and (2) to provide the general test conditions by which certain flavoring agents listed in *Section 3* are to be assayed (see *M-8a* and *M-8b* on page 424 of *Section 4*).

The procedures described below define and explain the apparatus, methods, and calculations necessary for the gas-liquid chromatographic analysis of the flavoring agents as developed by the Essential Oil Association (EOA). All of the material in this section has been adapted from "First Supplement to the EOA Book of Standards and Specifications" (January 1979). The permission granted by the Essential Oil Association for use of this material in the *Food Chemicals Codex* is gratefully acknowledged.

INTRODUCTION

The analyst following this procedure and performing the test should obtain sufficient resolution of major and even trace components of a mixture to calculate accurately the concentration of the desired component. He should be familiar with the general principles, usual techniques, and instrumental variables normally met in gas chromatographic analysis. He should pay particular attention to the following:

1. Stability of baseline, return to baseline before and after each peak of interest, and minimum use of recorder attenuation.
2. Any incompatibility between a sensitive sample component and column support, liquid substrate, or construction material.
3. The activity of the column and its resolution parameters must be checked periodically using a test mixture of the following composition by weight percent:

Acetophenone	15%
Linalool	25%
Benzyl Alcohol	5%
Phenylethanol	10%
Hydroxycitronellal	45%

The resolution obtained from the column under test should be comparable to that exhibited in the chromatograms shown on page 438. The quantitative composition of the sample as determined by area normalization (response factor of 1) should not deviate from the weight percent composition by more than a 10% relative error. Losses due to column activity will be especially apparent in the quantitation of benzyl alcohol and acetophenone.

4. The response to different components of the same or different detectors. Since sizable errors may be encountered in correlating area percent directly to weight percent, the methods for calculating response factors should be known.

I GLC CONDITIONS FOR ANALYSIS

A. Column Information:

1. Construction Material: Stainless steel tubing, alloy #304
   Length: 10 ft or 3 m
   Diameters: od, 0.125 in. or 3.18 mm;
   id, 0.101 to 0.085 in. or 2.57 to 2.16 mm
2. Substrate: Either polar or nonpolar, as specified for each individual substance in the tables (pages 439–457)
   Polar Column: Carbowax 20M
   Nonpolar Column: SE-30, GC grade, or OV-1 or OV-101
3. Substrate Concentration: 10% by weight

4. Solid Support: Any 10- or 20-mesh range of flux-calcined diatomaceous earth silanized and acid washed, having an approximate free-fall density of 0.2 g per cm^3, a minimum mesh of 120, and a maximum mesh of 80
5. Packing Density: At least 0.24 g per cm^3

B. Carrier Gas: Helium

Flow: The optimum flow rate is specified for each individual substance in the tables (pages 439–457), usually within the range of 25 to 50 ml per min.

C. Analytical Conditions: As specified for each substance
1. Column Temperature: Isothermal or programmed temperature
   a. Polar Column:
      Initial temperature: 75°C minimum
      Final temperature: 225°C maximum
      Rate of increase: 2° to 8°C per min, as specified for each substance
   b. Nonpolar Column:
      Initial temperature: 75°C minimum
      Final temperature: 275°C maximum
      Rate of increase: 2° to 8°C per min, as specified for each substance
2. Inlet Temperature: 225° to 250°C
3. Sample Size: 0.1 to 1 μl
4. Detector: Thermal conductivity cell; detector operating conditions consistent with good analytical procedure and manufacturer's recommendations
5. Sample size and attenuation should be adjusted to produce an approximate half-scale deflection for a 5% component.

The directions given for each substance in the tables on pages 439–457 indicate any variations from the general conditions provided above, including the following:

1. Name of substance
2. Flow rate of the carrier gas (helium)
3. Column temperature, its rate of increase, if programmed
4. Sample size
5. Recorder: millivolt range, chart width and speed
6. Minimum attenuation
7. Results of GLC analysis:
   a. Identification of sample components
   b. Retention times relative to that of a particular component, and time of elution of the last component
   c. Area percent of the peaks

It is not suggested that the instrumental conditions described herein are the only ones possible. Any gas chromatographic conditions that give equal or improved separations and (or) quantification may be substituted for those listed herein. However, in the event of dispute, the conditions listed herein will stand.

II MEASUREMENTS

Measurements may be made with a suitable measuring rule graduated in units of 0.5 mm or fiftieths of an inch. A magnifying glass should be used in reading the rule to ensure the greatest accuracy. For more precise work, the height of small peaks may be measured with a vernier caliper that allows estimation to 0.1 mm. Bandwidths may be read to 0.1 mm with a measuring magnifier (e.g., the Bausch and Lomb No. 81-34-35 fitted with a 20-mm reticle).

A. *Peak height* is defined as the height of a peak, measured from a drawn baseline to the top of a peak, multiplied by the attenuation.

B. *Bandwidth* is defined as the width of a peak measured at half height.

C. *Retention distance* is defined as the horizontal distance measured from the injection point or air peak to the peak apex.

D. *Relative Retention* The retention distance of one of the constituents of the sample is arbitrarily assigned a relative retention of 1, and all other constituents are assigned relative retentions by direct ratio of their retention distances to the retention distance of the constituent assigned a relative retention of 1.

III CALCULATIONS AND METHODS

A. Peak Area

1. Peak Height by Bandwidth

   This is the most commonly used method. Each peak is treated as a triangle and its area calculated by multiplying the peak height times the bandwidth. To determine the individual component's percentage, multiply the individual area by 100 and divide by the sum of all the areas (total area).

2. Automatic Integration

   Integrators are available that calculate the total area under each peak, expressed either by markers or in digital form. The areas are summed, and the individual area is multiplied by 100 and divided by the total to obtain the component's area percent.

   Integrators are most successfully applied to chromatograms with completely separated peaks and level baselines. Corrections must be made if these conditions are not maintained.

   Automatic integration controlled by either an analog or digital computer deals very successfully with partially resolved peaks and sloping baselines.

3. Internal Standard

   Results obtained using the previously described calculations are based on the assumption that the entire sample has eluted and the peaks of all of the components have been included in the calculation. They will be incorrect if any part of the sample does

not elute or if all the peaks are not measured. In such cases, and in all methods described above, the internal standard method may be used to determine percentages based on the total sample. For this method, measurements are required of the peaks of the component(s) being assayed and of the internal standard.

An accurately weighed mixture of the internal standard and the sample is prepared and chromatographed, the area ratio(s) of the component(s) to the standard is computed, and the percentage(s) of the component(s) is calculated.

If this calculation is to be applied, the substance used as the standard should be one that meets the following criteria:

a. Its detector response is similar to that of the component(s) to be determined. In general, the more nearly the chemical structure of the component resembles that of the standard, the closer will be the response.
b. Its retention distance is close to, but not identical with, that of the component(s).
c. Its elution time is different from that of any other component in the sample so that its peak does not superimpose on any other.

The weight ratio of the internal standard to the sample should be such that the internal standard and the component sought produce approximately equal peaks. This is, of course, not possible if several components of interest are at different levels of concentration.

If the internal standard method is applied properly, it may be assumed that the ratio of the weight of component to the weight of internal standard is exactly proportional to the peak area ratio, and under these conditions no correction factor is needed. The sample is first run by itself to determine whether the internal standard would mask any component by peak superposition. If there is no interference, a mixture is prepared of the sample and of the internal standard in the specified weight ratio, and the percentages of the internal standard and of the sample in the mixture are calculated. The mixture is chromatographed, and the areas of the component peak and the internal standard peak are calculated by one of the methods described above.

The calculations are as follows:

$$1a.\quad \frac{\%\ \text{Component in Mixture}}{\%\ \text{Internal Standard in Mixture}} = \frac{\text{Component Area}}{\text{Internal Standard Area}}$$

or

$$1b.\quad \%\ \text{Component in Mixture} = \%\ \text{Int. Std. in Mixture} \times \frac{\text{Component Area}}{\text{Int. Std. Area}}$$

$$2.\quad \%\ \text{Component in Sample} = \frac{\%\ \text{Component in Mixture} \times 100}{\%\ \text{Sample in Mixture}}$$

Should calibration be necessary, mixtures should be prepared of internal standard and component, of either 100% or of known purity. The number of mixtures and the weight ratios to be used depend upon the component being analyzed. Usually, three mixtures will be required. The weight ratio of one is chosen so that the heights of component and standard are equal. The ratios of the other two may be two thirds and four thirds of this value. Each mixture should be chromatographed at least three times, and areas calculated. The factor for each chromatograph should be calculated as specified below, and the averages taken for each mixture. An overall average factor is calculated from them. The calibration should be performed periodically.

$$1.\quad \text{Factor} = \frac{\text{Wt. Component} \times \%\ \text{Purity}}{\text{Wt. Int. Std.} \times \%\ \text{Purity}} \times \frac{\text{Int. Std. Area}}{\text{Component Area}}$$

$$2.\quad \%\ \text{Component in Sample Mixture} = \frac{\text{Component Area} \times \text{Factor} \times \%\ \text{Int. Std. in Sample Mixture}}{\text{Int. Std. Area}}$$

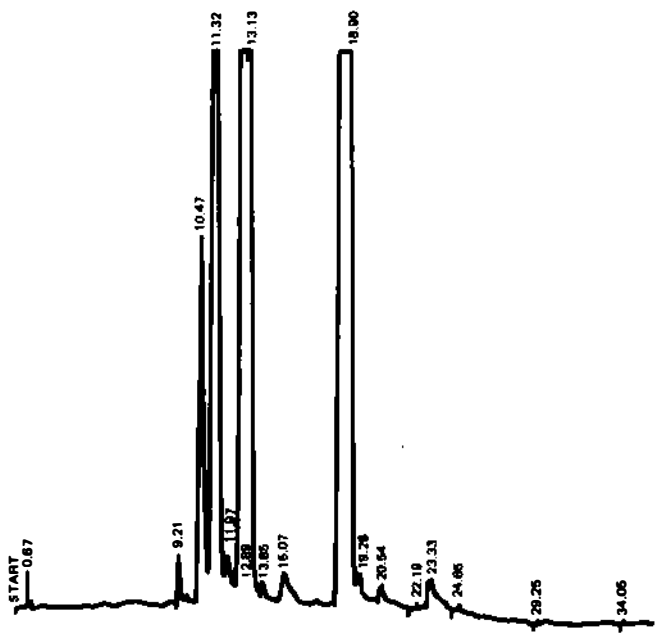
$$3.\quad \%\ \text{Component in Sample} = \frac{\%\ \text{Component in Sample Mixture} \times 100}{\%\ \text{Sample in Sample Mixture}}$$

EOA Polar Test Mixture

Column:	EOA polar
Carrier gas:	Helium
Carrier gas flow:	38 ml per min
Column temperature:	75° to 225°C @ 4°C per min
Detector:	Thermal conductivity
Sample size:	0.5 µl
Recorder:	1 mV; chart speed, 0.25 in. per min; chart width, 10 in.
Attenuation:	4
Method of calculation:	Area normalization

GLC Analysis

Component	Retention Relative to Major Peak	Area Percent
1 Linalool	0.58	24.6
2 Acetophenone	0.70	12.7
3 Benzyl Alcohol	0.93	5.2
4 Phenylethanol	0.96	10.8
5 Hydroxycitronellal	1.00	44.7
6 Unknown	1.13	2.0

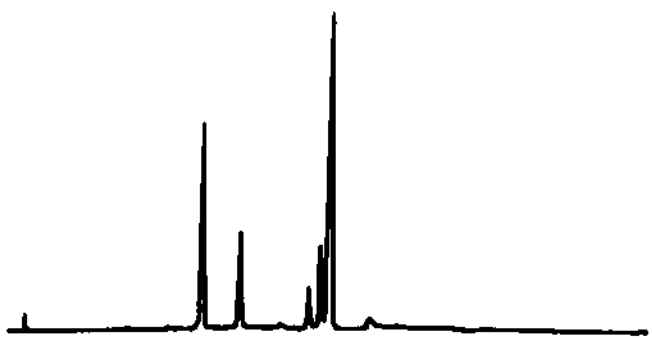


EOA Nonpolar Test Mixture

Column:	EOA nonpolar
Carrier gas:	Helium
Carrier gas flow:	38 ml per min
Column temperature:	75° to 225°C @ 4°C per min
Detector:	Thermal conductivity
Sample size:	0.5 µl
Recorder:	H.P. Printer Plotter; chart speed, 0.198 in. per min; chart width, 8.4 in.
Attenuation:	2
Method of calculation:	Area normalization

GLC Analysis

Component	Retention Relative to Major Peak	Area Percent
1 Unknowns	0.04–0.05	0.4
2 Benzyl Alcohol	0.49	5.1
3 Acetophenone	0.55	12.6
4 Unknowns	0.63	0.6
5 Linalool	0.68	26.6
6 Phenylethanol	0.69	9.8
7 Unknowns	0.73–0.80	0.2
8 Hydroxycitronellal	1.00	44.4
9 Unknowns	1.02–1.23	0.3



Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
Acetanisole	polar	30	200° isothermal	1	1; 0.25; 10	2	1	0.44	0.1
							2	0.57	0.2
							3 Acetanisole	1.00	99.2
							4	1.10	0.5
Acetophenone	polar	30	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.04	<0.1
							2	0.50	<0.1
							3	0.59	<0.1
							4 Acetophenone	1.00	99.5
							5	1.08	0.3
							6	1.15	<0.1
							7	1.23	<0.1
							8	1.26	0.1
							9	1.36	<0.1
Allyl Cyclohexanepropionate (Allyl Cyclohexyl Propionate)	polar	38	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1 Propyl Phenyl Propionate	0.92	1.7
							2 Allyl Cyclohexyl Propionate	1.00	94.8
							3	1.14	<0.1
							4 Allyl Phenyl Propionate	1.19	2.8
							5	1.32	0.2
							6	1.46	0.3
Allyl Hexanoate (Allyl Caproate)	polar	40	75° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.66	<0.1
							2	0.74	0.3
							3	0.85	0.4
							4 Allyl Caproate	1.00	99.2
α-Amylcinnamaldehyde (Amyl Cinnamic Aldehyde)	nonpolar	30	100° to 220° @ 4°/min	1	1; 0.25; 10.5	4	1	0.75	0.1
							2	0.88	0.3
							3 Amyl Cinnamic Aldehyde	1.00	99.6
Amyl Cinnamate	nonpolar	40	190° isothermal	0.6	1; 0.15; 10	1	1	0.29	<0.1
							2	0.52	0.4
							3	0.60	0.1
							4	0.82	0.1
							5 Amyl Cinnamate	1.00	96.8
							6	1.07	0.2
							7 Isoamyl-3(isoamyloxy)-3-phenylpropionate	1.66	2.4
Amyl Propionate	nonpolar	30	75° to 225° @ 4°/min	1	printer plotter; 0.198; 8	2	1 Isoamyl Propionate	0.87	31.6
							2 *n*-Amyl Propionate	1.00	68.4
Anisyl Acetate	nonpolar	34	160° isothermal	0.2	1; 0.25; 10	1	1	0.74	0.4
							2 Anisyl Acetate	1.00	99.6
Anisyl Alcohol	polar	40	75° to 225° @ 4°/min	1	1; 0.25; 10	1	1	0.34	<0.1
							2	0.65	<0.1
							3	0.81	0.7

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
							4	0.89	<0.1
							5 Anisyl Alcohol	1.00	98.0
							6	1.01	0.9
							7	1.04	0.3
Benzophenone	nonpolar	30	180° isothermal	1[a]	1; 0.25; 11	2	1 Benzophenone	1.00	99.6
							2	1.70	0.3
Benzyl Acetate	polar	40	120° to 225° @ 4°/min	1.1	1; 0.25; 10	2	1 Benzyl Acetate	1.00	98.2
							2 Benzyl Alcohol	1.29	1.7
							3	1.90	0.1
Benzyl Butyrate	polar	37	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.85	0.7
							2 Benzyl Isobutyrate	0.90	1.2
							3 Benzyl Propionate	0.91	0.7
							4 Benzyl Butyrate	1.00	96.4
							5	1.29	0.5
							6	1.43	0.2
Benzyl Isobutyrate	polar	40	175°	1	1; 0.25; 10	1	1 Benzyl Isobutyrate	1.00	98.9
							2	1.10	0.5
							3	1.21	0.6
Benzyl Isovalerate	polar	40	75° to 225° @ 4°/min	0.6	1; 0.25; 10	1	1	0.86	0.4
							2	0.89	<0.1
							3	0.97	0.8
							4 Benzyl Isovalerate	1.00	98.4
							5	1.06	0.3
Benzyl Propionate	polar	40	80° to 225° @ 6°/min	0.5	1; 0.25; 10	1	1	0.87	<0.1
							2	0.91	<0.1
							3 Benzyl Propionate	1.00	99.7
							4	1.06	<0.1
							5	1.17	<0.1
Benzyl Salicylate	nonpolar	32	100° to 275° @ 8°/min	1	1; 0.4; 10	1	1	0.04	<0.1
							2	0.05	<0.1
							3	0.28	<0.1
							4	0.57	<0.1
							5 Benzyl Salicylate	1.00	99.7
							6	1.03	0.2
Bornyl Acetate	polar	40	140° to 225° @ 6°/min	1.1	1; 0.25; 10	2	1	0.10	0.1
							2	0.19	<0.1
							3	0.54	0.3
							4 Bornyl Acetate	1.00	98.4
							5	1.11	<0.1
							6	1.17	0.1
							7 Terpene Acetate (mol wt 196)	1.27	1.0
							8	1.82	<0.1

[a] 20% w/w in ethanol

Butyl Isobutyrate (*n*-Butyl Isobutyrate)	polar	35	50° to 225° @ 5°/min	0.5	printer plotter; 0.25; 8	4	1		0.8	<0.1
							2		0.85	<0.1
							3	*n*-Butyl Isobutyrate	1.00	98.4
							4		1.08	<0.1
							5		1.12	0.9
							6		1.51	0.4
							air peak at 2.49 min excluded			
d-Carvone	nonpolar	34	160°	0.2	1; 0.25; 10	1	1		0.49	0.3
							2		0.58	0.7
							3		0.83	0.9
							4	*d*-Carvone	1.00	98.0
							5		1.35	<0.1
l-Carvone (Carvone)	polar	50	80° to 225° @ 6°/min	0.5	1; 0.25; 10	1	1		0.92	<0.1
							2	Carvone	1.00	99.9
							3		1.15	<0.1
Cinnamaldehyde (Cinnamic Aldehyde)	nonpolar	20	100° to 225° @ 4°/min	0.7	1; 0.25; 10.5	2	1		0.40	0.1
							2		0.82	0.9
							3	Cinnamic Aldehyde	1.00	98.8
							4		1.07	0.2
Cinnamyl Alcohol (Cinnamic Alcohol)	polar	30	200° isothermal	1	1; 0.25; 11	2	1	Benzaldehyde	0.19	0.1
							2	Cinnamic Aldehyde	0.57	0.4
							3	*cis*-Cinnamic Alcohol	0.69	1.2
							4	*trans*-Cinnamic Alcohol	1.00	98.3
Citral	polar	30	75° to 225° @ 4°/min	0.25	1; 0.25; 10	4	1		0.72	<0.1
							2		0.76	0.3
							3		0.84	0.4
							4	Neral	0.93	36.0
							5	Geranial	1.00	62.7
							6		1.05	0.1
							7		1.10	0.4
Citronellal	nonpolar	35	100° to 250° @ 4°/min	0.2	1; 0.25; 10	1	1		0.29	<0.1
							2		0.40	0.6
							3		0.54	<0.1
							4		0.67	<0.1
							5		0.80	<0.1
							6	Citronellal	1.00	88.1
							7	Terpene Alcohol (mol wt 154.2)	1.12	1.8
							8	Isopulegol	1.25	7.6
							9		1.41	0.1
							10		1.53	0.1
							11	Citronellol	1.82	1.3
							12		2.11	0.2

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
Citronellol (EOA Type A)	polar	35	100° to 200° @ 4°/min	1	1; 0.25; 10	2	1	0.58	<0.1
							2	0.65	0.1
							3 Dimethyl Octanol	0.71	1.1
							4	0.80	0.3
							5	0.82	0.3
							6 Citronellol	1.00	95.5
							7 Nerol	1.03	0.9
							8 Terpene Alcohol (mol wt 154)	1.04	0.8
							9 Geraniol	1.09	0.5
							10	1.12	<0.1
							11	1.21	0.2
							12	1.35	0.1
Citronellol (EOA Type B)	polar	35	100° to 200° @ 4°/min	1	1; 0.25; 10	2	1	0.71	0.3
							2	0.78	0.8
							3 Dimethyl Octanol	0.82	8.2
							4	0.90	0.6
							5 Citronellol	1.00	78.3
							6 Nerol	1.03	3.0
							7 Terpene Alcohol (mol wt 154)	1.04	2.1
							8 Geraniol	1.09	6.3
							9	1.43	0.4
Citronellyl Acetate	polar	38	75° to 225° @ 4°/min	0.2	1; 0.25; 10	1	1	0.44	0.2
							2	0.81	0.1
							3	0.84	<0.1
							4 Dimethyl Octanyl Acetate	0.87	5.2
							5	0.90	0.1
							6	0.93	0.5
							7 Citronellyl Acetate	1.00	83.9
							8	1.02	0.2
							9 Neryl Acetate	1.04	1.6
							10	1.06	0.7
							11 Geranyl Acetate	1.07	7.4
							12	1.38	0.1
							13	1.52	<0.1
Citronellyl Formate (EOA Type A)	polar	40	100° to 200° @ 4°/min	1	1; 0.25; 10	2	1 Dimethyl Octanyl Formate (+ terpene ester)	0.86	3.1
							2	0.92	0.2
							3 Citronellyl Formate	1.00	79.9
							4 Neryl Formate	1.03	1.5
							5 Geranyl Formate	1.07	10.9
							6 Citronellol	1.10	3.5
							7 Nerol	1.15	0.3
							8 Geraniol	1.19	0.5
							9 Terpene Alcohol	1.40	0.6

Citronellyl Formate (EOA Type B)	polar	40	100° to 200° @ 4°/min	1	1; 0.25; 10	2	1 Dimethyl Octanyl Formate	0.87	12.7
							2	0.90	0.3
							3	0.93	0.9
							4 Citronellyl Formate	1.00	78.3
							5 Neryl Formate	1.03	0.9
							6 (Terpene) Formate	1.05	1.2
							7 Geranyl Formate	1.07	0.9
							8 Citronellol	1.10	2.5
							9 Terpene Alcohol	1.40	1.8
							10	1.44	0.4
Citronellyl Propionate	polar	38	75° to 225° @ 4°/min	0.3	printer plotter; 0.2; 8	4	1 Dimethyl Octanyl Propionate	0.84	15.9
							2 α-Citronellyl Propionate	0.88	1.2
							3 unknown propionate	0.92	0.9
							4 Citronellyl Propionate	1.00	81.7
							5	1.54	0.2
Cresyl Acetate (*para*-Cresyl Acetate)	polar	30	140° to 225° @ 8°/min	0.2	1; 0.39; 10	2	1	0.45	<0.1
							2	0.92	0.2
							3 *para*-Cresyl Acetate	1.00	98.9
							4	1.08	0.2
							5	1.50	0.7
Cyclamen Aldehyde	polar	40	100° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.80	0.2
							2	0.88	0.4
							3 *ortho*-Cyclamen Aldehyde	0.92	11.1
							4 *para*-Cyclamen Aldehyde	1.00	87.5
							5	1.29	0.5
							6	2.11	0.3
Decanal (Aldehyde C-10)	nonpolar	30	100° to 220° @ 4°/min	1	1; 0.25; 10.5	4	1	0.40	0.2
							2	0.67	0.5
							3	0.86	<0.1
							4 Aldehyde C-10	1.00	99.1
							5	1.13	0.2
1-Decanol (Alcohol C-10)	nonpolar	20	75° to 225° @ 4°/min	0.5	printer plotter; 0.5; 8.5	1	1	0.64	0.3
							2	0.90	0.1
							3 Alcohol C-10	1.00	99.4
							4	1.20	0.1
Diethyl Succinate	nonpolar	32	100° to 275° @ 8°/min	1	1; 0.4; 10	1	1	0.08	<0.1
							2	0.11	0.6
							3	0.77	0.4
							4 Diethyl Succinate	1.00	98.2
							5	1.02	0.7
Dimethyl Anthranilate	nonpolar	34	160° to 230° @ 2°/min	0.2	1; 0.25; 10	1	1 Methyl Anthranilate	0.80	1.3
							2 Dimethyl Anthranilate	1.00	98.7
Dimethyl Benzyl Carbinol	nonpolar	40	100° to 220° @ 4°/min	1	1; 0.25; 10.5	4	1	1.00	100
Dimethyl Benzyl Carbinyl Acetate	nonpolar	34	160° to 230° @ 2°/min	0.2	1; 0.25; 10	1	1 Dimethyl Benzyl Carbinyl Acetate	1.00	99.3
							2	2.32	0.6

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
3,7-Dimethyl-1-octanol (Dimethyl Octanol)	polar	30	100° to 225° @ 4°/min	0.2	1; 0.25; 10	2	1 mass spectra inconclusive mixture	0.64	1.2
							2	0.71	0.5
							3	0.75	0.7
							4 apparent mol wt 208	0.77	3.7
							5	0.81	0.5
							6 Dimethyl Octanol	1.00	93.4
Ethyl Benzoate	nonpolar	30	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.04	<0.1
							2	0.60	<0.1
							3	0.80	<0.1
							4 Ethyl Benzoate	1.00	99.9+
Ethyl Butyrate	nonpolar	30	100° to 200° @ 6°/min	1	1; 0.25; 10.5	4	1	0.63	<0.1
							2	0.77	<0.1
							3 Ethyl Butyrate	1.00	99.9
Ethyl Decanoate	polar	30	75° to 225° @ 4°/min	1	printer plotter; 0.198; 8	1	1	0.05	<0.1
							2 Ethyl Decanoate	1.00	98.1
							3	1.08	<0.1
							4	1.21	0.9
							5	1.26	0.9
							6	1.46	<0.1
Ethyl Formate	polar	30	70° isothermal	1	printer plotter; 0.198; 8	2	1	0.50	<0.1
							2	0.59	<0.1
							3 Ethyl Formate	1.00	99.2
							4	1.73	0.8
Ethyl Heptanoate (Ethyl Heptoate)	polar	38	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.05	<0.1
							2	0.25	<0.1
							3	0.77	0.6
							4	0.89	<0.1
							5 Ethyl Heptoate	1.00	99.1
							6	1.05	<0.1
							7	1.63	<0.1
							8	1.68	<0.1
							9	1.70	<0.1
Ethyl Hexanoate (Ethyl Caproate)	nonpolar	34	160°	0.2	1; 0.25; 10	1	1	0.73	0.7
							2 Ethyl Caproate	1.00	99.2
Ethyl Isovalerate	polar	30	75° to 225° @ 4°/min	1	printer plotter; 0.198; 8	1	1	0.43	0.1
							2	0.56	0.1
							3 Ethyl Isovalerate	1.00	99.6
							4	1.20	0.1
Ethyl Methyl Phenylglycidate (Aldehyde C-16)	polar	40	120° to 225° @ 4°/min	0.3	1; 0.25; 10	2	1	0.43	0.3
							2	0.53	0.5

							3	0.83	0.3
							4 Aldehyde C-16 (*cis*-isomer)	0.87	39.2
							5	0.95	0.4
							6 Aldehyde C-16 (*trans*-isomer)	1.00	58.4
							7	1.14	0.9
Ethyl Octanoate (Ethyl Caprylate)	polar	30	75° to 225° @ 4°/min	0.4	printer plotter; 0.198; 8	2	1	0.48	0.2
							2 Ethyl Caprylate	1.00	99.5
							3	1.49	0.3
Ethyl Phenylacetate (Ethyl Phenyl Acetate)	nonpolar	30	150° to 225° @ 4°/min	0.2	1; 0.4; 10	2	1	0.27	0.3
							2	0.30	<0.1
							3	0.66	0.4
							4 Methyl Phenyl Acetate	0.77	1.2
							5 Ethyl Phenyl Acetate	1.00	97.9
Ethyl Phenylglycidate (Ethyl Phenyl Glycidate)	polar	38	75° to 225° @ 4°/min	0.5	printer plotter; 0.198; 8	2	1 *cis*-Ethyl Phenyl Glycidate	0.93	8.3
							2 *trans*-Ethyl Phenyl Glycidate (mol wt 192)	1.00	86.0
							3	1.01	4.2
							4	1.05	0.2
							5	1.07	0.7
							6	1.09	0.2
							7	1.15	0.6
Ethyl Salicylate	nonpolar	30	100° to 220° @ 4°/min	1	1; 0.25; 10.5	4	1	0.78	<0.1
							2	0.88	<0.1
							3 Ethyl Salicylate	1.00	99.9
Eucalyptol	nonpolar	20	75° to 225° @ 4°/min	0.5	printer plotter; 0.5; 8.5	1	1	0.72	0.2
							2 Eucalyptol	1.00	99.7
							3	1.28	0.1
Geranyl Acetate	polar	38	75° to 225° @ 4°/min	0.2	1; 0.25; 10	1	1	0.77	0.1
							2 Dimethyl Octanyl Acetate	0.79	1.8
							3	0.85	0.2
							4 Citronellyl Acetate	0.90	17.3
							5	0.93	0.2
							6 Neryl Acetate	0.95	16.0
							7 Geranyl Acetate	1.00	64.3
Geranyl Butyrate (EOA Type A)	polar	40	200°	0.5	1; 0.25; 10	2	1	0.43	0.1
							2	0.49	<0.1
							3	0.57	0.4
							4	0.62	0.3
							5 Citronellyl Butyrate	0.71	22.1
							6 Neryl Butyrate	0.83	0.7
							7 Geranyl Butyrate	1.00	76.4
Geranyl Butyrate (EOA Type B)	polar	40	200°	0.5	1; 0.25; 10	2	1	0.1	<0.1
							2	0.12	0.2
							3	0.14	<0.1
							4	0.32	<0.1

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
							5	0.40	0.1
							6	0.44	0.1
							7	0.52	0.5
							8	0.60	0.1
							9 Citronellyl Butyrate	0.71	4.9
							10 Neryl Butyrate	0.83	14.7
							11 Geranyl Butyrate	1.00	79.1
Geranyl Formate (EOA Type A)	polar	40	100° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.66	1.0
							2	0.76	0.2
							3	0.77	0.3
							4	0.83	0.1
							5 Neryl Formate	0.92	12.6
							6 Geranyl Formate	1.00	79.3
							7 formate ester	1.16	1.8
							8 formate ester	1.24	4.5
Geranyl Formate (EOA Type B)	polar	40	100° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.23	0.1
							2	0.79	0.9
							3 Citronellyl Formate	0.83	23.3
							4 Neryl Formate	0.92	2.9
							5 Geranyl Formate	1.00	70.6
							6 formate ester	1.09	1.2
							7	1.25	0.7
							8	1.88	0.2
							9	1.99	0.1
Geranyl Phenylacetate (Geranyl Phenyl Acetate)	polar	37	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.01	<0.1
							2	0.43	<0.1
							3	0.46	0.1
							4	0.58	<0.1
							5 Citronellyl Phenyl Acetate	0.87	24.1
							6	0.93	<0.1
							7 Geranyl Phenyl Acetate	1.00	75.6
Geranyl Propionate (EOA Type A)	polar	30	100° to 225° @ 4°/min	0.2	1; 0.25; 10	2	1	0.92	0.6
							2 Neryl Propionate	0.96	35.6
							3 Geranyl Propionate	1.00	61.1
							4	1.04	0.6
							5 unknown terpene ester	1.06	1.0
							6 unknown terpene ester	1.09	1.1
Geranyl Propionate (EOA Type B)	polar	30	100° to 225° @ 4°/min	0.2	1; 0.25; 10	2	1	0.72	0.7
							2	0.79	0.2
							3	0.83	0.9
							4	0.85	0.4
							5 Citronellyl Propionate	0.88	34.6
							6	0.93	0.8
							7 Neryl Propionate	0.95	0.7
							8 Geranyl Propionate	1.00	61.6

Geraniol (EOA Type A)	polar	40	75° to 225° @ 4°/min	0.2	1; 0.25; 1	1	1		0.80	0.4
							2		0.82	0.5
							3		0.85	<0.1
							4		0.88	<0.1
							5	Citronellol	0.91	14.8
							6	Nerol	0.94	3.7
							7		0.96	0.4
							8	Geraniol	1.00	80.2
							9		1.20	0.2
Geraniol (EOA Type B)	polar	40	75° to 225° @ 4°/min	0.2	printer plotter; 0.198; 8	4	1		0.78	0.3
							2		0.89	<0.1
							3	Nerol	0.94	1.3
							4	Geraniol	1.00	98.3
Heptanal (Aldehyde C-7)	polar	30	75° to 220° @ 4°/min	0.2	1; 0.25; 10	2	1-15	sum of 15 components—no single component greater than 0.3%	(0.33 to 0.99)	1.7
							16	Aldehyde C-7	1.00	93.5
							17	Aldehyde (unknown)	1.07	2.2
							18	unknown ester	1.24	1.1
							19	unknown ester	1.41	1.5
α-Hexylcinnamaldehyde (Hexyl Cinnamic Aldehyde)	polar	50	75° to 225° @ 8°/min	0.4	1; 0.25; 10	2	1	Hexyl Phenyl Propyl Aldehyde	0.84	2.1
							2		0.91	<0.1
							3	*cis*-Hexyl Cinnamic Aldehyde	0.92	0.3
							4	*trans*-Hexyl Cinnamic Aldehyde	1.00	96.9
							5		1.04	0.3
							6		1.09	0.3
							7		1.11	<0.1
Hydroxycitronellal (Hydroxy Citronellal)	polar	30	200° isothermal	0.5	1; 0.25; 12	2	1		0.08	<0.1
							2		0.14	<0.1
							3		0.24	<0.1
							4		0.29	<0.1
							5		0.35	<0.1
							6		0.43	<0.1
							7		0.48	<0.1
							8		0.58	<0.1
							9	Hydroxy Citronellal	1.00	98.4
							10		1.24	<0.1
							11		1.41	0.5
							12		1.79	<0.1
							13		1.98	0.4
							14		2.45	<0.1

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
Hydroxycitronellal Dimethyl Acetal (Hydroxy Citronellal Dimethyl Acetal)	polar	40	100° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.06	0.8
							2	0.46	0.2
							3	0.50	0.3
							4	0.72	0.1
							5	0.76	0.2
							6	0.81	0.3
							7	0.85	0.6
							8 Hydroxy Citronellal Dimethyl Acetal	1.00	97.4
							9	1.25	<0.1
Indole	polar	37	75° to 225° @ 4°/min	0.4[b]	1; 0.25; 11	2	1 solvent	0.04	—
							2 Indole	1.00	100
α-Ionone (*alpha*-Ionone)	polar	38	75° to 225° @ 4°/min	0.2	printer plotter; 0.25; 8.5	1	1	0.82	0.1
							2	0.86	0.2
							3	0.92	0.2
							4 *alpha*-Ionone	1.00	86.4
							5 *gamma*-Ionone	1.02	8.1
							6 *beta*-Ionone	1.07	5.0
							7	1.11	0.1
β-Ionone (*beta*-Ionone)	polar	38	75° to 225° @ 4°/min	0.2	printer plotter; 0.2; 8.5	1	1	0.73	0.2
							2	0.90	0.2
							3	0.92	0.5
							4 *beta*-Ionone	1.00	98.7
							5	1.12	0.2
							6	1.15	0.2
Isoamyl Acetate (EOA Type A) (Isoamyl Acetate)	polar	40	75° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.54	0.2
							2	0.73	0.1
							3 Isoamyl Acetate	1.00	96.0
							4 *n*-Amyl Acetate	1.24	3.4
							5	1.69	0.3
Isoamyl Acetate (EOA Type B) (Isoamyl Acetate)	polar	40	75° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.74	<0.1
							2 Isoamyl Acetate	1.00	65.7
							3 *n*-Amyl Acetate	1.23	33.8
							4	1.56	0.3
Isoamyl Butyrate (EOA Type A) (Isoamyl Butyrate)	polar	40	75° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.14	0.2
							2 Isoamyl Butyrate	1.00	99.7
							3	1.10	<0.1
Isoamyl Butyrate (EOA Type B) (Isoamyl Butyrate)	polar	40	75° to 225° @ 4°/min	0.3	1; 0.25; 10	1	1	0.12	0.1
							2 Isoamyl Butyrate	0.79	35.7
							3 *n*-Amyl Butyrate	1.00	64.2

[b] 20% in acetone

Isoamyl Formate (EOA Type A) (Isoamyl Formate)	polar	40	75° to 225° @ 4°/min	0.3	1; 0.25; 10	1	1 Isoamyl Formate	0.81	25.0
							2 *n*-Amyl Formate	1.00	72.1
							3	1.35	0.84
							4 *n*-Amyl Alcohol	1.70	2.1
Isoamyl Formate (EOA Type B) (Isoamyl Formate)	polar	40	75° to 225° @ 4°/min	0.3	1; 0.25; 10	1	1	0.61	<0.1
							2	0.68	<0.1
							3	0.76	<0.1
							4	0.82	<0.1
							5	0.89	<0.1
							6 Isoamyl Formate	1.00	97.4
							7 *n*-Amyl Formate	1.19	1.14
							8 Isoamyl Alcohol	1.64	1.27
Isoamyl Formate (EOA Type C) (Isoamyl Formate)	polar	40	75° to 225° @ 4°/min	0.3	1; 0.25; 10	1	1	0.50	<0.1
							2	0.55	<0.1
							3	0.62	<0.1
							4	0.67	<0.1
							5 Isoamyl Formate	0.81	33.7
							6 *n*-Amyl Formate	1.00	66.1
							7	1.18	0.3
							8	1.24	0.9
							9 Isoamyl Alcohol	1.34	1.4
							10 *n*-Amyl Alcohol	1.69	2.0
Isoamyl Hexanoate (EOA Type A) (*n*-Amyl Caproate)	polar	35	100° to 220° @ 4°/min	0.5	1; 0.25; 10	1	1	0.63	0.1
							2	0.74	0.4
							3 2-Methyl Butyl Caproate + 3-Methyl Butyl Caproate	0.86	35.9
							4 *n*-Amyl Caproate	1.00	63.5
							5	1.19	<0.1
Isoamyl Hexanoate (EOA Type B) (*n*-Amyl Caproate)	polar	35	100° to 220° @ 4°/min	0.5	1; 0.25; 10	1	1	0.69	<0.1
							2	0.77	<0.1
							3	0.83	0.1
							4 2-Methyl Butyl Caproate + 3-Methyl Butyl Caproate	1.00	97.4
							5 *n*-Amyl Caproate	1.07	0.3
							6	1.19	<0.1
							7	1.25	0.2
							8	1.32	<0.1
							9 2-Methyl Butyl Caprylate + 3-Methyl Butyl Caprylate	1.44	1.8
Isoamyl Salicylate	nonpolar	38	75° to 225° @ 4°/min	0.5	printer plotter; 0.198; 8	2	1	0.77	0.3
							2	0.92	0.2
							3 2-Methyl Butyl Salicylate + 3-Methyl Butyl Salicylate	0.95	34.5
							4 *n*-Amyl Salicylate	1.00	64.9
Isobornyl Acetate	polar	30	100° to 220° @ 4°/min	1	1; 0.25; 10.5	2	1	0.77	0.4
							2 mixture of two terpene acetates (mol wt 196)	0.82	3.9
							3	0.88	0.1
							4 Isobornyl Acetate	1.00	93.5
							5 Terpene Acetate (mol wt 196)	1.08	2.1

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
Isobutyl Butyrate	polar	35	50° to 225° @ 5°/min	0.5	printer plotter; 0.25; 8	4	1	0.73	<0.1
							2	0.89	<0.1
							3 Isobutyl Butyrate	1.00	99.0
							4 *n*-Butyl *n*-Butyrate	1.16	0.9
							air peak at 2.47 min excluded		
Isobutyl Phenylacetate (Isobutyl Phenyl Acetate)	polar	30	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.86	0.3
							2	0.89	0.2
							3	0.94	<0.1
							4	0.96	0.2
							5 Isobutyl Phenyl Acetate	1.00	97.9
							6	1.01	0.9
							7	1.04	0.4
Isobutyl Salicylate	nonpolar	30	100° to 220° @ 4°/min	1	1; 0.25; 10.5	4	1	0.88	<0.1
							2 Isobutyl Salicylate	1.00	99.9
							3	1.03	<0.1
Isoeugenol	nonpolar	34	160° to 230° @ 2°/min	0.2	1; 0.25; 10	1	1	0.78	0.1
							2 *cis*-Isoeugenol	0.85	15.7
							3 *trans*-Isoeugenol	1.00	83.6
							4	1.27	0.4
Isopulegol	polar	37	75° to 225° @ 4°/min	0.4	printer plotter; 0.198; 8	2	1 Terpene Alcohol (mol wt 154)	0.82	<0.1
							2 Terpene Alcohol (mol wt 154)	0.84	<0.1
							3 Terpene Alcohol (mol wt 154)	0.92	1.2
							4 Terpene Alcohol (mol wt 154)	0.95	1.3
							5 Isopulegol	1.00	94.4
							6 Terpene Alcohol (mol wt 154)	1.03	1.3
							7 Terpene Alcohol (mol wt 154)	1.04	1.7
							8	1.72	<0.1
Lauryl Alcohol	polar	50	80° to 225° @ 6°/min	0.5	1; 0.25; 10	1	1	0.69	0.8
							2	0.81	<0.1
							3 Lauryl Alcohol	1.00	99.0
							4	1.16	<0.1
							5	1.31	<0.1
Lauryl Aldehyde (Aldehyde C-12)	polar	40	100° to 220° @ 4°/min	1	1; 0.25; 10.5	2	1	0.72	0.2
							2	0.78	0.2
							3	0.83	0.1
							4	0.88	0.4
							5 Aldehyde C-12	1.00	97.2
							6	1.07	0.1
							7	1.10	0.3
							8 Alcohol C-12	1.16	1.1
							9	1.23	0.3
							10	1.28	0.1

d-Limonene (Limonene)	polar	30	100° to 225° @ 4°/min	0.2	1; 0.25; 10	2	1	0.48	0.4
							2	0.70	0.3
							3 Myrcene	0.78	1.7
							4 Limonene	1.00	93.9
							5	1.22	0.1
							6	1.90	0.2
							7	1.95	0.2
							8	2.08	0.2
							9	2.21	0.7
							10	2.26	0.3
							11	2.60	0.2
							12	2.79	0.1
							13	2.90	0.4
							14	3.14	0.7
							15	3.47	0.2
							16	3.60	0.1
Linalool	polar	38	140° to 220° @ 4°/min	0.4	1; 0.25; 11	1	1 Linalool Oxide (*cis*)	0.76	1.6
							2 Linalool Oxide (*trans*)	0.83	1.5
							3 Linalool	1.00	91.3
							4	1.12	0.2
							5	1.22	<0.2
							6	1.26	0.1
							7	1.35	<0.1
							8 Terpineol	1.39	3.4
							9	1.49	0.1
							10	1.54	0.4
							11	1.65	0.2
							12	1.77	0.5
							13	1.86	0.5
Linalyl Acetate	polar	35	100° to 225° @ 4°/min	1	1; 0.1; 10	2	1	0.30	0.6
							2	0.39	0.2
							3	0.42	0.5
							4 Linalool	0.91	1.2
							5 Linalyl Acetate	1.00	96.8
							6	1.22	0.2
							7	1.27	0.2
							8	1.31	0.3
Linalyl Acetate, Synthetic	polar	40	70° to 225° @ 4°/min	2	1; 0.25; 10.5	1	1 Linalyl Acetate	1.00	98.7
							2	1.08	<0.1
							3	1.14	<0.1
							4	1.20	<0.1
							5	1.25	<0.1
							6	1.33	0.2
							7	1.44	<0.1
							8	1.53	0.8
							9	1.78	<0.1
Linalyl Formate	polar	40	125° isothermal	1	1; 0.25; 10	1	1	0.21	0.1
							2	0.27	0.1
							3	0.66	0.9
							4 Linalool	0.78	1.5
							5 Linalyl Formate	1.00	97.1
							6	1.21	0.1
							7	1.38	0.2

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
Linalyl Isobutyrate	polar	38	100° to 225° @ 4°/min	0.5	1; 0.2; 9.5	1	1	0.05	0.1
							2	0.29	0.1
							3	0.40	0.1
							4	0.81	0.1
							5	0.82	0.1
							6	0.87	0.5
							7	0.92	3.2
							8 Linalyl Isobutyrate	1.00	93.3
							9	1.08	0.1
							10	1.10	0.2
							11	1.12	0.2
							12	1.16	0.1
							13	1.26	0.1
							14	1.31	0.2
							15	1.38	0.8
							16	1.48	0.1
							17	1.59	0.4
							18	1.83	0.1
Linalyl Propionate	nonpolar	40	100° to 200° @ 4°/min	1	1; 0.25; 10	2	1 Linalool	0.50	0.8
							2 (Terpene) Propionate	0.92	1.6
							3 Linalyl Propionate	1.00	92.8
							4 (Terpene) Propionate	1.02	2.7
							5	1.14	0.6
							6	1.18	0.5
							7	1.22	0.9
p-Methoxybenzaldehyde (Anisic Aldehyde)	polar	38	100° to 225° @ 6°/min	1	1; 0.25; 10	2	1	0.85	0.9
							2 Anisic Aldehyde	1.00	98.5
							3	1.16	0.4
							4	1.18	0.2
Methyl Benzoate	polar	38	100° to 225° @ 6°/min	1	1; 2; 10	2	1 Methyl Benzoate	1.00	100
α-Methylbenzyl Alcohol (Methyl Phenyl Carbinol)	polar	38	80° to 220° @ 5°/min	0.2	1; 0.25; 10	2	1	0.81	0.8
							2 Methyl Phenyl Carbinol	1.00	99.1
							3	1.34	0.1
4'-Methyl Acetophenone (Methyl Acetophenone)	polar	15	80° to 225° @ 4°/min	0.4	1; 0.25; 10	2	1 *ortho*-Methyl Acetophenone	0.86	3.2
							2	0.91	<0.1
							3 *meta*-Methyl Acetophenone	0.94	2.9
							4 *para*-Methyl Acetophenone	1.00	94.0
							5	1.03	<0.1
							6	1.04	<0.1
							7	1.09	<0.1
p-Methyl Anisole (*para*-Cresyl Methyl Ether)	nonpolar	30	75° to 225° @ 4°/min	0.4	printer plotter; 0.2; 8	2	1 *para*-Cresyl Methyl Ether	1.00	100

Methyl Anthranilate	nonpolar	34	160°	0.2	1; 0.25; 10	1	1	Methyl Anthranilate	1.00	99.9
							2		1.14	0.1
α-Methylbenzyl Acetate (Methyl Benzyl Acetate)	nonpolar	38	75° to 225° @ 4°/min	0.5	printer plotter; 0.198; 8	2	1		0.73	<0.1
							2	Methyl Benzyl Acetate	1.00	98.9
							3		1.10	0.1
							4		1.54	0.4
							5		1.55	0.5
α-Methylcinnamaldehyde (Methyl Cinnamic Aldehyde)	polar	30	75° to 225° @ 4°/min	1	printer plotter; 0.198; 8	1	1		0.48	<0.1
							2		0.75	0.3
							3	*cis*-Methyl Cinnamic Aldehyde	0.79	1.6
							4		0.89	0.2
							5	*trans*-Methyl Cinnamic Aldehyde	1.00	97.9
Methyl Cinnamate	nonpolar	34	160°	1.5[c]	1; 0.25; 10	1	1	solvent	0.09	—
							2	Methyl Cinnamate	1.00	99.1
							3		1.05	0.9
Methyl Eugenol	polar	30	200° isothermal	0.5	1; 0.06; 10	2	1		0.09	<0.1
							2		0.11	<0.1
							3		0.41	0.3
							4		0.48	<0.1
							5		0.55	<0.1
							6		0.68	<0.1
							7		0.78	<0.1
							8	Methyl Eugenol	1.00	97.0
							9		1.07	0.2
							10		1.12	0.5
							11	*trans*-Methyl Isoeugenol	1.41	1.6
							12		1.60	<0.1
							13		2.03	<0.1
							14		2.22	<0.1
							15		2.46	<0.1
6-Methyl-5-hepten-2-one (Methyl Heptenone)	polar	32	75° to 225° @ 4°/min	1	printer plotter; 0.198; 8	2	1		0.05	<0.1
							2		0.13	0.2
							3		0.50	<0.1
							4		0.57	<0.1
							5		0.59	0.1
							6		0.74	<0.1
							7		0.86	<0.1
							8		0.90	<0.1
							9	Methyl Heptenone	1.00	98.8
							10		1.06	<0.1
							11		1.23	0.3
							12		1.72	0.2
							13		1.74	0.1
Methyl Isoeugenol	polar	40	100° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	Methyl Eugenol	0.83	0.9
							2	*cis*-Methyl Isoeugenol	0.91	8.9
							3	*trans*-Methyl Isoeugenol	1.00	90.2

[c] 150 mg/ml of acetone

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (μl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
Methyl β-Naphthyl Ketone (Methyl Naphthyl Ketone)	nonpolar	30	150° to 225° @ 4°/min	0.3	1; 0.4; 10	2	1	0.39	<0.1
							2	0.90	0.3
							3 Methyl Naphthyl Ketone	1.00	99.7
Methyl 2-Octynoate (Methyl Heptine Carbonate)	polar	40	100° to 220° @ 4°/min	0.3	1; 0.25; 10	1	1	0.32	<0.1
							2	0.72	<0.1
							3	0.83	<0.1
							4	0.90	0.3
							5 Methyl Heptine Carbonate	1.00	99.4
Methyl Phenylacetate (Methyl Phenyl Acetate)	nonpolar	34	160°	0.2	1; 0.25; 10	1	1	0.13	0.2
							2 Methyl Phenyl Acetate	1.00	99.8
2-Methylundecenal (Aldehyde C-12 MNA)	polar	39	75° to 225° @ 4°/min	0.5	printer plotter; 0.198; 8	2	1 Methyl Nonyl Ketone	0.94	3.6
							2 Aldehyde C-12 MNA	1.00	95.2
							3	1.10	0.9
							4	1.21	0.2
Nerolidol	polar	40	75° to 225° @ 4°/min	1	1; 0.25; 10	1	1	0.74	0.4
							2	0.78	0.8
							3	0.80	0.2
							4 *cis*-Nerolidol	0.95	37.8
							5 *trans*-Nerolidol	1.00	60.4
							6	1.13	<0.1
							7	1.17	0.1
							8	1.20	0.1
γ-Nonalactone (*gamma*-Nonalactone)	polar	38	75° to 225° @ 4°/min	0.5	printer plotter; 0.198; 8	2	1	0.86	0.1
							2	0.90	<0.1
							3	0.94	0.2
							4 *gamma*-Nonalactone	1.00	95.3
							5 *delta*-Nonalactone	1.02	3.8
							6	1.08	0.3
							7	1.11	0.2
Nonanal (Aldehyde C-9)	polar	37	75° to 225° @ 4°/min	0.25	printer plotter; 0.198; 8	2	1	0.06	<0.1
							2	0.12	<0.1
							3	0.75	<0.1
							4	0.92	<0.1
							5 Aldehyde C-9	1.00	96.5
							6	1.13	0.5
							7 Nonyl Alcohol (+ unknown)	1.20	1.2
							8 Nonanoic Acid	2.19	1.4
							9	2.38	0.1
							10	3.17	0.1
Octanal (Aldehyde C-8)	polar	30	100° to 225° @ 5°/min	1	1; 0.3; 11	4	1	0.09	<0.1
							2	0.13	<0.1
							3	0.25	<0.1

							4	0.65	<0.1
							5 Aldehyde C-8	1.00	99.4
							6	1.80	0.1
							7	1.99	0.1
l-Octanol (Alcohol C-8)	polar	30	100° to 225° @ 4°/min	1	1; 0.25; 10.5	4	1	0.82	<0.1
							2 Alcohol C-8	1.00	99.9
							3	1.47	<0.1
Octyl Formate	polar	38	80° to 220° @ 5°/min	0.2	1; 0.25; 10	2	1	0.50	0.07
							2	0.55	0.06
							3	0.79	0.11
							4 Octyl Formate	1.00	98.7
							5	1.21	0.4
							6	1.75	0.5
Phenethyl Acetate (Phenyl Ethyl Acetate)	polar	38	75° to 225° @ 4°/min	0.5	1; 0.25; 10	1	1	0.66	0.2
							2	0.76	<0.1
							3	0.82	0.2
							4	0.89	0.2
							5 Phenyl Ethyl Acetate	1.00	98.0
							6	1.06	0.2
							7	1.08	0.2
							8	1.10	0.1
							9	1.11	0.1
							10	1.15	0.2
							11	1.31	0.3
Phenethyl Alcohol (Phenyl Ethyl Alcohol)	polar	35	75° to 225° @ 4°/min	0.6	1; 0.25; 10	1	1	0.71	0.2
							2	0.87	0.1
							3	0.91	0.2
							4 Phenyl Ethyl Alcohol	1.00	99.5
Phenylacetaldehyde (Phenyl Acetaldehyde)	polar	38	80° to 150° @ 4°/min	0.6	1; 0.25; 10	2	1	0.05	<0.1
							2	0.65	<0.1
							3	0.82	0.2
							4 Phenyl Acetaldehyde	1.00	99.5
							5	1.63	0.3
Phenylacetaldehyde Dimethyl Acetal (Phenylacetaldehyde DMA)	polar	32	100° to 225° @ 8°/min	0.7	1; 0.4; 10	1	1	0.51	<0.1
							2	0.53	<0.1
							3	0.71	0.2
							4	0.74	0.3
							5	0.86	<0.1
							6	0.89	<0.1
							7 Phenylacetaldehyde DMA	1.00	97.3
							8	1.04	0.3
							9	1.08	<0.1
							10	1.14	<0.1
							11	1.17	0.2
							12	1.24	0.3
							13	1.29	<0.1
							14	1.38	0.5
							15	1.48	<0.1
							16	1.52	<0.1
							17	1.63	<0.1
							18	1.66	<0.1
							19	1.82	<0.1

Specific Test Conditions for Determination of "GLC Profile" of Substances Listed in Section 3 (*continued*)

Substance	Column Type	Gas Flow Rate (ml/min)	Column Temperature (°C)	Sample Size (µl)	Recorder (mV); Speed (in./min); Width (in.)	Attenuation	GLC Analysis		
							Component	Retention Relative to Major Peak	Area (%)
3-Phenyl-1-propanol (Phenyl Propyl Alcohol)	nonpolar	30	100° to 220° @ 4°/min	t	1; 0.25; 11	2	1 Phenyl Propyl Alcohol	1.00	99.8
							2	1.15	<0.1
							3	1.31	<0.1
2-Phenylpropionaldehyde (Hydratropic Aldehyde)	nonpolar	40	100° to 225° @ 6°/min	t	1; 0.25; 10	2	1	0.02	0.1
							2	0.08	0.2
							3 Acetophenone	0.88	11.3
							4 Hydratropic Aldehyde	1.00	88.2
3-Phenylpropionaldehyde (Phenyl Propyl Aldehyde)	polar	35	75° to 225° @ 5°/min	0.4	printer plotter; 0.2; 8.5	2	1 Phenyl Propyl Aldehyde	1.00	99.9
							2	1.21	<0.1
Piperonal (Heliotropine)	nonpolar	34	160°	0.2	1; 0.25; 10	1	1 solvent	0.09	—
							2 Heliotropine	1.00	99.9
p-Propyl Anisole (Dihydro Anethole)	polar	40	100° to 225° @ 4°/min	0.3	1; 0.25; 10	2	1 Dihydro Anethole	1.00	100
Rhodinol	polar	35	75° to 225° @ 5°/min	0.3	printer plotter; 0.25; 8.5	1	1	0.77	0.4
							2	0.82	0.6
							3 Citronellyl Formate	0.87	1.1
							4	0.89	0.7
							5 3,7-Guaiadiene	0.90	2.6
							6	0.93	0.6
							7 Citronellol	1.00	62.0
							8	1.02	0.5
							9 Nerol	1.03	2.3
							10	1.04	0.6
							11 Geraniol	1.06	27.9
							12	1.11	0.2
							13	1.12	0.2
							14	1.33	0.3
Rhodinyl Acetate	polar	30	120° to 200° @ 4°/min	0.2	1; 0.25; 10	2	1	0.75	0.9
							2	0.77	0.2
							3	0.82	0.2
							4	0.84	0.4
							5 Terpene Acetate	0.89	1.6
							6	0.93	0.2
							7 Citronellyl Acetate	1.00	54.6
							8	1.05	0.9
							9 Terpene Acetate	1.08	1.9
							10 Neryl Acetate	1.14	4.3
							11 Geranyl Acetate	1.22	33.5
							12	1.36	0.9

Terpineol	polar	35	100° to 220° @ 4°/min	1	1; 0.25; 10	2	1	0.72	0.1
							2 Terpin-1-ene-ol	0.77	3.6
							3	0.83	0.7
							4 *trans*-β-Terpineol	0.86	11.8
							5 *cis*-β-Terpineol	0.94	2.1
							6 α-Terpineol + unresolved γ-Terpineol	1.00	81.4
							7	1.56	0.3
Terpinyl Acetate	polar	35	75° to 225° @ 5°/min	0.3	printer plotter; 0.25; 8.5	1	1	0.46	0.5
							2 *cis*-Dihydro α-Terpinyl Acetate	0.85	2.2
							3 *trans*-Dihydro α-Terpinyl Acetate	0.87	1.5
							4 *cis*-β-Terpinyl Acetate	0.88	3.4
							5 *trans*-Dihydro β-Terpinyl Acetate	0.90	1.2
							6	0.93	0.2
							7 *trans*-β-Terpinyl Acetate	0.95	2.4
							8 γ-Terpinyl Acetate	0.97	5.0
							9 α-Terpinyl Acetate	1.00	83.6
p-Tolyl Isobutyrate (*para*-Cresyl Isobutyrate)	polar	30	75° to 225° @ 4°/min	1	printer plotter; 0.198; 8	1	1	0.89	0.2
							2 *para*-Cresyl Isobutyrate	1.00	99.6
							3	1.06	0.2
γ-Undecalactone (*gamma*-Undecalactone)	polar	32	100° to 225° @ 8°/min	0.8	1; 0.4; 10	1	1	0.75	<0.1
							2	0.79	<0.1
							3	0.82	0.2
							4	0.86	0.2
							5	0.88	0.2
							6	0.91	0.2
							7	0.94	1.1
							8 *gamma*-Undecalactone	1.00	95.9
							9 *delta*-Undecalactone	1.02	1.3
							10	1.07	0.5
							11	1.10	0.1
							12	1.13	0.1
Undecanal (Aldehyde C-11 Undecylic)	polar	40	80° to 225° @ 6°/min	0.5	1; 0.25; 10	1	1 Aldehyde C-11 Undecylic	1.00	99.1
							2	1.03	<0.1
							3	1.15	0.5
							4	1.30	<0.1
							5	1.86	0.2
							6	2.28	<0.1
10-Undecenal (Undecylenic Aldehyde)	nonpolar	35	100° to 250° @ 4°/min	0.2	1; 0.25; 10	1	1 Undecylenic Aldehyde	1.00	99.5
							2	1.12	0.5

6/ *General Tests and Apparatus*

Contents

List of Figures

Alginates Assay

Apparatus The apparatus required is shown in Fig. 1. It consists essentially of a soda lime column, *A*, a mercury valve, *B*, connected through a side arm, *C*, to a reaction flask, *D*, by means of a rubber connection. Flask *D* is a 100-ml round-bottomed, long-neck boiling flask, resting in a suitable heating mantle, *E*.

The reaction flask is provided with a reflux condenser, *F*, to which is fitted a delivery tube, *G*, of 40-ml capacity, having a stopcock, *H*. The reflux condenser terminates in a trap, *I*, containing 25 g of 20-mesh zinc or tin, which can be connected with an absorption tower, *J*.

The absorption tower consists of a 45-cm tube fitted with a medium-porosity fritted glass disk sealed to the inner part above the side arm and having a delivery tube sealed to it extending down to the end of the tube. A trap, consisting of a bulb of approximately 100-ml capacity, is blown above the fritted disk and the outer portion of a ground spherical joint is sealed on above the bulb. A 250-ml Erlenmeyer flask, *K*, is connected to the bottom of the absorption tower. The top of the tower is connected to a soda lime tower, *L*, which is connected to a suitable pump to provide vacuum and air supply, the choice of which is made by a three-way stopcock, *M*. The volume of air or vacuum is controlled by a capillary-tube regulator or needle valve, *N*.

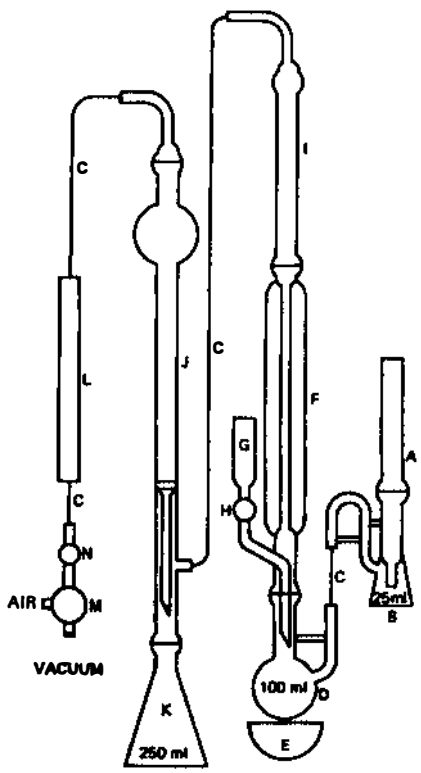


FIGURE 1 Apparatus for Alginates Assay

All joints are size 35/25, ground spherical type.

Procedure Transfer about 250 mg of the sample, accurately weighed, into the reaction flask, *D*, add 25 ml of 0.1 *N* hydrochloric acid, insert several boiling chips, and connect the flask to the reflux condenser, *F*, using syrupy phosphoric acid as a lubricant. (NOTE: Stopcock grease may be used for the other connections.) Check the system for air leaks by forcing mercury up into the inner tube of the mercury valve, *B*, to a height of about 5 cm. Turn off the pressure using the stopcock, *M*. If the mercury level does not fall appreciably after 1 to 2 min, the apparatus may be considered to be free from leaks. Draw carbon dioxide–free air through the apparatus at a rate of 3000 to 6000 ml per h. Raise the heating mantle, *E*, to the flask, heat the sample to boiling, and boil gently for 2 min. Turn off and lower the mantle, and allow the sample to cool for 15 min. Charge the delivery tube, *G*, with 23 ml of hydrochloric acid. Disconnect the absorption tower, *L*, rapidly transfer 25.0 ml of 0.25 *N* sodium hydroxide into the tower, add 5 drops of *n*-butanol, and again connect the absorption tower. Draw carbon dioxide–free air through the apparatus at the rate of about 2000 ml per h, add the hydrochloric acid to the reaction flask through the delivery tube, raise the heating mantle, and heat the reaction mixture to boiling. After 2 h, discontinue the current of air and heating. Force the sodium hydroxide solution down into the flask, *K*, using gentle air pressure, and then rinse down the absorption tower with three 15-ml portions of water, forcing each washing into the flask with air pressure. Remove the flask, and add to it 10 ml of a 10% solution of barium chloride ($BaCl_2.2H_2O$). Stopper the flask, shake gently for about 2 min, add phenolphthalein TS, and titrate with 0.1 *N* hydrochloric acid. Perform a blank determination (see page 2). Each ml of 0.25 *N* sodium hydroxide consumed is equivalent to 5.5 mg of carbon dioxide (CO_2). Calculate the results on the dried basis.

Alkali Salts of Organic Acids Assay

This assay is not applicable to organic alkali salts containing sulfur or halogens.

Unless otherwise directed, transfer about 2 g of the sample,

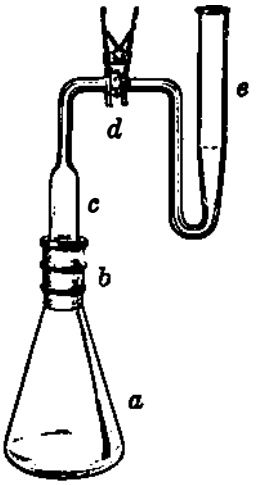


FIGURE 2 General Apparatus for Arsenic Test (Courtesy of the Fisher Scientific Co., Pittsburgh, Pa.)

accurately weighed, to a porcelain crucible, and carefully ignite until the material is completely charred.

Caution: The ignited salt should be protected from contact with the free flame at all times.

After cooling, place the crucible in a beaker, add 50 ml of water and 50.0 ml of 0.5 *N* sulfuric acid, and disperse the carbonized mass with a glass rod. Cover the beaker, and boil the mixture for 30 min. Filter, wash the residue with hot water until the washings are neutral to litmus, and cool. To the combined filtrate and washings add phenolphthalein TS, and titrate the excess acid with 0.5 *N* sodium hydroxide. The weight of the alkali salt is obtained by multiplying the volume of the acid consumed by the equivalence factor of the salt being analyzed.

A 400-mg sample and 0.1 *N* acid and sodium hydroxide may be employed satisfactorily in this assay.

Arsenic Test

Silver Diethyldithiocarbamate Colorimetric Method

NOTE: All reagents used in this test should be very low in arsenic content.

Apparatus The general apparatus, shown in Fig. 2, is to be used unless otherwise specified in an individual monograph. It consists of a 125-ml arsine generator flask (*a*) fitted with a scrubber unit (*c*) and an absorber tube (*e*), with a 24/40 standard-taper joint (*b*) and a ball-and-socket joint (*d*), secured with a No. 12 clamp, connecting the units. The tubing between *d* and *e* and between *d* and *c* is a capillary having an inside diameter of 2 mm and an outside diameter of 8 mm. Alternatively, an apparatus embodying the principle of the general assembly described and illustrated may be used.

NOTE: The special assemblies shown in Figs. 3, 4, and 5 are to be used only when specified in certain monographs.

Standard Arsenic Solution Weigh accurately 132.0 mg of arsenic trioxide that has been previously dried at 105° for 1 h, and dissolve it in 5 ml of sodium hydroxide solution (1 in 5). Neutralize the solution with diluted sulfuric acid TS, add 10 ml in excess, and dilute to 1000.0 ml with recently boiled water. Transfer 10.0 ml of this solution into a 1000-ml volumetric flask, add 10 ml of diluted sulfuric acid TS, dilute to volume with recently boiled water, and mix. Use this final solution, which contains 1 μg of arsenic (As) in each ml, within 3 days.

Silver Diethyldithiocarbamate Solution Dissolve 1 g of recrystallized silver diethyldithiocarbamate, $(C_2H_5)_2NCSSAg$, in 200 ml of reagent-grade pyridine. Store this solution in a light-resistant container and use within 1 month.

Silver diethyldithiocarbamate is available commercially or may be prepared as follows: Dissolve 1.7 g of reagent grade silver nitrate in 100 ml of water. In a separate container, dissolve 2.3 g of sodium diethyldithiocarbamate, $(C_2H_5)_2NCSSNa.3H_2O$), in 100 ml of water, and filter. Cool both solutions to about 15°, mix the two solutions, while stirring, collect the yellow precipitate in a medium-porosity sintered-glass crucible or funnel, and wash with about 200 ml of cold water.

Recrystallize the reagent, whether prepared as directed above or obtained commercially, as follows: Dissolve in freshly distilled pyridine, using about 100 ml of solvent for each g of reagent, and filter. Add an equal volume of cold water to the pyridine solution, while stirring. Filter off the precipitate, using suction, wash with cold water, and dry in vacuum at room temperature for 2 to 3 h. The dry salt is pure yellow in color and should show no change in character after 1 month when stored in a light-resistant container. Discard any material that changes in color or develops a strong odor.

Stannous Chloride Solution Dissolve 40 g of reagent-grade stannous chloride dihydrate, $SnCl_2.2H_2O$, in 100 ml of hydrochloric acid. Store the solution in glass containers and use within 3 months.

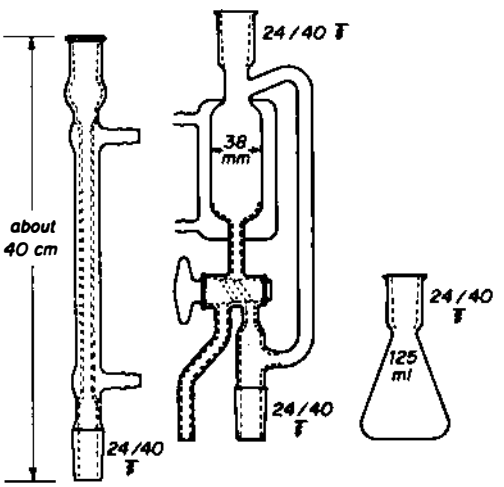


FIGURE 3 Modified Bethge Apparatus for the Distillation of Arsenic Tribromide

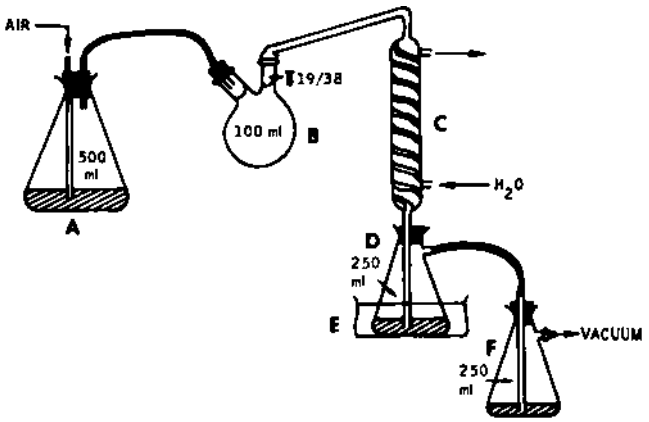


FIGURE 4 Special Apparatus for the Distillation of Arsenic Trichloride (Flask *A* contains 150 ml of hydrochloric acid; flasks *D* and *F* contain 20 ml of water. Flask *D* is placed in an ice water bath, *E*.)

Lead Acetate–Impregnated Cotton Soak cotton in a saturated solution of reagent-grade lead acetate, squeeze out the excess solution, and dry in a vacuum at room temperature.

Sample Solution The solution obtained by treating the sample as directed in an individual monograph is used directly as the *Sample Solution* in the *Procedure*. Sample solutions of organic compounds are prepared in the generator flask (*a*), unless otherwise directed, according to the following general procedure:

Caution: Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Appropriate safety precautions must be employed at all times.

NOTE: If halogen-containing compounds are present, use a lower temperature while heating the sample with sulfuric acid, do not boil the mixture, and add the peroxide, with caution, before charring begins, to prevent loss of trivalent arsenic.

Transfer 1.0 g of the sample into the generator flask, add 5 ml of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, using preferably a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 ml.) After the sample has been initially decomposed by the acid, add with caution, dropwise, 30% hydrogen peroxide, allowing the reaction to subside and reheating between drops. The first few drops must be added very slowly with sufficient mixing to prevent a rapid reaction, and heating should be discontinued if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during digestion. *Maintain oxidizing conditions at all times during the digestion by adding small quantities of the peroxide whenever the mixture turns brown or darkens.* Continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250°–300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, add cautiously 10 ml of water, again evaporate to strong fuming, and cool. Add cautiously 10 ml of water, mix, wash the sides of the flask with a few ml of water, and dilute to 35 ml.

Procedure If the *Sample Solution* was not prepared in the generator flask, transfer to the flask a volume of the solution, prepared as directed, equivalent to 1.0 g of the substance being tested, and add water to make 35 ml. Add 20 ml of dilute sulfuric acid (1 in 5), 2 ml of potassium iodide TS, and 0.5 ml of *Stannous Chloride Solution*, and mix. Allow the mixture to stand for 30 min at room temperature. Pack the scrubber tube (*c*) with two plugs of *Lead Acetate–Impregnated Cotton*, leaving a small air space between the two plugs, lubricate joints *b* and *d* with stopcock grease, if necessary, and connect the scrubber unit with the absorber tube (*e*). Transfer 3.0 ml of *Silver Diethyldithiocarbamate Solution* to the absorber tube, add 3.0 g of granular zinc (20-mesh) to the mixture in the flask, and immediately insert the standard-taper joint in the flask. Allow the evolution of hydrogen and color development to proceed at room temperature (25° ± 3°) for 45 min, swirling the flask gently at 10-min intervals. (The addition of a small amount of isopropanol to the generator flask may improve the uniformity of the rate of gas evolution.) Disconnect the absorber tube from the generator and scrubber units, and transfer the *Silver Diethyldithiocarbamate Solution* to a 1-cm absorption cell. Determine the absorbance at the wavelength of maximum absorption between 535 nm and 540 nm, with a suitable spectrophotometer or colorimeter, using *Silver Diethyldithiocarbamate Solution* as the blank. The absorbance due to any red color from the solution of the sample does not exceed that produced by 3.0 ml of *Standard Arsenic Solution* (3 µg As) when treated in the same manner and under the same conditions as the sample. The room temperature during the

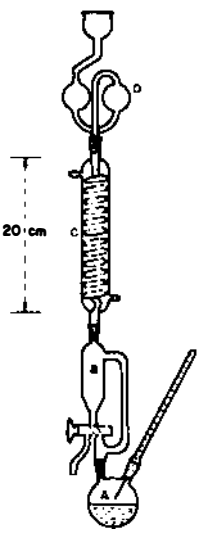


FIGURE 5 Special Apparatus for the Determination of Inorganic Arsenic (*A*, 250-ml distillation flask; *B*, receiver chamber, approximately 50-ml capacity; *C*, reflux condenser; *D*, splash head.)

generation of arsine from the standard should be held to within ±2° of that observed during the determination of the sample.

Interferences Metals or salts of metals such as chromium, cobalt, copper, mercury, molybdenum, nickel, palladium, and silver are said to interfere with the evolution of arsine. Antimony, which forms stibine, is the only metal likely to produce a positive interference in the color development with the silver diethyldithiocarbamate. Stibine forms a red color that has a maximum absorbance at 510 nm, but at 535 to 540 nm the absorbance of the antimony complex is so diminished that the results of the determination would not be altered significantly.

Ash (Acid-Insoluble)

Boil the ash obtained as directed under *Total Ash*, below, with 25 ml of diluted hydrochloric acid TS for 5 min, collect the insoluble matter on a tared Gooch crucible or ashless filter, wash with hot water, ignite, and weigh. Calculate the percentage of acid-insoluble ash from the weight of sample taken.

Ash (Total)

Unless otherwise directed, weigh accurately about 3 g of the sample in a tared crucible, ignite at a low temperature (about 550°), not to exceed very dull redness, until free from carbon, cool in a desiccator, and weigh. If a carbon-free ash is not obtained, wet the charred mass with hot water, collect the insoluble residue on an ashless filter paper, and ignite the residue and filter paper until the ash is white or nearly so. Finally, add the filtrate, evaporate it to dryness, and heat the whole to a dull redness. If a carbon-free ash is still not obtained, cool the crucible, add 15 ml of alcohol, break up the ash with a glass rod, then burn off the alcohol, again heat the whole to a dull redness, cool, and weigh.

Calcium Pantothenate Assay

Standard Stock Solution of Calcium Pantothenate Dissolve 50 mg of USP Calcium Pantothenate Reference Standard, previously dried and stored in the dark over phosphorus pentoxide and accurately weighed while protected from absorption of moisture during the weighing, in about 500 ml of water in a 1000-ml volumetric flask. Add 10 ml of 0.2 *N* acetic acid and 100 ml of sodium acetate solution (1 in 60), then add water to volume. Each ml represents 50 µg of USP Calcium Pantothenate Reference Standard. Store under toluene in a refrigerator.

Standard Preparation On the day of the assay, dilute a measured volume of *Standard Stock Solution of Calcium Pantothenate* with sufficient water to contain, in each ml, between 0.01 and 0.04 µg of calcium pantothenate, the exact concentration being such that the responses obtained as directed under *Procedure*, using 2.0 and 4.0 ml of the *Standard Preparation*, are within the linear portion of the log-concentration response curve.

Assay Preparation Prepare a solution with water containing approximately the equivalent of the calcium pantothenate concentration in the *Standard Preparation*.

Basal Medium Stock Solution

Acid-Hydrolyzed Casein Solution	25	ml
Cystine-Tryptophan Solution	25	ml
Polysorbate 80 Solution	0.25	ml
Dextrose, Anhydrous	10	g
Sodium Acetate, Anhydrous	5	g
Adenine-Guanine-Uracil Solution	5	ml
Riboflavin-Thiamin Hydrochloride-Biotin Solution	5	ml
para-Aminobenzoic Acid–Niacin–Pyridoxine Hydrochloride Solution	5	ml
Salt Solution A	5	ml
Salt Solution B	5	ml

Dissolve the anhydrous dextrose and sodium acetate in the solutions previously mixed, and adjust to a pH of 6.8 with sodium hydroxide TS. Finally, add water to make 250 ml.

Acid-Hydrolyzed Casein Solution Mix 100 g of vitamin-free casein with 500 ml of dilute hydrochloric acid (1 in 2), and reflux the mixture for 8 to 12 h. Remove the hydrochloric acid from the mixture by distillation under reduced pressure until a thick paste remains. Redissolve the resulting paste in water, adjust the solution to a pH of 3.5 ± 0.1 with sodium hydroxide solution, and add water to make 1000 ml. Add 20 g of activated charcoal, stir for 1 h, and filter. Repeat the treatment with activated charcoal. Store under toluene in a refrigerator at a temperature not below 10°. Filter the solution if a precipitate forms under storage.

Cystine-Tryptophan Solution Suspend 4.0 g of L-cystine and 1.0 g of L-tryptophan (or 2.0 g of DL-tryptophan) in 700 to 800 ml of water, heat to 70° to 80°, and add dilute hydrochloric acid (1 in 2) dropwise, with stirring, until the solids are dissolved. Cool, and add water to make 1000 ml. Store under toluene in a refrigerator at a temperature not below 10°.

Adenine-Guanine-Uracil Solution Dissolve 200 mg each of adenine sulfate, guanine hydrochloride, and uracil, with the aid of heat, in 10 ml of dilute hydrochloric acid (1 in 3), cool, and add water to make 200 ml. Store under toluene in a refrigerator.

Salt Solution A Dissolve 25 g of monobasic potassium phosphate and 25 g of dibasic potassium phosphate in water to make 500 ml. Add 5 drops of hydrochloric acid, and store under toluene.

Salt Solution B Dissolve 10 g of magnesium sulfate, 500 mg of sodium chloride, 500 mg of ferrous sulfate, and 500 mg of manganese sulfate in water to make 500 ml. Add 5 drops of hydrochloric acid, and store under toluene.

Polysorbate 80 Solution Dissolve 25 g of polysorbate 80 in sufficient alcohol to make 250 ml.

Riboflavin–Thiamin Hydrochloride–Biotin Solution Prepare a solution containing in each ml 20 μg of riboflavin, 10 μg of thiamin hydrochloride, and 0.04 μg of biotin by dissolving riboflavin, crystalline thiamin hydrochloride, and biotin in 0.02 *N* acetic acid. Store, protected from light, under toluene in a refrigerator.

para-Aminobenzoic Acid–Niacin–Pyridoxine Hydrochloride Solution Prepare a solution in neutral 25% alcohol to contain 10 μg of para-aminobenzoic acid, 50 μg of niacin, and 40 μg of pyridoxine hydrochloride in each ml. Store in a refrigerator.

Stock Culture of Lactobacillus Plantarum Dissolve 2.0 g of water-soluble yeast extract in 100 ml of water, add 500 mg of anhydrous dextrose, 500 mg of anhydrous sodium acetate, and 1.5 g of agar, and heat the mixture, with stirring, on a steam bath until the agar dissolves. Add approximately 10-ml portions of the hot solution to test tubes, suitably close or cover the tubes, sterilize at 121°, and allow the tubes to cool in an upright position. Prepare stab cultures in three or more of the tubes, using a pure culture of *Lactobacillus plantarum*,* incubating for 16 to 24 h at any selected temperature between 30° and 37° but held constant to within ±0.5°, and finally store in a refrigerator. Prepare a fresh stab of the stock culture every week, and do not use for inoculum if the culture is more than 1 week old.

Culture Medium To each of a series of test tubes containing 5.0 ml of the *Basal Medium Stock Solution* add 5.0 ml of water containing 0.2 μg of calcium pantothenate. Plug the tubes with cotton, sterilize in an autoclave at 121°, and cool.

Inoculum Make a transfer of cells from the *Stock Culture of Lactobacillus Plantarum* to a sterile tube containing 10 ml of *Culture Medium*. Incubate this culture for 16 to 24 h at any selected temperature between 30° and 37° but held constant to within ±0.5°. The cell suspension so obtained is the inoculum.

Procedure To similar test tubes add, in duplicate, 1.0 and/or 1.5, 2.0, 3.0, 4.0, and 5.0 ml, respectively, of the *Standard Preparation*. To each tube and to four similar tubes containing no *Standard Preparation* add 5.0 ml of *Basal Medium Stock Solution* and sufficient water to make 10 ml.

To similar test tubes add, in duplicate, volumes of the *Assay Preparation* corresponding to three or more of the levels listed above for the *Standard Preparation*, including the levels of 2.0, 3.0, and 4.0 ml. To each tube add 5.0 ml of the *Basal Medium Stock Solution* and sufficient water to make 10 ml. Place one complete set of standard and assay tubes together in one tube rack and the duplicate set in a second rack or section of a rack.

Cover the tubes of both series suitably to prevent contamination, and sterilize in an autoclave at 121° for 5 min. Cool, and add 1 drop of *Inoculum* to each tube, except two of the four tubes containing no *Standard Preparation* (to serve as the uninoculated blanks). Incubate the tubes at a temperature between 30° and 37°, held constant to within ±0.5° until, following 16 to 24 h of incubation, there has been no substantial increase in turbidity in the tubes containing the highest level of standard during a 2-h period.

Determine the transmittance of the tubes in the following manner. Mix the contents of each tube, and transfer to an optical container if necessary. Place the container in a spectrophotometer that has been set at a specific wavelength between 540 and 660 nm, and read the transmittance when a steady state is reached. This steady state is observed a few seconds after agitation when the galvanometer reading remains constant for 30 s or more. Allow approximately the same time interval for the reading on each tube.

With the transmittance set at 1.00 for the uninoculated blank, read the transmittance of the inoculated blank. With the transmittance set at 1.00 for the inoculated blank, read the transmittance for each of the remaining tubes. If there is evidence of contamination with a foreign microorganisms, disregard the results of the assay.

Calculation Prepare a standard concentration-response curve as follows: For each level of the standard, calculate the response from the sum of the duplicate values of the transmittance as the difference $y = 2.00 - \Sigma$ (of transmittance). Plot this response on the ordinate of cross-section paper against the logarithm of the ml of *Standard Preparation* per tube on the abscissa, using for the ordinate either an arithmetic or a logarithmic scale, whichever gives the better approximation to a straight line. Draw the straight line or smooth curve that best fits the plotted points.

Calculate the response, y, adding together the two transmittances for each level of the *Assay Preparation*. Read from the standard curve the logarithm of the volume of the *Standard Preparation* corresponding to each of those values of y that fall within the range of the lowest and highest points plotted for the standard. Subtract from each logarithm so obtained the logarithm of the volume, in ml, of the *Assay Preparation* to obtain the difference, x, for each level. Average the values of x for each of three or more levels to obtain $\bar{x} = M'$, the log-relative potency of the *Assay Preparation*. Determine the quantity, in mg, of USP Calcium Pantothenate Reference Standard corresponding to the calcium pantothenate taken for assay as antilog M = antilog (M' + log R), where R is the number of mg of calcium pantothenate taken for assay.

*American Type Culture Collection No. 8014 is suitable. This strain formerly was known as *Lactobacillus arabinosus* 17.5.

Chewing Gum Base

BOUND STYRENE

Abbé-Type Refractometer Use an instrument, having fourth decimal place accuracy, that can be placed in a nearly

horizontal position for measurement of the refractive index of solids. An Amici-type compensating prism for achromatization is necessary unless a sodium vapor lamp is used as a light source.

Ethanol-Toluene Azeotrope Mix 70 volumes of ethanol or of formula 2B ethanol with 30 volumes of toluene, reflux for 4 h over calcium oxide, and then distil, discarding the first and last portions and collecting only that portion distilling within a range of 1°. (NOTE: Refluxing and distilling are not necessary if anhydrous 2B ethanol or absolute grain alcohol is used.)

Sample Preparation Sheet out a sample of the polymer to a thickness of 0.5 mm, and cut the sheeted sample into strips approximately 13 mm wide and 25 mm long. Fasten one strip to each leg of a "spider," consisting of a 13-mm square of sheet aluminum or stainless steel having a Nichrome wire leg about 38 mm long attached to each corner. Place the spider and strips in a 400-ml flask containing 60 ml of *Ethanol-Toluene Azeotrope*, positioning the spider so that each sample strip is in contact on all sides with the solvent. Extract for 1 h at a temperature at which the solvent boils gently, then replace the solvent with another 60-ml portion of *Ethanol-Toluene Azeotrope*, and extract for an additional hour. Remove the spider and sample strips from the flask, and dry them at 100° to constant weight in a vacuum oven at a pressure of about 10 mm of Hg. (*Caution:* The samples must be extracted and dried thoroughly, but overheating, which would cause plastication, must be avoided.)

Remove the extracted and dried strips from the spider, and press the strips between aluminum foil (0.025 to 0.08 mm thick, having good tear strength) at 100° for 3 to 10 min (preferably not more than 5 min), using any suitable apparatus to produce a uniform thickness not exceeding 0.5 mm. If the pressing is done between flat platens, without a cavity, use a force between about 500 and 1500 lb, increasing the applied force proportionally if several strips are pressed at one time; if cavity pressing plates are used, close the platens without applying pressure and preheat for 1 min, then apply a force of about 11 tons for 3 min, remove the specimens from the press, and allow them to cool.

Procedure Cut the pressed sample in half with sharp scissors, and peel off one piece of the foil. Cut off a strip about 6 mm wide and 12 mm long with the scissors so that one of the narrower ends is freshly cut.

Check the adjustment of the refractometer by means of a glass test plate pressed firmly against the prism, using a drop of α-bromo-naphthalene as the contact liquid. The small light source should be collimated. The best readings are obtained with the glass test piece if the light is diffused through crumpled tissue paper. After this adjustment, clean the prism well with lens paper moistened with alcohol. The refractive index of the glass test piece and of the test specimen must be measured at a known constant temperature, preferably 25°.

Place the test sample on the prism with the cut edge toward the light source approximately where the edge of the glass test piece was positioned. Remove the tissue paper from the light source, press the specimen firmly on the prism, and wait at least 1 min for the sample to attain temperature equilibrium. The upper prism may be closed lightly on the specimen if adequate light can still be focused on the end of the specimen. Unless the specimen is very thin, however, this operation can damage the prism or its mounting. Adjust the compensating prism until a sharp dividing line between light and dark fields with minimum color is obtained. Test the contact between the specimen and the prism by pressing the test specimen against the prism: There should be no change in the position of the boundary line during this test. Move the hand control, from the light into the dark field, until the boundary line just reaches the cross hairs, and make at least three readings. If the readings differ by more than 0.0001 refractive index unit, further readings should be made. Repeat the process of obtaining readings with another portion of the sample having a freshly cut edge, and average the mean values of the two sets of readings thus obtained. If the two mean values do not differ by more than 0.0002 refractive index unit, report the average as the results of the calculations. If necessary, correct the refractive index measurements to 25° using the formula

$$n_{25} = n_t + 0.00037(t - 25),$$

in which n_{25} is the refractive index at 25°, and n_t is the refractive index at the temperature, t, of measurement.

Calculate the percentage of bound styrene in emulsion-polymerized samples by the formula

$$23.50 + 1164(n_{25} - 1.53456) - 3497(n_{25} - 1.53456)^2.$$

Calculate the percentage of bound styrene in solution-polymerized samples by the formula

$$(1212.1212)(n_{25}) - 1838.3636.$$

Alternatively, the percentage of bound styrene may be determined by reference to suitable tables.

MOLECULAR WEIGHT

Polyethylene

Sample Solutions Dissolve 1 g of the sample, accurately weighed, in 95 ml of tetrahydronaphthalene, filter into a 100-ml volumetric flask, dilute to volume with the solvent, and mix (*Solution 1*). Transfer 50.0 ml of *Solution 1* into a tared dish, evaporate on a steam bath for about 1 h, and then complete the evaporation to dryness by heating in a vacuum oven at 70° for 2 h or to constant weight. Calculate the concentration, C_1, in g per 100 ml, of *Solution 1*. Prepare *Solutions 2* and *3*, respectively, by diluting 5.0-ml and 10.0-ml portions of *Solution 1* to 50.0 ml with the solvent, and then calculate the concentration of each (C_2 and C_3, respectively).

Procedure Determine the flow time, in seconds, of the solvent (t_0) and of the three *Sample Solutions* (t_1, t_2, and t_3, respectively) in a Cannon-Fenske viscometer immersed in a constant-temperature bath maintained at 130°. Calculate the specific viscosity, η_{sp}, of each *Sample Solution* by the formula $(t/t_0) - 1$, and then calculate the reduced viscosity of each by the formula η_{sp}/C. Plot the reduced viscosity of each solution against concentration, and extrapolate to zero concentration to obtain the intrinsic viscosity, $[\eta]$. Finally, calculate the molecu-

lar weight of the polyethylene by the formula $([\eta]/K)^{1/a}$, in which K is 5.1×10^{-4}, and a is 0.725.

Polyisobutylene (Flory Method)

Sample Solutions Dissolve 1 g of the sample, accurately weighed, in 95 ml of diisobutylene, filter into a 100-ml volumetric flask, dilute to volume with the solvent, and mix (*Solution 1*). Transfer 50.0 ml of *Solution 1* into a tared dish, evaporate on a steam bath for about 1 h, and then complete the evaporation to dryness by heating in a vacuum oven at 70° for 2 h or to constant weight. Calculate the concentration, C_1, in g per 100 ml, of *Solution 1*. Prepare *Solutions 2* and *3*, respectively, by diluting 5.0-ml and 10.0-ml portions of *Solution 1* to 50.0 ml with the solvent, and then calculate the concentration of each (C_2 and C_3, respectively).

Procedure Determine the flow time, in seconds, of the solvent (t_0) and of the three *Sample Solutions* (t_1, t_2, and t_3, respectively) in a Cannon-Fenske viscometer immersed in a constant-temperature bath maintained at 20°. Calculate the specific viscosity, η_{sp}, of each *Sample Solution* by the formula $(t/t_0) - 1$, and then calculate the reduced viscosity of each by the formula η_{sp}/C. Plot the reduced viscosity of each solution against concentration, and extrapolate to zero concentration to obtain the intrinsic viscosity, $[\eta]$. Finally, calculate the molecular weight of the polyisobutylene by the formula $([\eta]/K)^{1/a}$, in which K is 3.60×10^{-4}, and a is 0.64.

Polyvinyl Acetate

Sample Solutions Dissolve 1 g of the sample, accurately weighed, in 95 ml of acetone, filter into a 100-ml volumetric flask, dilute to volume with the solvent, and mix (*Solution 1*). Transfer 50.0 ml of *Solution 1* into a tared dish, evaporate on a steam bath for about 1 h, and then complete the evaporation to dryness by heating in a vacuum oven at 70° for 2 h or to constant weight. Calculate the concentration, C_1, in g per 100 ml, of *Solution 1*. Prepare *Solutions 2* and *3*, respectively, by diluting 5.0-ml and 10.0-ml portions of *Solution 1* to 50.0 ml with the solvent, and then calculate the concentration of each (C_2 and C_3, respectively).

Procedure Determine the flow time, in seconds, of the solvent (t_0) and of the three *Sample Solutions* (t_1, t_2, and t_3, respectively) in a Cannon-Fenske viscometer immersed in a constant-temperature bath maintained at 25°. Calculate the specific viscosity, η_{sp}, of each *Sample Solution* by the formula $(t/t_0) - 1$, and then calculate the reduced viscosity of each by the formula η_{sp}/C. Plot the reduced viscosity of each solution against concentration, and extrapolate to zero concentration to obtain the intrinsic viscosity, $[\eta]$. Finally, calculate the molecular weight of the polyvinyl acetate by the formula $([\eta]/K)^{1/a}$, in which K is 1.88×10^{-4}, and a is 0.69.

QUINONES

Standard Preparations Transfer 25.0 mg of hydroquinone into a 100-ml volumetric flask, dissolve in water, dilute to volume with water, and mix. Transfer 1.0-, 2.0-, 3.0-, 4.0-, and 6.0-ml aliquots of this solution into a series of 100-ml volumetric flasks, dilute each to volume with water, and mix. Transfer 2.0 ml of each of these solutions and 3.0 ml of water into a series of 25-ml graduates, add 0.5 ml of 0.1 *N* sodium carbonate to each, and continue as directed under *Sample Preparations*, beginning with " . . . shake immediately, then add 1.0 ml of 15% sulfuric acid. . . . "

Sample Preparations Place 30 g of freshly coagulated and washed sample in a two-necked 250-ml flask, add 100 ml of water, and heat at 66° for 2 h. (*Caution:* Do not boil.) Cool to room temperature, and transfer 5.0 ml of the extract into a 25-ml glass-stoppered graduate. Transfer 5.0 ml of water into a second graduate to serve as the blank. To each graduate add 1.0 ml of 15% sulfuric acid. To the graduate containing the sample extract add 0.5 ml of 0.1 *N* sodium carbonate, shake immediately, and then add 1.0 ml of 15% sulfuric acid. (NOTE: The elapsed time for this operation should not exceed 15 s.) To each graduate add 1.0 ml of 2,4-dinitrophenylhydrazine solution (dissolve 100 mg of 2,4-dinitrophenylhydrazine in 50 ml of carbonyl-free methanol, add 4 ml of hydrochloric acid, and dilute to 100 ml with water), stopper, and heat at 70° in a water bath for 1 h. Cool to room temperature, then add to each graduate 13 ml of water and 5.0 ml of benzene, stopper, and shake vigorously. Allow the phases to separate, and pipet 2.0 ml of the benzene layer from each graduate into corresponding test tubes containing 10 ml of a 1 in 100 solution of diethanolamine in pyridine. Shake each tube, and allow the color to develop for 10 min.

Procedure Determine the absorbance of the *Sample Preparation* in a 1-cm cell at 620 nm, with a suitable spectrophotometer, against the reagent blank. Determine the absorbance of each *Standard Preparation* in the same manner. Prepare a *Standard Curve* by plotting absorbance of each *Standard Preparation* against μg of quinone. From the *Standard Curve*, read the quantity, in μg, of quinones (as benzoquinone) in the *Sample Preparation*, and record the value thus obtained as Q. Calculate the quantity of quinones (as benzoquinone), in ppm, in the sample by the formula $20Q/W$, in which W is the weight of the sample taken, in g.

RESIDUAL STYRENE

Standard Preparation Place 25 ml of carbon disulfide in a 100-ml volumetric flask, cap with a serum stopper, and tare the flask to the nearest 0.1 mg. Inject, with 50-μg syringes, 15 μl each of styrene and of alpha-methylstyrene (AMS), reweighing after each addition to obtain the weight of each solution injected. Record the weight, in mg, of styrene as w_1 and that of AMS as w_2. Dilute to volume with carbon disulfide, and mix. Pipet 2 ml of this solution into a second 100-ml volumetric flask, dilute to volume with carbon disulfide, and mix. Finally, pipet 25 ml of the diluted solution into a third 100-ml volumetric flask, dilute to volume with carbon disulfide, and mix.

AMS-Solvent Solution Place 25 ml of carbon disulfide in a

100-ml volumetric flask, cap with a serum stopper, and tare the flask to the nearest 0.1 mg. Using a 50-µl syringe, inject 15 µl of AMS, and reweigh to obtain the weight of AMS injected. Dilute to volume with carbon disulfide, and mix. Pipet 2 ml of this solution into a second 100-ml volumetric flask, dilute to volume with carbon disulfide, and mix. Finally, pipet 25 ml of the diluted solution into a third 100-ml volumetric flask, dilute to volume, and mix. Calculate the weight, in g, of AMS in each ml of the final solution, and record the result as w' (approximately 7.5×10^{-7}).

Sample Preparation

Latex Samples Add, with agitation, 100 ml of the latex to a mixture consisting of 15 ml of glacial acetic acid and 10 g of sodium chloride in 500 ml of hot water. Coagulation starts almost immediately. When coagulation is complete, collect the coagulum on a coarse filter or cheesecloth, and wash with 1000 ml of a hot solution prepared with 5.6 g of sodium hydroxide and 1000 ml of water. Wash with hot water until the wash water is free of alkali, then cut the coagulum into small pieces, and dry at 105° for 4 h. Continue as directed under *Solid Samples*, beginning with "Transfer 1.5 g, accurately weighed. . . . "

Solid Samples Cut a piece approximately 2 in. × 3 in. × 5 in. from the corner of a polymer bale, and pass it through a cold mill, set at least 1/4 in. open, four times, reversing the sample on each pass. Cut the sample into two pieces at least 1 in. from the edge to expose clean polymer, and then dice or cut into small strips approximately 2 g of the clean polymer. Transfer 1.5 g, accurately weighed, into a 4-oz bottle fitted with a polyethylene cap, add 25.0 ml of the *AMS-Solvent Solution*, cap tightly, and agitate on a mechanical shaker until the polymer dissolves. (NOTE: Some polymers tend to swell and form viscous cements instead of dissolving cleanly. If this occurs, add 5- to 10-ml increments of carbon disulfide to obtain a mobile slurry, and in the next step increase the volume of methanol by a proportional amount.) Add 25 ml of methanol, cap the bottle, and shake vigorously on the shaker for 30 min. After the contents have settled, decant 10 ml of the coagulant serum into a 1-oz bottle, add 10 ml of water, and stopper with a serum cap. Shake vigorously for 1 min, then turn the bottle upside down, and allow the layers to separate. Withdraw by syringe 1 to 2 ml of the lower (carbon disulfide) layer, and transfer it into a 10-dram vial filled 1/4 in. with anhydrous sodium sulfate. Seal with a polyethylene cap, shake to mix, and allow to settle.

Procedure Inject a 10-µl portion of the *Sample Preparation* into a suitable gas chromatograph in which the detector is the hydrogen flame-ionization type and the column is 10-ft × 3/16-in. stainless steel tubing packed with 25% Ucon 50 HB 2000 on 60/80-mesh acid-washed DMCS Chromosorb W, or with equivalent packing materials. The carrier is nitrogen or helium flowing at 40 ml per min. The injection port temperature is 240°; the column temperature, 170° isothermal; and the detector temperature, 250°. Adjust the sensitivity of the instrument to give as large a signal as possible for styrene and AMS as is consistent with an acceptable background level. Measure the styrene and AMS peaks by any convenient method, recording the area of the styrene peak as A_1 and that of the AMS peak as A_2.

In the same manner, inject a 10-µl portion of the *Standard Preparation* into the chromatograph, obtain the chromatogram, and record the area of the styrene peak as a_1 and that of the AMS peak as a_2. Calculate the styrene factor, F, by the formula $(w_1/w_2) \times (a_2/a_1)$.

Calculate the content of residual styrene in the sample taken, in ppm, by the formula

$$(A_1/A_2) \times F \times 25 \times (w'/W) \times 10^6,$$

in which W is the weight of the sample taken, in g.

SAMPLE SOLUTION FOR ARSENIC TEST

Transfer a 1-g sample, accurately weighed, into a Kjeldahl flask, rest the open end of the flask in a Kjeldahl fume bulb attached to a water aspirator, add 5 ml of sulfuric acid and 4 ml of 30% hydrogen peroxide, and digest over a small flame. (See *Caution* statement under *Arsenic Test*, page 465.) Continue adding the peroxide in 2-ml portions, allowing the reaction to subside between additions, until all organic matter is destroyed, fumes of sulfuric acid are copiously evolved, and the solution becomes colorless. *Maintain oxidizing conditions at all times during the digestion by adding peroxide whenever the mixture turns brown or darkens.* (The amount of peroxide required to completely digest the samples will vary, but as much as 200 ml may be required in some cases, depending upon the nature of the material.) Cool, cautiously add 10 ml of water, again evaporate to strong fuming, and cool. Transfer the solution into an arsine generator flask, wash the Kjeldahl flask and bulb with water, adding the washings to the generator flask, and dilute to 35 ml with water.

SAMPLE SOLUTION FOR LEAD LIMIT TEST

Transfer a 3.3-g sample, accurately weighed, into a porcelain dish or casserole, heat on a hot plate until completely charred, then heat in a muffle furnace at 480° for 8 h or overnight, and cool. Cautiously add 5 ml of nitric acid, evaporate to dryness on a hot plate, then heat again in the muffle furnace at 480° for exactly 15 min, and cool. Extract the ash with two 10-ml portions of water, filtering each extract into a separator. Leach any insoluble material on the filter with 6 ml of *Ammonium Citrate Solution*, 2 ml of *Hydroxylamine Hydrochloride Solution*, and 5 ml of water (see *Lead Limit Test*, page 518, for preparation of these solutions), adding the filtered washings to the separator. Continue as directed under *Procedure* in the *Lead Limit Test*, page 518, beginning with "To the separator add 2 drops of phenol red TS. . . . "

TOTAL UNSATURATION

Transfer about 500 mg of the sample, accurately weighed, into a 500-ml iodine flask containing 100 ml of filtered carbon tetrachloride. Stopper the flask securely, cover to protect the mixture from light, and shake in a mechanical shaker until the sample is completely dissolved (about 1.5 h). Remove the flask from the shaker and the cover from the flask, and then add 5 ml of a 1 in 5 solution of trichloroacetic acid in carbon tetrachloride, 25.0 ml of 0.1 N iodine in carbon tetrachloride, and 25 ml of a 3 in 10 solution of mercuric acetate in glacial acetic acid.

Stopper the flask, mix the contents thoroughly by shaking, and allow to stand in a dark place for exactly 30 min. Add 50 ml of potassium iodide TS, immediately titrate with 0.1 *N* sodium thiosulfate, using starch TS as indicator, and record the volume, in ml, required as *S*. Perform a blank determination (see page 2), and record the volume of 0.1 *N* sodium thiosulfate as *B*. Calculate the percent total unsaturation by the formula

0.250 N Sodium Thiosulfate Dissolve 62.5 g of sodium thiosulfate, $Na_2S_2O_3.5H_2O$, in 750 ml of recently boiled and in which *N* is the exact normality of the 0.1 *N* sodium thiosulfate solution, *W* is the weight of the sample taken, in g, and 1.87 is an equivalence factor for isobutylene, which is the chief contributor of olefin linkages in the copolymer. (NOTE: The percentage thus obtained will be high if the copolymer contains an antioxidant that reacts as an unsaturated compound in this test procedure; in this case, the weight percent of the antioxidant must be determined by appropriate means and subtracted from the percent total unsaturation as determined herein.)

Chloride and Sulfate Limit Tests

Where limits for chloride and sulfate are specified in the monograph, the sample solution and control should be compared in appropriate glass cylinders of the same dimensions and matched as closely as practicable with respect to their optical characteristics.

If the solution is not perfectly clear after acidification, filter through filter paper that has been washed free of chloride and sulfate. Add identical quantities of the precipitant (silver nitrate TS or barium chloride TS) in rapid succession to both the sample solution and the control solution.

Experience has shown that visual turbidimetric comparisons are best made between solutions containing from 10 to 20 μg of chloride ion (Cl) or from 200 to 400 μg of sulfate ion (SO_4) in 50 ml. Weights of samples are specified on this basis in the monographs in which these limits are included.

Chloride Test

Standard Chloride Solution Dissolve 165 mg of sodium chloride in water and dilute to 100.0 ml. Transfer 10.0 ml of this solution to a 1000-ml volumetric flask, dilute to volume with water, and mix. Each ml of the final solution contains 10 μg of chloride ion (Cl).

Procedure Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 ml of water, neutralize to litmus external indicator with nitric acid, if necessary, then add 1 ml in excess. To the clear solution or filtrate add 1 ml of silver nitrate TS, dilute to 50 ml with water, mix, and allow to stand for 5 min protected from direct sunlight. Compare the turbidity, if any, with that produced similarly in a control solution containing the required volume of *Standard Chloride Solution* and the quantities of the reagents used for the sample.

Sulfate Test

Standard Sulfate Solution Dissolve 148 mg of anhydrous sodium sulfate in water and dilute to 100.0 ml. Transfer 10.0 ml of this solution to a 1000-ml volumetric flask, dilute to volume with water, and mix. Each ml of the final solution contains 10 μg of sulfate (SO_4).

Procedure Unless otherwise directed, dissolve the specified amount of the test substance in 30 to 40 ml of water, neutralize to litmus external indicator with hydrochloric acid, if necessary, then add 1 ml of diluted hydrochloric acid TS. To the clear solution or filtrate add 3 ml of barium chloride TS, dilute to 50 ml with water, and mix. After 10 min compare the turbidity, if any, with that produced in a solution containing the required volume of *Standard Sulfate Solution* and the quantities of the reagents used for the sample.

Chromatography

For the purposes of the *Food Chemicals Codex*, chromatography is defined as an analytical technique whereby a mixture of chemicals may be separated by virtue of their differential affinities for two immiscible phases. One of these, the stationary phase, consists of a fixed bed of small particles with a large surface area, while the other, the mobile phase or "eluant," is a fluid that moves constantly through, or over the surface of, the fixed phase. Chromatographic systems achieve their ability to separate mixtures of chemicals by selectively retarding the passage of some compounds through the stationary phase while permitting others to move more freely. Therefore, the chromatogram may be evaluated qualitatively by determining the R_f, or retardation factor, for each of the eluted substances. The R_f is a measure of that fraction of its total elution time that any compound spends in the mobile phase. Since this fraction is directly related to the fraction of the total amount of the solute that is in the mobile phase, the R_f can be expressed as

$$R_f = V_m C_m / (V_m C_m + V_s C_s),$$

where V_m and V_s are the volumes of the mobile and stationary phase, respectively, and C_m and C_s are the concentrations of the solute in either phase at any time. This can be simplified to

$$R_f = V_m / (V_m + K V_s),$$

where $K = C_s/C_m$ and is an equilibrium constant that indicates this differential affinity of the solute for the phases. Alternatively, a new constant k', the capacity factor, may be introduced, giving another form of the expression:

$$R_f = 1/(1 + k'),$$

where $k' = KV_s/V_m$. The capacity factor, k', which is normally constant for small samples, is a parameter that expresses the ability of a particular chromatographic system to interact with a solute. The larger the k' value, the more the sample is retarded.

Both the retardation factor and the capacity factor may be used for qualitative identification of a solute or for developing

strategies for improving separation. In terms of parameters easily obtainable from the chromatogram, the R_f is defined as the ratio of the distance traveled by the solute band to the distance traveled by the mobile solvent in a particular time. The capacity factor, k', can be evaluated by the expression

$$k' = (t_r - t_o)/t_o,$$

where t_r, the retention time, is the elapsed time from the start of the chromatogram to the elution maximum of the solute, and t_o is the retention time of a solute that is not retained by the chromatographic system.

Retardation of the solutes by the stationary phase may be achieved by one or a combination of mechanisms. Certain substances, such as alumina or silica gel, interact with the solutes primarily by *adsorption*, either *physical adsorption*, in which the binding forces are weak and easily reversible, or *chemisorption*, where strong bonding to the surface can occur. Another important mechanism of retardation is *partition*, which occurs when the solute dissolves in the stationary phase, usually a liquid coated as a thin layer on the surface of an inert particle or chemically bonded to it. If the liquid phase is a polar substance (e.g., polyethylene glycol) and the mobile phase is nonpolar, the process is termed *normal-phase chromatography*. When the stationary phase is nonpolar (e.g., octadecylsilane) and the mobile phase is polar, the process is *reverse-phase chromatography*. For the separation of mixtures of ionic species, insoluble polymers called *ion exchangers* are used as the stationary phase. Ions of the solutes contained in the mobile phase are adsorbed onto the surface of the ion exchanger while at the same time displacing an electrically equivalent amount of less strongly bound ions in order to maintain the electroneutrality of both phases. The chromatographic separation of mixtures of large molecules such as proteins may be accomplished by a mechanism called *exclusion chromatography* or *gel chromatography*. The stationary phases used are highly cross-linked polymers that have imbibed a sufficient amount of solvent to form a gel. The separation is based on the physical size of the solutes; those that are too large to fit within the interstices of the gel are eluted rapidly, while the smaller molecules follow an irregular path through the pores of the gel and are eluted later. In any chromatographic separation, more than one of the above mechanisms may be occurring simultaneously.

Chromatographic separations may also be characterized according to the type of instrumentation or apparatus used. The types of chromatography that may be used in the FCC are column, paper, thin-layer, gas, and high-pressure liquid chromatography.

COLUMN CHROMATOGRAPHY

Apparatus The equipment needed for column chromatography is not elaborate, consisting only of a cylindrical glass or Teflon tube that has a restricted outflow orifice. The dimensions of the tube are not critical and may vary from 10 to 40 mm in inside diameter and from 100 to 600 mm in length. For a given separation, greater efficiency may be obtained with a long narrow column, but the resultant flow rate will be lower. A fritted-glass disk may be seated in the end of the tube to act as a support for the packing material. The column is fitted at the end with a stopcock or other flow-restriction device in order to control the rate of delivery of the eluant.

Procedure The stationary phase is introduced into the column either as a dry powder or as a slurry in the mobile phase. Since a homogeneous bed free of void spaces is necessary to achieve maximum separation efficiency, the packing material is introduced in small portions and allowed to settle before further additions are made. Settling may be accomplished by allowing the mobile phase to flow through the bed, by tapping or vibrating the column if a dry powder is used, or by compressing each added portion using a tamping rod. The rod can be a solid glass, plastic, or metal cylinder whose diameter is slightly smaller than the column, or it can be a thinner rod onto the end of which has been attached a disk of suitable diameter. Ion-exchange resins and exclusion polymers are never packed as dry powders since after introduction of the mobile phase they will swell and create sufficient pressure to shatter the column. When the packing has been completed, the sample is introduced onto the top of the column. If the sample is soluble, it is dissolved in a mimimum amount of the mobile phase, pipetted onto the column, and allowed to percolate into the top of the bed. If it is not soluble or if the volume of solution is too large, it may be mixed with a small amount of the column packing. This material is then transferred to the chromatographic tube to form the top of the bed.

The chromatogram is then developed by adding the mobile phase to the column in small portions and allowing it to percolate through the packed bed either by gravity or under the influence of pressure or vacuum. Development of the chromatogram takes place by selective retardation of the components of the mixture as a result of their interaction with the stationary phase. In column chromatography, the stationary phase may act by adsorption, partition, ion exchange, exclusion of the solutes, or a combination of these effects.

When the development is complete, the components of the sample mixture may be detected and isolated by either of two procedures. The entire column may be extruded carefully from the tube, and if the compounds are colored or fluorescent under ultraviolet light, the appropriate segments may be cut from the column using a razor blade. If the components are colorless, they may be visualized by painting or spraying a thin longitudinal section of the surface of the chromatogram with color-developing reagents. The chemical may then be separated from the stationary phase by extraction with a strong solvent such as methanol and subsequently quantitated by suitable methods.

In the second procedure, the mobile phase may be allowed to flow through the column until the components of the mixture successively appear in the effluent. This eluate may be collected in fractions and the mobile phase evaporated if desired. The chemicals present in each fraction may then be determined by suitable analytical techniques.

PAPER CHROMATOGRAPHY

In this type of chromatography, the stationary phase ordinarily consists of a sheet of paper of suitable texture and thickness. The paper used is made from highly purified cellulose, which has a great affinity for water and other polar solvents since it

has many hydroxyl functional groups. The tightly bound water acts as the stationary phase, and therefore the mechanism that predominates is liquid-liquid or partition chromatography. Adsorption of solutes to the cellulose surface may also occur, but this is of lesser importance. Papers especially impregnated to permit ion-exchange or reverse-phase chromatography are also available.

Apparatus The essential equipment for paper chromatography consists of the following:

Vapor-Tight Chamber The chamber is constructed preferably of glass, stainless steel, or porcelain. It is provided with inlets for the addition of solvent or for releasing internal pressure, and it is designed to permit observation of the progress of the chromatographic run without being opened. Tall glass cylinders are convenient if they are made vapor-tight with suitable covers and a sealing compound.

Supporting Rack The rack serves as a support for the solvent troughs and antisiphoning rods. It is constructed of a corrosion-resistant material about 5 cm shorter than the inside height of the chamber.

Solvent Troughs The troughs, made of glass, are designed to be longer than the width of the chromatographic sheets and to contain a volume of solvent greater than that required for one chromatographic run.

Antisiphoning Rods Constructed of heavy glass, the rods are placed on the rack and arranged to run outside of, parallel to, and slightly above the edge of the glass trough.

Chromatographic Sheets Special chromatographic filter paper is cut to length approximately equal to the height of the chamber. The sheet is at least 2.5 cm wide but not wider than the length of the trough. A fine pencil line is drawn horizontally across the filter paper at a distance from one end such that when the sheet is suspended from the antisiphoning rods with the upper end of the paper resting in the trough and the lower portion hanging free into the chamber, the line is located a few cm below the rods. Care is necessary to avoid contaminating the paper by excessive handling or by contact with dirty surfaces.

Procedure for Descending Chromatography Separation of substances by descending chromatography is accomplished by allowing the mobile phase to flow downward on the chromatographic sheet.

The substance or substances to be analyzed are dissolved in a suitable solvent. Convenient volumes of the resulting solution, normally containing 1 to 20 μg of the compound, are placed in 6- to 10-mm spots along the pencil line not less than 3 cm apart. If the total volume to be applied would produce spots of a diameter greater than 6 to 10 mm, it is applied in separate portions to the same spot, each portion being allowed to dry before the next is added.

The spotted chromatographic sheet is suspended in the chamber by use of the antisiphoning rod and an additional heavy glass rod that holds the upper end of the sheet in the solvent trough. The bottom of the chamber is covered with a mixture containing both phases of the prescribed solvent system. It is important to ensure that the portion of the sheet hanging below the rods is freely suspended in the chamber without touching the rack or the chamber walls. The chamber is sealed to allow equilibration (saturation) of the chamber and the paper with solvent vapor. Any excess pressure is released as necessary. For large chambers, equilibration overnight may be necessary.

A volume of the mobile phase in excess of the volume required for complete development of the chromatogram is saturated with the immobile phase. After equilibration of the chamber, the prepared mobile solvent is introduced into the trough through the inlet. The inlet is closed, and the mobile phase is allowed to travel down the paper the desired distance. Precautions must be taken against allowing the solvent to run down the sheet when opening the chamber and removing the chromatogram. The location of the solvent front is quickly marked, and the sheets are dried.

The chromatogram is observed and measured directly or after suitable development to reveal the location of the spots of the isolated components of the mixture.

Procedure for Ascending Chromatography In ascending chromatography, the lower edge of the sheet (or strip) is dipped into the mobile phase to permit the mobile phase to rise on the chromatographic sheet.

The test materials are applied to the chromatographic sheet as directed under *Procedure for Descending Chromatography*. Enough of both phases of the solvent mixture to cover the bottom of the chamber is added. Empty solvent troughs are placed on the bottom of the chamber, and the chromatographic sheet is suspended so that the end near which the spots have been added hangs free inside the empty trough.

The chamber is sealed, and equilibration is allowed to proceed as described under *Procedure for Descending Chromatography*. Then the solvent is added through the inlet to the trough in excess of the quantity of solvent required for complete moistening of the chromatographic sheet. The chamber is resealed. When the solvent front has reached the desired height, the chamber is opened and the sheet is removed, the location of the solvent front is quickly marked, and the sheet is dried.

Small cylinders may be used without troughs so that only the mobile phase is placed on the bottom. The chromatographic sheet is suspended during equilibration with the lower end just above the solvent, and chromatography is started by lowering the sheet so that it touches the solvent.

Detection of Chromatographic Bands After the chromatogram has been fully developed, the bands corresponding to the various solutes may be detected by means similar to those described in *Column Chromatography*. If the compounds are colored or fluorescent under ultraviolet light, they may be visualized directly. Colorless compounds may be detected by spraying the paper with color-developing reagents. The bands corresponding to the individual components can be cut from the paper, and the chemical substances eluted from the cellulose by the use of a strong solvent such as methanol.

Identification of Solutes Since the chromatographic mobilities of the solutes may change from run to run due to varying experimental conditions, presumptive identification of a substance should be based on comparison with a reference standard. The R_f values of the unknown substance and the standard on the same chromatogram must be identical. Alternatively, the ratio between the distances traveled by a given

compound and a reference substance, the R_f value, must be 1.0. Identification may also be made by mixing a small amount of the reference substance with the unknown and chromatographing. The resulting chromatogram should contain only one spot. Definitive identification of solutes may be achieved by eluting them from the paper and subjecting them to IR, NMR, or mass spectrometry.

THIN-LAYER CHROMATOGRAPHY

In thin-layer chromatography (TLC), the stationary phase is a uniform layer of a finely divided powder that has been coated on the surface of a glass or plastic sheet and that is held in place by a binder. The capacity of the system is dependent on the thickness of the layer, which may range from 0.1 to 2.0 mm. The thinner layers are used primarily for analytical separations, while the thicker layers, because of their greater sample-handling ability, are useful for preparative work.

Substances that are used as coatings in TLC include silica gel, alumina, powdered glass, or cellulose. Separations occur due to adsorption of the solutes from the mobile phase onto the surface of the thin layer. However, adsorption of water from the air or solvent components from the mobile phase can give rise to partition or liquid-liquid chromatography. Specially coated plates are available that permit ion exchange or reverse-phase separations.

Apparatus Acceptable apparatus and materials for thin-layer chromatography consist of the following:

Glass Plates Flat glass plates of uniform thickness throughout their areas. The most common sizes are 20 × 20 cm and 5 × 20 cm.

Aligning Tray An aligning tray or other suitable flat surface is used to align and hold plates during application of the adsorbent.

Adsorbent The adsorbent may consist of finely divided adsorbent materials for chromatography. It can be applied directly to the glass plate, or it can be bonded to the plate by means of plaster of Paris or with starch paste. Pretreated chromatographic plates are available commercially.

Spreader A suitable spreading device that when moved over the glass plate applies a uniform layer of adsorbent of desired thickness over the entire surface of the plate.

Storage Rack A rack of convenient size to hold the prepared plates during drying and transportation.

Developing Chamber A glass chamber that can accommodate one or more plates and can be properly closed and sealed as described under *Paper Chromatography*. It is fitted with a plate-support rack that can support the plates when the lid of the chamber is in place.

An ultraviolet light source suitable for observations with short (254 nm) and long (360 nm) ultraviolet wavelengths may be required.

Procedure Clean the plates scrupulously, as by immersion in a chromic acid cleansing mixture, and rinse them with copious quantities of water until the water runs off the plates without leaving any visible water or oily spots, and then dry.

Arrange the plate or plates on the aligning tray, and secure them so that they will not slip during the application of the adsorbent. Mix an appropriate quantity of adsorbent and liquid, usually water, which when shaken for 30 s gives a smooth slurry that will spread evenly with the aid of a spreader. Transfer the slurry to the spreader, and apply the coating at once before the binder begins to harden. Move the spreader smoothly over the plates from one end of the tray to the other. Remove the spreader, and wipe away excess slurry. Allow the plates to set for 10 min, and then place them in the storage rack and dry at 105° for 30 min or as directed in the monograph. Store the finished plates in a desiccator.

Equilibrate the atmosphere in the chamber as described under *Procedure for Descending Chromatography* in the section on *Paper Chromatography*.

Apply the *Sample Solution* and the *Standard Solution* at points about 1.5 cm apart and about 2 cm from the lower edge of the plate (the lower edge is the first part over which the spreader moves in the application of the adsorbent layer), and allow to dry. A template will aid in determining the spot points and the 10- to 15-cm distance through which the solvent front should move.

Arrange the plate on the supporting rack (sample spots on the bottom), and introduce the rack into the developing chamber. The solvent in the chamber must be deep enough to reach the lower edge of the adsorbent, but must not touch the spot points. Seal the cover in place, and maintain the system until the solvent ascends to a point 10 to 15 cm above the initial spots, this usually requiring from 15 min to 1 h. Remove the plates, and dry them in air. Measure and record the distance of each spot from the point of origin. If so directed, spray the spots with the reagent specified, observe, and compare the sample with the standard chromatogram.

Detection and Identification Detection and identification of solute bands is done by methods essentially the same as those described in *Paper Chromatography* and *Column Chromatography*. However, in TLC an additional method called *fluorescence quenching* is also used. In this procedure, an inorganic phosphor is mixed with the adsorbent before it is coated on the plate. When the developed chromatogram is irradiated with ultraviolet light, the surface of the plate fluoresces with a characteristic color except in those places where ultraviolet-absorbing solutes are situated. These quench the fluorescence and are detectable as dark spots.

Quantitative Analysis Two methods are available if quantitation of the solute is necessary. In the first, the bands are detected and their positions marked. Those areas of adsorbent containing the compounds of interest are scraped from the surface of the plate into a centrifuge tube. The chemicals are extracted from the adsorbent with the aid of a suitable strong solvent, the suspension is centrifuged, and the supernatant layer is subjected to appropriate methods of quantitative analysis.

The second method involves the use of a scanning densitometer. This is a spectrophotometric device that directs a beam of monochromatic radiation across the surface of the plate. After interaction with the solutes in the adsorbent layer, the radiation is detected as transmitted or reflected light and a recording of light intensity versus distance traveled is produced. The concentration of a particular species is proportional to the area

under its peak and can be determined accurately by comparison with standards.

GAS CHROMATOGRAPHY

This type of chromatography differs from the others in that the mobile phase is a gas and therefore the solutes must be vaporized in order to allow movement through the column. The stationary phases that are used are solids (gas-solid chromatography, GSC) or liquids coated as a thin layer on an inert solid or on the walls of the column (gas-liquid chromatography, GLC).

In gas-solid chromatography, the passage of a solute through the column will be retarded by adsorption or exclusion mechanisms. In gas-liquid chromatography, the solutes will partition between the gaseous mobile phase and the stationary liquid. In either case, if the rate of flow of the mobile phase is constant throughout the chromatogram, all solutes will spend the same time in the gas phase. Therefore, the efficiency of a separation depends on the time spent in the stationary phase, and those solutes that have a greater affinity for the stationary phase (larger K or k') will elute later in the chromatogram (lower R_f). The value of the k' and therefore the success of the separation depend on a number of parameters that are within the control of the analyst. These include (1) carrier gas flow rate, (2) column length and diameter, (3) particle size of the solid support or adsorbent, (4) the particular liquid phase used, (5) the amount of liquid phase relative to the amount of solid support, and (6) the temperature.

Apparatus The essential components of a basic gas chromatograph are a carrier gas supply, a sample injection port, a column, a detector, and a suitable data-recording device. The carrier gas supply system consists of a tank of highly compressed inert gas, a pressure regulator to reduce the pressure to operating levels, and a flow meter to permit reproducible flow rates to be achieved. Because of their inertness and availability, the gases most commonly used are helium or nitrogen. The regulated carrier gas then flows through an injection port into which the analytical samples are introduced. Since the solutes to be chromatographed must be vaporized, the injection port is heated to a temperature high enough to cause rapid vaporization but not thermal degradation. Samples are introduced into the gas stream through a silicone rubber septum with the aid of a microliter syringe. The samples may be injected into a mixing area within the injector port or, in certain instances, directly onto the head of the column. The vaporized sample is carried into the column, where it is separated into its various components. The column is contained in an oven that is usually maintained at a constant temperature suitable to the particular analysis. Where a mixture contains solutes of widely diverse volatility, a temperature-programming device may be used to vary the oven temperature during the run. When the solutes leave the end of the column in the effluent, they enter a detector that produces an electrical signal proportional to the mass or concentration of the solute in the eluate. In order to prevent condensation of the solutes, the detector is heated. The two types of detectors most commonly used are the thermal conductivity detector, which detects changes in the thermal conductivity of the gas stream as solutes are eluted, and the flame-ionization detector, in which the eluting solutes are burned in a hydrogen flame, producing a small electrical current. After amplification, the electrical signal is conducted to a suitable recording device, which produces the chromatogram as a detector response versus time plot. The chromatogram consists of a series of bell-shaped curves, each representing a particular solute. The areas under the curves are proportional to the concentrations of the solutes.

Columns Gas chromatographic columns consist of tubes of stainless steel, aluminum, copper, tin, or glass filled with stationary phase. Glass, tin, or Teflon-lined metal columns are used where degradation of sensitive compounds might occur on hot metal column walls. Columns of various dimensions may be used, but they range usually from 0.6 to 1.8 m in length and from 2 to 4 mm in inside diameter. Capillary columns, whose inside diameters may be 0.25 mm and whose lengths may be 50 m or more, are sometimes used for separations where very high efficiencies are required.

Solid support materials must be as inert as possible in order to prevent interaction of the solutes with active surfaces, resulting in degradation, rearrangement, or loss of peak symmetry (tailing). The most commonly used supports are derived from silicates, usually diatomaceous earth. Before use they are acid-washed, calcined, and treated with a silanizing reagent to render surface hydroxyls inactive. They are available in various particle sizes from 30- to 120-mesh, with the 80- to 100-mesh and 100- to 120-mesh fractions most often used. Porous polymeric materials, which may be coated if desired or used as supplied, are available for the separation of low-molecular-weight compounds.

Liquid phases for partition chromatography may be chosen from a large variety of compounds, ranging from the very polar polyethylene glycols to the nonpolar methyl silicone gums. The choice of a liquid phase for a particular separation is mainly empirical, but usually polar phases are used for the analysis of mixtures of polar compounds.

Before use, a packed column should be conditioned in the chromatograph to reduce the level of extraneous detector signals produced by the bleeding of volatile substances from the support and the liquid phase. This can be accomplished by heating the column at a temperature slightly above its expected operating temperature while maintaining a low flow of carrier gas through it. During this process, the column should be disconnected from the detector to prevent its contamination.

Qualitative Analysis Since it is impractical in gas chromatography to measure the R_f, presumptive identification of a solute should be done by comparing its position on the chromatogram with that of a reference standard. The position of a solute is characterized by its *retention time*, the time from injection to the peak maximum; its *retention volume*, the product of retention time and carrier gas flow rate; or its *retention distance*, the distance from injection to the peak maximum. Since conditions may vary between determinations, it is more appropriate to identify a substance by its *relative retention*,

$$\alpha = (x_2 - x_0)/(x_1 - x_0),$$

where x_2 is the retention time, volume, or distance of the desired chemical, x_1 is that of the reference compound, preferably determined on the same chromatogram, and x_0 is the

retention of an inert compound that is not retarded by the column.

A method of definitive identification is to trap and condense the effluent for each peak and subject the condensate to analysis by IR, NMR, mass spectrometry, or other suitable methods.

A measure of the efficiency of the separation of two adjacent peaks is given by the dimensionless constant R, the resolution factor, which can be calculated by the equation

$$R = 2(t_2 - t_1)/(w_1 - w_2),$$

where t_2 and t_1 are the retention times of the two peaks, and w_1 and w_2 are the baseline widths determined by the intersection of the tangents of the inflection points of the peaks with the baseline. A resolution of 1.0 corresponds to a peak overlap of approximately 2% and is usually considered to be adequate for analytical purposes.

A measure of the efficiency of a column is the number, N, of theoretical plates it contains for a given compound:

$$N = 16(t_r/w_b)^2,$$

where t_r is the retention time of the peak, and w_b is its width in units of time at the baseline.

Quantitative Analysis In a gas chromatogram, the parameter that is proportional to the concentration of any solute is the area under its peak. The determination of exact areas requires the use of an automatic electronic integrator or computer. For economic reasons, manual methods, based on the approximately triangular shape of the peak, are used more frequently. The most common of these calculates the area as the product of the peak height times its width at half the height. For isothermal systems, where the experimental parameters can be rigorously controlled, peak heights may be substituted for areas.

In order to convert the peak areas to the amount or percentage of the solute in the sample, three different methods may be used.

1. *Area Normalization* This method is based on the assumption that a peak is obtained on the chromatogram for each component of the mixture. The areas of all the peaks, each corrected by multiplying by its response factor, are added together to obtain the total area. Then the percentage of any component is equal to its corrected area divided by the total area and multiplied by 100. This method is reliable only if all components of the sample give a peak and if the various response factors are known.

2. *External Standard* A series of samples containing known amounts of the analyte are chromatographed under identical conditions. From the data obtained, a standard or working curve can be constructed by plotting area versus amount of standard. After chromatographing the unknown under the same conditions, the area is measured, and by interpolation using the standard curve the amount of the unknown in the sample can be determined. This method is highly reliable if the volume of sample injected is controlled precisely.

3. *Internal Standard* In order to correct for errors that might occur when injection volumes vary or the chromatographic conditions change slightly from run to run, the internal standard method may be used. In this method, another standard, which is chemically similar to the unknown component and which elutes separately from all other peaks, is added in a constant amount to all standard and test solutions of the analyte. After chromatographing, a calibration curve is constructed by plotting the area ratios of the standard solutions (area of analyte standard per area of internal standard) versus the weight or concentration ratios of each standard. The unknown is then chromatographed, its area ratio is determined, and the corresponding weight ratio is found by interpolation using the calibration curve. Since the amount of internal standard is constant and known, the concentration of the unknown component can be calculated.

HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Many of the disadvantages of column chromatography, such as low efficiency, prolonged analysis time, nonreusable columns, and poor quantitative reproducibility, have been resolved by advances in column and instrument technology that have given rise to the technique of high-pressure liquid chromatography (HPLC).

In this type of analysis, which is also known as high-performance or high-speed liquid chromatography, the mobile phase is a liquid that is pumped at moderately high pressures through a narrow-bore column. The stationary phase consists of solid particles of very small size and large surface area. In theory, the method is exactly analogous to traditional column chromatography; however, the use of microparticulate packings and narrow columns give separation efficiencies much greater than those of any other chromatographic technique.

Interaction of the solutes with the stationary phases occurs by the same mechanisms that apply to traditional liquid chromatography, i.e., adsorption, partition, ion exchange, and exclusion. However, in HPLC the applicability of partition chromatography has been extended to a wider range of samples due to the development of packing materials that have the liquid phase permanently bonded to a solid support, thereby preventing inactivation of the column due to stripping of the liquid phase. Reverse-phase partition chromatography, in which the bonded material is a long-chain nonpolar substance (e.g., octadecylsilyl), is used extensively because its selectivity for solutes can be adjusted over a wide range by varying the polarity of the mobile phase. This mode may also be used as a substitute for ion-exchange resins for the separation of water-soluble ionic or ionizable substances. Three techniques are available to accomplish this: (1) ion suppression, in which the pH of the mobile phase is adjusted to prevent ionization of weak acids and bases; (2) ion pairing, in which an ionic reagent (e.g., heptane-sulfonate) is added to the mobile phase in order to form a less polar ion pair with a charged solute; and (3) "soap chromatography," in which the pairing reagent is an ionic detergent (e.g., sodium lauryl sulfate).

Apparatus The basic components of a liquid chromatograph are a solvent delivery system, a sample injection device, a column, a detector, and a suitable data-recording device.

The solvent delivery system consists of one or more pumps capable of delivering a pulseless flow of mobile phase at pressures ranging from 100 to 5000 psig. In the *isocratic* mode,

where a mobile phase of constant composition is used throughout the run, a single pump and solvent reservoir are required. For the separation of mixtures where the k' values vary over a wide range, *gradient-elution* analysis may be used. In this method, two pumps, each delivering a separate component of the mobile phase to a mixing chamber, are used. By varying the rate of delivery from each pump throughout the analysis, a solvent mixture of constantly changing composition will be delivered to the column.

Injection of the sample into the chromatograph may be done by means of a syringe or by using a rotary valve injector. Syringe injection is done through a rubber septum as in gas chromatography, or since diffusion of the sample in the mobile phase is negligible in HPLC, the flow may be stopped and a cap removed from the head of the column so that the sample may be directly deposited on the stationary phase. The cap is then replaced and the flow restarted. In the rotary valve injector, the sample is loaded into a calibrated loop of tubing by syringe or suction while the mobile phase is diverted so that it flows only through the column. When the injection valve is rotated, the mobile phase then flows through the calibrated tube and deposits the sample on the column.

The columns usually used for analytical separations have internal diameters ranging from 2 to 4 mm and lengths from 25 to 100 cm. The longer, narrower columns (2.1 mm × 100 cm) are packed with pellicular stationary phase material that has particle sizes ranging from 37 to 50 μm. Totally porous microparticulate packings, which are available in 5-, 10-, and 20-μm sizes, are used in the shorter columns (4 mm × 25 cm). Satisfactory columns containing the larger particles (> 30 μm) can be obtained by dry packing, using vibration or tapping to settle the bed after each addition of stationary phase. However, due to the static charge on the smaller particles, which causes them to clump together, dry packing is not feasible. Instead columns are filled using the *balanced density slurry* technique, in which the stationary phase material is suspended in a solvent mixture that has the same density as the solid. The slurry is then forced into the column under pressure. Microparticulate packings can give efficiencies of up to 40,000 plates per m, while 500 to 1000 plates per m can be expected from columns packed with pellicular phases.

The types of detectors most frequently used in HPLC are spectrophotometric, fluorometric, and refractometric detectors. The spectrophotometric detectors are fixed- or variable-wavelength photometers that operate in the ultraviolet and visible portions of the spectrum. The most commonly employed detectors of this type are those that use the mercury line at 254 nm as their source of radiation, since solutes containing benzenoid functions absorb strongly at this wavelength. In order to prevent remixing of separated solutes, the volume of the detector cell is very small, usually 8 μl. Detectors of this type are sensitive to as little as 1 ng of a strong ultraviolet absorber.

Fluorometric detectors are extremely sensitive to small quantities (< 1 ng) of naturally fluorescing compounds. They may also be used for the detection of nonfluorescent compounds after reaction with suitable fluorimetric reagents either prior to chromatography or after separation on the column.

In the case of chemicals that neither absorb effectively in the ultraviolet or visible regions nor fluoresce, the differential refractometer may be used. This instrument, which detects differences between the refractive index of the pure mobile phase and of the mobile phase containing the solutes, is less sensitive than the other detectors but responds to a wider range of chemicals. However, since refractive index varies significantly with temperature, drift is a problem and the detector must be thermostated. Moreover, it is not useful for gradient-elution chromatography.

Qualitative and Quantitative Analysis The signals delivered by HPLC detectors to the data-recording devices produce a chromatogram that is a plot of detector response versus time or distance, as in gas chromatography. Therefore the methods used for identifying and quantitating the various solutes in the sample are the same as those discussed under *Gas Chromatography*.

1,4-Dioxane Limit Test

Vacuum Distillation Apparatus Assemble a closed-system vacuum distillation apparatus, employing glass vacuum stopcocks (*A*, *B*, and *C*), as shown in Fig. 6. The concentrator tube (*D*) is made of borosilicate or quartz (not flint) glass, graduated precisely enough to measure the 0.9 ml or more of distillate and marked so that the analyst can dilute accurately to 2.0 ml (available as Chromaflex concentrator tube, Kontes Glass Co., Vineland, N.J., Catalog No. K42560-0000).

Standard Preparation Prepare a solution of 1,4-dioxane in water containing 100 μg per ml. Keep the solution refrigerated, and prepare fresh weekly.

Sample Preparation Transfer 20 g of the sample, accurately weighed, into a 50-ml round-bottom flask (*E*) having a 24/40 ground-glass neck. Semisolid or waxy samples should be liquefied by heating on a steam bath before making the transfer. Add 2.0 ml of water to the flask for crystalline samples, and 1.0 ml for liquid, semisolid, or waxy samples. Place a small Teflon-covered stirring bar in the flask, stopper, and stir to mix. Immerse the flask in an ice bath, and chill for about 1 min.

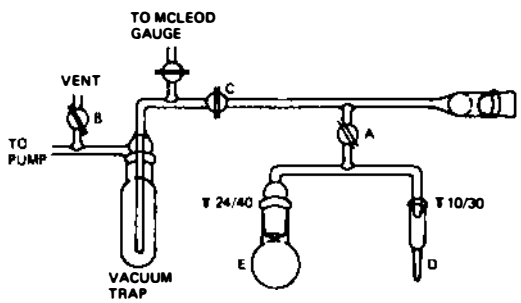


FIGURE 6 Closed-System Vacuum Distillation Apparatus for 1,4-Dioxane

Wrap heating tape around the tube connecting the Chromaflex tube (*D*) and the round-bottom flask (*E*), and apply about 10 V to the tape. Apply a light coating of high-vacuum silicone grease to the ground-glass joints, and connect the Chromaflex tube to the 10/30 joint and the round-bottom flask to the 24/40 joint. Immerse the vacuum trap in a Dewar flask filled with liquid nitrogen, close stopcocks *A* and *B*, open stopcock *C*, and begin evacuating the system with a vacuum pump. Prepare a slush bath from powdered dry ice and methanol, and raise the bath to the neck of the round-bottom flask. After freezing the contents of the flask for about 10 min, and when the vacuum system is operating at 0.05 mm pressure or lower, open stopcock *A* for 20 s, and then close it. Remove the slush bath, and allow the flask to warm in air for about 1 min. Immerse the flask in a water bath at 20° to 25°, and after about 5 min warm the water in the bath to 35° to 40° (sufficient to liquefy most samples) while stirring slowly but constantly with the magnetic bar. Cool the water in the bath by adding ice, and chill for about 2 min. Replace the water bath with the slush bath, freeze the contents of the flask for about 10 min, then open stopcock *A* for 20 s, and close it. Remove the slush bath, and repeat the heating steps as before, this time reaching a final temperature of 45° to 50° or a temperature necessary to melt the sample completely. If there is any condensation in the tube connecting the round-bottom flask to the Chromaflex tube, slowly increase the voltage to the heating tape and heat until condensation disappears.

Stir with the magnetic stirrer throughout the following steps: Very slowly immerse the Chromaflex tube in the Dewar flask containing liquid nitrogen.

> *Caution:* When there is liquid distillate in the Chromaflex tube, the tube must be immersed in the nitrogen *very slowly* or the tube will break.

Water will begin to distil into the tube. As ice forms in the tube, raise the Dewar flask to keep the liquid nitrogen level only slightly below the level of ice in the tube. When water begins to freeze in the neck of the 10/30 joint, or when liquid nitrogen reaches the 2.0-ml graduation mark on the Chromaflex tube, remove the Dewar flask and let the ice melt without heating. After the ice has melted, check the volume of water that has distilled, and repeat the sequence of chilling and thawing until at least 0.9 ml of water has been collected. Freeze the tube once again for about 2 min, and release the vacuum first by opening stopcock *B*, followed by stopcock *A*. Remove the Chromaflex tube from the apparatus, close it with a greased stopper, and let the ice melt without heating. Mix the contents of the tube by swirling, note the volume of distillate, and dilute to 2.0 ml with water, if necessary. Use this *Sample Preparation* as directed under *Chromatography*.

Chromatography Use a gas chromatograph equipped with a flame-ionization detector. Under typical conditions, the instrument contains a 4-mm (id) × 6-ft glass column packed with 80/100- or 100/120-mesh Chromosorb 104 or equivalent. The column is maintained isothermally at about 140°, the injection port at 200°, and the detector at 250°. Nitrogen is the carrier gas, flowing at a rate of about 35 ml per min. Install an oxygen scrubber between the carrier gas line and the column. The column should be conditioned for about 72 h at 250° with 30 to 40 ml per min carrier flow. (NOTE: Chromosorb 104 is oxygen-sensitive. Both new and used columns should be flushed with carrier gas for 30 to 60 min before heating each time they are installed in the gas chromatograph.)

Inject a volume of the *Standard Preparation*, accurately measured, to give about 20% of maximum recorder response. Where possible, keep the injection volume in the range of 2 to 4 µl, and use the solvent-flush technique to minimize errors associated with injection volumes. In the same manner, inject an identical volume of the *Sample Preparation*. The height of the peak produced by the *Sample Preparation* does not exceed that produced by the *Standard Preparation*.*

Distillation Range

Scope This method is to be used for determining the distillation range of pure or nearly pure compounds or mixtures having a relatively narrow distillation range of about 40°C or less. The result so determined is an indication of purity, not necessarily of identity. Products having a distillation range of greater than 40° may be determined by this method if a wide-range thermometer, such as ASTM-E-1-2C or 3C, is specified in the individual monograph.

Definitions

Distillation Range The difference between the temperature observed at the start of a distillation and that observed at which a specified volume has distilled, or at which the dry point is reached.

Initial Boiling Point The temperature indicated by the distillation thermometer at the instant the first drop of condensate leaves the end of the condenser tube.

Dry Point The temperature indicated at the instant the last drop of liquid evaporates from the lowest point in the distillation flask, disregarding any liquid on the side of the flask.

Apparatus

Distillation Flask A 200-ml round-bottomed distilling flask of heat-resistant glass is preferred when sufficient sample (in excess of 100 ml) is available for the test. If a sample of less than 100 ml must be used, a smaller flask having a capacity of at least double the volume of the liquid taken may be employed. The 200-ml flask has a total length of 17.9 cm, and the inside diameter of the neck is 2.1 cm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side arm 12.7 cm long and 5 mm in internal diameter, which is inclined downward at an angle of 75° from the vertical.

Condenser Use a straight glass condenser of heat-resistant tubing, 56 to 60 cm long and equipped with a water jacket so

*If the sample fails the test because of known or suspected interference, another aliquot may be run on a 6-ft x 2-mm (id) column of 0.2% Carbowax 1500 on Carbopak C, column operating at 100° isothermal, with 20 ml per min of helium carrier flow. Under these conditions, the 1, 4-dioxane elutes in about 4 min.

that about 40 cm of the tubing is in contact with the cooling medium. The lower end of the condenser may be bent to provide a delivery tube or it may be connected to a bent adapter that serves as the delivery tube.

NOTE: All-glass apparatus with standard-taper ground joints may be used alternatively if the assembly employed provides results equal to those obtained with the flask and condenser described above.

Receiver The receiver is a 100-ml cylinder that is graduated in 1-ml subdivisions and calibrated "to contain." It is used for measuring the sample as well as for receiving the distillate.

Thermometer An accurately standardized partial-immersion thermometer having the smallest practical subdivisions (not greater than 0.2°C) is recommended in order to avoid the necessity for an emergent stem correction. Suitable thermometers are available as the ASTM Series 37C through 41C, and 102C through 107C, or as the MCA types R-1 through R-4 (see *Thermometers*, page 547).

Source of Heat A Bunsen burner is the preferred source of heat. An electric heater may be used, however, if it is shown to give results comparable to those obtained with the gas burner.

Shield The entire burner and flask assembly should be protected from external air currents. Any efficient shield may be employed for this purpose.

Flask Support An asbestos board, 6.5 mm in thickness and having a 10-cm circular hole, is placed on a suitable ring or platform support and fitted loosely inside the shield to ensure that hot gases from the source of heat do not come in contact with the sides or neck of the flask. A second 6.5-mm asbestos board, at least 15 cm square and provided with a 30-mm circular hole, is placed on top of the first board. This board is used to hold the 200-ml distillation flask, which should be fitted firmly on the board so that direct heat is applied to the flask only through the opening in the board.

Procedure

NOTE: For materials boiling below 50°, cool the liquid to below 10° before sampling, receive the distillate in a water bath cooled to below 10°, and use water cooled to below 10° in the condenser.

Measure 100 ± 0.5 ml of the liquid in the 100-ml graduate, and transfer the sample together with an efficient antibumping device to the distilling flask. Do not use a funnel in the transfer or allow any of the sample to enter the side arm of the flask. Place the flask on the asbestos boards, which are supported on a ring or platform, and place in position the shield for the flask and burner. Connect the flask and condenser, place the graduate under the outlet of the condenser tube, and insert the thermometer. The thermometer should be located in the center of the neck end so that the top of the bulb (when present, auxiliary bulb) is just below the bottom of the outlet to the side arm. Regulate the heating so that the first drop of liquid is collected within 5 to 10 min. Read the thermometer at the instant the first drop of distillate falls from the end of the condenser tube, and record as the initial boiling point. Continue the distillation at the rate of 4 or 5 ml of distillate per min, noting the temperature as soon as the last drop of liquid evaporates from the bottom of the flask (dry point) or when the specified percentage has distilled over. Correct the observed temperature readings for any variation in the barometric pressure from the normal (760 mm) by allowing 0.1° for each 2.7 mm of variation, adding the correction if the pressure is lower, or subtracting if higher than 760 mm.

When a total-immersion thermometer is used, correct for the temperature of the emergent stem by the formula

$$0.00015 \times N(T - t),$$

in which N represents the number of degrees of emergent stem from the bottom of the stopper, T, the observed temperatures of distillation, and t, the temperature registered by an auxiliary thermometer the bulb of which is placed midway of the emergent stem, adding the correction to the observed readings of the main thermometer.

Enzyme Assays

A list of the enzymes covered by the general monograph on *Enzyme Preparation*, page 107, is shown in the accompanying table. Also incorporated in the table are the trivial names by which each is commonly known, as well as the systematic names, in accordance with the *Recommendations (1978) of the Nomenclature Committee of the International Union of Biochemistry on the Nomenclature and Classification of Enzymes*, of the major component or of the enzyme for which the preparation is standardized.

The following procedures are provided for application as necessary in determining compliance with the vendor's declared representations for enzyme activity:

ALPHA-AMYLASE ACTIVITY (NONBACTERIAL)

Application and Principle This procedure is for the determination of α-amylase activity of enzyme preparations derived from *Aspergillus niger*, var.; *Aspergillus oryzae*, var.; *Rhizopus oryzae*, var.; and barley malt. The assay is based on the time required to obtain a standard degree of hydrolysis of a starch solution at 30° ± 0.1°. The degree of hydrolysis is determined by comparing the iodine color of the hydrolysate with that of a standard.

Apparatus

Reference Color Standard Use a special Alpha-Amylase Color Disk available as Catalog No. 620-S5 from Hellige, Inc., 3718 Northern Boulevard, Long Island City, N.Y. Alternatively, a color standard may be prepared by dissolving 25.0 g of cobaltous chloride ($CoCl_2.6H_2O$) and 3.84 g of potassium dichromate in 100 ml of 0.01 N hydrochloric acid. This standard is stable indefinitely when stored in a stoppered bottle or comparator tube.

Enzyme Preparations Used in Food Processing

Trivial Name	Classification	Source	Systematic Name (IUB)[a]	IUB No.[a]
α-Amylase	carbohydrase	(1) *Aspergillus niger*, var. (2) *Aspergillus oryzae*, var. (3) *Rhizopus oryzae*, var. (4) *Bacillus subtilis*, var. (5) barley malt (6) *Bacillus licheniformis*, var.	1,4-α-D-glucan glucanohydrolase	3.2.1.1
β-Amylase	carbohydrase	barley malt	1,4-α-D-glucan maltohydrolase	3.2.1.2
Bromelain	protease	pineapples: *Ananas comosus*, *Ananas bracteatus* (L)	none	3.4.22.4
Catalase	oxidoreductase	(1) *Aspergillus niger*, var. (2) bovine liver (3) *Micrococcus lysodeikticus*	hydrogen peroxide: hydrogen peroxide oxidoreductase	1.11.1.6
Cellulase	carbohydrase	(1) *Aspergillus niger*, var. (2) *Trichoderma reesei*	1,4-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase	3.2.1.4
Ficin	protease	figs: *Ficus* sp.	none	3.4.22.3
β-Glucanase	carbohydrase	(1) *Aspergillus niger*, var. (2) *Bacillus subtilis*, var.	1,3-(1,3;1,4)-β-D-glucan 3(4)-glucanohydrolase	3.2.1.6
Glucoamylase (Amyloglucosidase)	carbohydrase	(1) *Aspergillus niger*, var. (2) *Aspergillus oryzae*, var. (3) *Rhizopus oryzae*, var.	1,4-α-D-glucan glucohydrolase	3.2.1.3
Glucose Isomerase	isomerase	(1) *Actinoplanes missouriensis* (2) *Bacillus coagulans* (3) *Streptomyces olivaceus* (4) *Streptomyces olivochromogenes* (5) *Streptomyces rubiginosus*	D-xylose ketolisomerase	5.3.1.5
Glucose Oxidase	oxidoreductase	*Aspergillus niger*, var.	β-D-glucose: oxygen oxidoreductase	1.1.3.4
Hemicellulase	carbohydrase	*Aspergillus niger*, var.	none	none
Invertase	carbohydrase	*Saccharomyces* sp. *(Kluyveromyces)*	β-D-fructofuranoside fructohydrolase	3.2.1.26
Lactase	carbohydrase	(1) *Aspergillus niger*, var. (2) *Aspergillus oryzae*, var. (3) *Saccharomyces* sp.	β-D-galactoside galactohydrolase	3.2.1.23
Lipase	lipase	(1) edible forestomach tissue of calves, kids, and lambs (2) animal pancreatic tissues (3) *Aspergillus oryzae*, var. (4) *Aspergillus niger*, var.	carboxylic-ester hydrolase triacylglycerol acylhydrolase	3.1.1.1 3.1.1.3
Papain	protease	papaya: *Carica papaya* (L)	none	3.4.22.2
Pectinase[b]	carbohydrase	(1) *Aspergillus niger*, var. (2) *Rhizopus oryzae*, var.	poly(1,4-α-D-galacturonide) glycanohydrolase pectin pectylhydrolase poly(1,4-α-D-galacturonide) lyase	3.2.1.15 3.1.1.11 4.2.2.2

Enzyme Preparations Used in Food Processing (*continued*)

Trivial Name	Classification	Source	Systematic Name (IUB)[a]	IUB No.[a]
Pepsin	protease	porcine or other animal stomachs	none	3.4.23.1
Protease (general)	protease	(1) *Aspergillus niger*, var. (2) *Aspergillus oryzae*, var. (3) *Bacillus subtilis*, var. (4) *Bacillus licheniformis*, var.	none	3.4.21.14 3.4.24.4
Rennet	protease	(1) fourth stomach of ruminant animals	none	3.4.23.4
		(2) *Endothia parasitica*		3.4.23.6
		(3) *Mucor miehei, M. pusillus*		3.4.23.6
Trypsin	protease	animal pancreas	none	3.4.21.4

[a] *Enzyme Nomenclature: Recommendations (1978) of the Nomenclature Committee of the International Union of Biochemistry*, Academic Press, New York, 1979.
[b] Usually a mixture of polygalacturonase, pectin methylesterase, and pectate lyase.

Comparator Use either the standard Hellige comparator (Catalog No. 607) or the pocket comparator (Catalog No. 605) with prism attachment (Catalog No. 605A). The comparator should be illuminated with a 100-W frosted lamp placed 6 in. from the rear opal glass of the comparator and mounted so that direct rays from the lamp do not shine into the operator's eyes.

Comparator Tubes Use the precision-bored square tubes with a 13-mm viewing depth that are supplied with the Hellige comparator. Suitable tubes are also available from other apparatus suppliers (e.g., Coleman Universal Distributors).

Reagents and Solutions

Buffer Solution (pH 4.8) Dissolve 164 g of anhydrous sodium acetate in about 500 ml of water, add 120 ml of glacial acetic acid, and adjust the pH to 4.8 with glacial acetic acid. Dilute to 1000.0 ml with water, and mix.

β-Amylase Solution Dissolve 250 mg of β-amylase, free from α-amylase, in 5 ml of water. The enzyme, which has been standardized to 2000° diastatic power, is distributed by Sturge Enzymes, Div. of Henley and Co., Inc., 750 Third Ave., New York, N.Y. 10017. (NOTE: The enzyme should be stored in a refrigerator, and it should be allowed to warm to room temperature before opening, in order to prevent condensation of moisture.)

Special Starch Use starch designated as "Starch (Lintner) Soluble," Baker Analyzed Reagent Catalog No. 4010. Before using new batches, test them in parallel with previous lots known to be satisfactory. Variations of more than ±3° diastatic power in the averages of a series of parallel tests indicate an unsuitable batch.

Buffered Substrate Solution Disperse 10.0 g (dry-weight basis) of *Special Starch* in 100 ml of cold water, and pour slowly into 300 ml of boiling water. Boil with stirring for 1 to 2 min, then cool, and add 25 ml of *Buffer Solution*, followed by all of the *β-Amylase Solution*. Quantitatively transfer the mixture into a 500-ml volumetric flask with the aid of water saturated with toluene, dilute to volume with the same solvent, and mix. Store the solution at 30° ± 2° for not less than 18 or more than 72 h before use. (This solution is also known as "buffered limit dextrin substrate.")

Stock Iodine Solution Dissolve 5.5 g of iodine and 11.0 g of potassium iodide in about 200 ml of water, dilute to 250 ml with water, and mix. Store in a dark bottle and make a fresh solution every 30 days.

Dilute Iodine Solution Dissolve 20 g of potassium iodide in 300 ml of water, and add 2.0 ml of *Stock Iodine Solution*. Quantitatively transfer into a 500-ml volumetric flask, dilute to volume with water, and mix.

Sample Preparation Prepare a solution of the sample so that 5 ml of the final dilution will give an endpoint between 10 and 30 min under the conditions of the assay.

For barley malt, finely grind 25 g of the sample in a Miag-Seck mill, available from the Schock Gusmer Division of the Pfaudler Co., 1000 West Avenue, Rochester, N.Y. 10003, or from Ludwig Baer Machinery, Inc., 270 Madison Avenue, New York, N.Y. 10016. Quantitatively transfer the powder into a 1000-ml Erlenmeyer flask, add 500 ml of a 0.5% solution of sodium chloride, and allow the infusion to stand for 2.5 h at 30° ± 0.2°, agitating the contents by gently rotating the flask at 20-min intervals. (*Caution:* The infusion must not be mixed by inverting the flask, and the quantity of the grist left adhering to the inner walls of the flask as a result of agitation must be as small as possible.) Filter the infusion through a 32-cm fluted filter of Whatman No. 1, or equivalent, paper on a 20-cm funnel, returning the first 50 ml of filtrate to the filter. Collect the filtrate until 3 h have elapsed from the time the sodium chloride solution and the sample were first mixed. Pipet 20.0 ml of the filtered infusion into a 100-ml volumetric flask, dilute to volume with the 0.5% sodium chloride solution, and mix.

Procedure Pipet 5.0 ml of *Dilute Iodine Solution* into a series of 13- × 100-mm test tubes, and place them in a water bath maintained at 30° ± 0.1°, allowing 20 tubes for each assay.

Pipet 20.0 ml of the *Buffered Substrate Solution*, previously

heated in the water bath for 20 min, into a 50-ml Erlenmeyer flask, and add 5.0 ml of 0.5% sodium chloride solution, also previously heated in the water bath for 20 min. Place the flask in the water bath.

At zero time, rapidly pipet 5.0 ml of the *Sample Preparation* into the equilibrated substrate, mix immediately by swirling, and then stopper the flask, which should be placed back in the water bath. After 10 min, transfer 1.0 ml of the reaction mixture from the 50-ml flask into one of the test tubes containing the *Dilute Iodine Solution*, shake the tube, then pour its contents into a *Comparator Tube*, and immediately compare with the *Reference Color Standard* in the *Comparator*, using a tube of water behind the color disk. (NOTE: Be certain that the pipet tip does not touch the iodine solution; carryback of iodine to the hydrolyzing mixture will interfere with enzyme action and will affect the results of the determination.) In the same manner, repeat the transfer and comparison procedure at accurately timed intervals until the α-amylase color is reached, at which time the elapsed time should be recorded. In cases where two comparisons 30 s apart show that one is darker and the other lighter than the *Reference Color Standard*, the endpoint is recorded to the nearest quarter minute. The 13-mm *Comparator Tube* should be shaken out between successive readings. Slight differences in color discrimination between operators may be minimized by the use of a prism attachment and by maintaining a 6- to 10-in. distance between the *Comparator* and the operator's eye.

Calculation One α-amylase dextrinizing unit (DU) is defined as the quantity of α-amylase that will dextrinize soluble starch in the presence of an excess of β-amylase at the rate of 1 g per h at 30°.

Calculate the α-amylase dextrinizing units in the sample as follows:

$$\text{DU (solution)} = 24/(W \times T),$$

and

$$\text{DU (dry basis)} = \text{DU (solution)} \times 100/(100 - M),$$

in which W is the weight, in g, of the enzyme sample added to the incubation mixture in the 5-ml aliquot of the *Sample Preparation* used; T is the elapsed dextrinizing time, in min; 24 is the product of the weight of the starch substrate (0.4 g) and 60 min; and M is the percent moisture in the sample, determined by suitable means.

BACTERIAL ALPHA-AMYLASE ACTIVITY (BAU)

Application and Principle This procedure is for the determination of α-amylase activity, expressed as bacterial amylase units (BAU), of enzyme preparations derived from *Bacillus subtilis*, var., and *Bacillus licheniformis*, var. It is not applicable to products that contain β-amylase. The assay is based on the time required to obtain a standard degree of hydrolysis of a starch solution at 30° ± 0.1°. The degree of hydrolysis is determined by comparing the iodine color of the hydrolysate with that of a standard.

Apparatus Use the *Reference Color Standard*, the *Comparator*, and the *Comparator Tubes* as described under *Alpha-Amylase Activity (Nonbacterial)*, page 479, but use either daylight or daylight-type fluorescent lamps as the light source for the *Comparator*. (Incandescent lamps give slightly lower results.)

Reagents and Solutions

pH 6.6 Buffer Solution A: Dissolve 9.1 g of monobasic potassium phosphate (KH_2PO_4) in sufficient water to make 1000 ml. *Solution B:* Dissolve 9.5 g of dibasic sodium phosphate (Na_2HPO_4) in sufficient water to make 1000 ml. Add 400 ml of *Solution A* to 600 ml of *Solution B*, mix, and adjust the pH to 6.6, if necessary, by the addition of *Solution A* or *Solution B* as required.

Dilute Iodine Solution Prepare as directed under *Alpha-Amylase Activity (Nonbacterial)*, page 481.

Special Starch Use the material described under *Alpha-Amylase Activity (Nonbacterial)*, page 481.

Starch Substrate Solution Disperse 10.0 g (dry-weight basis) of *Special Starch* in 100 ml of cold water, and pour slowly into 300 ml of boiling water. Boil with stirring for 1 to 2 min, and then cool with continuous stirring. Quantitatively transfer the mixture into a 500-ml volumetric flask with the aid of water, add 10 ml of *pH 6.6 Buffer*, dilute to volume with water, and mix.

Sample Preparation Prepare a solution of the sample so that 10 ml of the final dilution will give an endpoint between 15 and 35 min under the conditions of the assay.

Procedure Pipet 5.0 ml of *Dilute Iodine Solution* into a series of 13- × 100-mm test tubes, and place them in a water bath maintained at 30° ± 0.1°, allowing 20 tubes for each assay.

Pipet 20.0 ml of the *Starch Substrate Solution* into a 50-ml Erlenmeyer flask, stopper, and allow to equilibrate for 20 min in the water bath at 30°.

At zero time, rapidly pipet 10.0 ml of the *Sample Preparation* into the equilibrated mixture, and continue as directed in the *Procedure* under *Alpha-Amylase Activity (Nonbacterial)*, page 481, beginning with " . . . mix immediately by swirling, and then stopper the flask. . . . "

Calculation One bacterial amylase unit (BAU) is defined as that quantity of enzyme that will dextrinize 1 mg of starch per min under the specified test conditions.

Calculate the α-amylase activity of the sample, expressed as BAU, by the formula

$$\text{BAU/g} = 40F/T,$$

in which 40 is a factor (400/10) derived from the 400 mg of starch (20 ml of a 2% solution) and the 10-ml aliquot of *Sample Preparation* used; F is the dilution factor (total dilution volume/sample weight, in g); and T is the dextrinizing time, in min.

CATALASE ACTIVITY

Application and Principle This procedure is for the determination of catalase activity, expressed as Baker Units, of

preparations derived from *Aspergillus niger*, var.; bovine liver; or *Micrococcus lysodeikticus*. The assay is an exhaustion method based on the breakdown of hydrogen peroxide by catalase, and the simultaneous breakdown of the catalase by the peroxide, under controlled conditions.

Reagents and Solutions

0.250 N Sodium Thiosulfate Dissolve 62.5 g of sodium thiosulfate, $Na_2S_2O_3.5H_2O$, in 750 ml of recently boiled and cooled water, add 3.0 ml of 0.2 *N* sodium hydroxide as a stabilizer, dilute to 1000 ml with water, and mix. Standardize as directed for *0.1 N Sodium Thiosulfate*, page 567, and adjust to exactly 0.250 *N* if necessary.

Peroxide Substrate Solution Dissolve 25.0 g of anhydrous dibasic sodium phosphate (Na_2HPO_4), or 70.8 g of $Na_2HPO_4.12H_2O$, in about 1500 ml of water, and adjust to pH 7.0 ± 0.1 with 85% phosphoric acid. Cautiously add 100 ml of 30% hydrogen peroxide, dilute to 2000 ml in a graduate, and mix. Store in a clean amber bottle, loosely stoppered. The solution is stable for more than 1 week if kept at 5° in a full container. (With freshly prepared substrate, the blank will require about 16 ml of 0.250 *N* sodium thiosulfate. If the blank requires less than 14 ml, the substrate solution is unsuitable and should be prepared fresh again. It is essential that the sample titration is between 50% and 80% of that required for the blank.)

Procedure Pipet an aliquot of not more than 1.0 ml of the sample, previously diluted to contain approximately 3.5 Baker Units of catalase, into a 200-ml beaker. Rapidly add 100 ml of *Peroxide Substrate Solution*, previously adjusted to 25°, and stir immediately for 5 to 10 s. Cover the beaker, and incubate at 25° ± 1° until the reaction is completed. Stir vigorously for 5 s, and then pipet 4.0 ml from the beaker into a 50-ml Erlenmeyer flask. Add 5 ml of 2 *N* sulfuric acid to the flask, mix, then add 5.0 ml of 40% potassium iodide solution, freshly prepared, and 1 drop of 1% ammonium molybdate solution, and mix. While continuing to mix, titrate rapidly to a colorless endpoint with *0.250 N Sodium Thiosulfate*, recording the volume, in ml, required as *S*. Perform a blank determination with 4.0 ml of *Peroxide Substrate Solution*, and record the volume required, in ml, as *B*. (NOTE: When preparations derived from beef liver are tested, the reaction is complete within 30 min. Preparations derived from *Aspergillus* and other sources may require up to 1 h. In assaying an enzyme of unknown origin, a titration should be run after 30 min and then at 10-min intervals thereafter. The reaction is complete when two consecutive titrations are the same.)

Calculation One Baker Unit is the amount of catalase that will decompose 266 mg of hydrogen peroxide under the conditions of the assay.

Calculate the activity of the sample by the formula

$$\text{Baker Units/g or ml} = 0.4(B - S) \times (1/C),$$

in which *C* is the ml of aliquot of original enzyme preparation added to each 100 ml of *Peroxide Substrate Solution*, or, when 1 ml of diluted enzyme is used, *C* is the dilution factor.

CELLULASE ACTIVITY

Application and Principle This procedure is for the determination of cellulase enzymes derived from *Aspergillus niger*, var., and *Trichoderma reesei*. The assay is based on the time required to reduce the viscosity of a soluble cellulose from 400 centipoises to 300 centipoises at pH 5.0.

Apparatus

Viscometer Use a Brookfield Model LVF or equivalent-type viscometer, with a No. 1 Spindle, capable of rotating at 12 rpm and of being read in centipoises. A suitable viscometer is available from Brookfield Engineering Laboratories, Inc., 240 Cushing Street, Stoughton, Mass. 02072.

Sample Container Use a 250-ml beaker, or equivalent container, designed for use with the Brookfield viscometer. Berzelius beakers, available as Corning Catalog No. 1140, are suitable for this purpose.

Beater Use a wire whip hand beater, such as the Ekco Presto-Whip with a spiral cone (available at hardware stores).

Reagents and Solutions

Sodium Acetate Buffer, pH 5.0 Dissolve 34 g of sodium acetate, $NaC_2H_3O_2.3H_2O$, in about 800 ml of water, and adjust the pH to 5.0 with glacial acetic acid. Quantitatively transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard Solution Weigh accurately 1 g of a standard cellulase preparation (available as Cellase 1000 Reference Standard, from G. B. Fermentation Industries, Inc., 1 North Broadway, Des Plaines, Ill. 60016), and dissolve it in 100 ml of water. Quantitatively transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix. Each ml of this solution contains 2.6 cellulase activity (CA) units.

Substrate Solution Sift 132 g of sodium carboxymethylcellulose (cellulose gum, Hercules Type 7-LF) through a household-type tea strainer or 40-mesh screen, and add with continuous stirring to approximately 2125 ml of water. Add 375 ml of *Sodium Acetate Buffer*, and continue stirring until most of the gum has gone into solution. Allow the mixture to stand at room temperature for 2 to 3 h, stirring frequently to assure uniform and complete dispersion of the gum. (NOTE: Use only gentle mixing so as not to shear the polymer mechanically.)

Since the substrate may vary from lot to lot, each lot should be checked, by the *Procedure* below, before use in assaying the enzyme unknown. The viscosity of the *Substrate Solution* should be reduced from 400 cps to 300 cps in 277 ± 10 s by 5.0 ml of the *Standard Solution*. If the viscosity-reduction time does not fall within this range, appropriate dilutions of the *Substrate Solutions* should be made.

Sample Preparation Prepare a solution of the enzyme preparation in water so that each 5 ml of the final dilution contains between 2 and 10 cellulase activity (CA) units.

Procedure Transfer 200 g of the *Substrate Solution* into a *Sample Container*, and equilibrate for 15 min in a water bath maintained at 35° ± 0.1°. At zero time, rapidly pipet 5.0 ml of the *Sample Preparation* into the equilibrated substrate, mix immediately for 15 s with the *Beater*, and then lower the viscometer spindle as rapidly as possible into the mixture. *Do*

not remove the Sample Container from the water bath at any time during the determination. Begin stirring at 12 rpm, and start timing with a stopwatch when the reading indicates a viscosity of 400 cps. Continue timing until the viscosity is reduced to 300 cps, and record the elapsed time, T_U, in seconds. (NOTE: The elapsed time should fall between 150 and 600 s; if longer times are required, use a higher concentration of enzyme in the *Sample Preparation*.)

In the same manner, treat 200 g of the *Substrate Solution* with 5.0 ml of the *Standard Solution*, and record the elapsed time.

Calculation One cellulase activity (CA) unit is defined as that quantity of enzyme required to reduce the viscosity of 200 g of a 5% solution of the specified sodium carboxymethyl cellulose substrate from 400 to 300 cps at 35° ± 0.1° and pH 5.0, in 1 h.

Calculate the activity of the enzyme preparation taken for analysis by the formula

$$\text{CA, units/g} = 1000 \times 60 \times 60/(W \times T_U),$$

in which W is the weight, in mg, of cellulase contained in the 5-ml aliquot of the *Sample Preparation* used.

DIASTASE ACTIVITY (DIASTATIC POWER, DP)

Application and Principle This procedure is for the determination of amylase activity of barley malt and other enzyme preparations. The assay is based on a 30-min hydrolysis of a starch substrate at pH 4.6 and 20°. The reducing sugar groups produced on hydrolysis are measured in a titrimetric procedure using alkaline ferricyanide.

Apparatus

Mill Use a laboratory mill of the type Miag-Seck, for fine grinding of malt, available from the Schock Gusmer Division of the Pfaudler Co., 1000 West Avenue, Rochester, N.Y. 10003, or from Ludwig Baer Machinery, Inc., 270 Madison Avenue, New York, N.Y. 10016.

Reagents and Solutions

Acetate Buffer Solution Dissolve 68 g of sodium acetate, $NaC_2H_3O_2.3H_2O$, in 500 ml of 1 *N* acetic acid in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Special Starch Use the material described under *Alpha-Amylase Activity (Nonbacterial)*, page 481.

Starch Substrate Solution Disperse 20.0 g (dry-weight basis) of *Special Starch* in 50 ml of water, mix to a fine paste, and pour slowly into 750 ml of boiling water. Boil with stirring for 2 min, cool, add 20 ml of *Acetate Buffer Solution*, and mix. Quantitatively transfer into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Acetic Acid–Potassium Chloride–Zinc Sulfate Solution (A-P-Z) Dissolve 70 g of potassium chloride and 20 g of zinc sulfate ($ZnSO_4.7H_2O$) in 700 ml of water in a 1000-ml volumetric flask, add 200 ml of glacial acetic acid, dilute to volume with water, and mix.

Alkaline Ferricyanide Solution, 0.05 N Dissolve 16.5 g of potassium ferricyanide, $K_3Fe(CN)_6$, and 22 g of anhydrous sodium carbonate in 800 ml of water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Potassium Iodide Solution Dissolve 50 g of potassium iodide in 50 ml of water in a 100-ml volumetric flask, dilute to volume with water, and mix. Add 2 drops of 50% sodium hydroxide solution, and mix. The solution should be colorless.

Sample Preparation

Malt Samples Grind 30 g of the sample to a fine powder in a Maig-Seck mill. Accurately weigh 25 g of the powder, and transfer it into a 1000-ml Erlenmeyer flask. Add 500 ml of a 0.5% sodium chloride solution, and allow the infusion to stand for 2.5 h at 20° ± 0.2°, agitating the contents by gently rotating the flask at 20-min intervals. (NOTE: The infusion must not be mixed by inverting the flask, and the quantity of grist left adhering to the inner walls of the flask as a result of agitation must be as small as possible. Gentle swirling of the contents of the flask without splashing against the walls will produce sufficient mixing.) Filter the infusion through a 32-cm fluted filter of Whatman No. 1, or equivalent, paper on a 20-cm funnel, returning the first 50 ml of filtrate to the filter. Place a watch glass over the funnel, and use a suitable cover around the stem and over the receiver to reduce evaporation losses during filtration. Collect the filtrate until 30 min of filtration time have elapsed. Pipet 20.0 ml of the filtrate into a 100-ml volumetric flask, dilute to volume with 0.5% sodium chloride solution, and mix.

Other Enzyme Preparations Prepare a solution so that 10 ml of the final dilution will give a diastatic power (DP) value between 2° and 150°.

Procedure Pipet 10.0 ml of the *Sample Preparation* into a 250-ml volumetric flask, and at zero time add 200 ml of *Starch Substrate Solution*, previously equilibrated for 30 min in a water bath maintained at 20° ± 0.2°. Start the stopwatch at zero time.

Place the mixture in the water bath at 20°, and allow it to cool for exactly 30 min, then add 20.0 ml of 0.5 *N* sodium hydroxide, dilute to volume with water, and mix.

Prepare a blank by adding 20.0 ml of 0.5 *N* sodium hydroxide to a 250-ml volumetric flask, followed by 10.0 ml of the *Sample Preparation*. Swirl to mix, add 200 ml of *Starch Substrate Solution*, dilute to volume with water, and mix.

Pipet 5.0 ml of the sample digestion mixture into a 125-ml Erlenmeyer flask, add 10.0 ml of *Alkaline Ferricyanide Solution*, and swirl to mix. Heat the flask for exactly 20 min in a boiling water bath, and then cool to room temperature. Add 25 ml of *A-P-Z Solution*, followed by 1 ml of *Potassium Iodide Solution*, and swirl to mix. Titrate with 0.05 *N* sodium thiosulfate to the complete disappearance of the blue color, recording the volume, in ml, of 0.05 *N* sodium thiosulfate required as *S*.

Treat the blank solution in the same manner as described for the sample, recording the volume, in ml, of 0.05 *N* sodium thiosulfate required as *B*.

Calculation One unit of diastase activity, expressed as degrees diastatic power (DP°), is defined as that amount of enzyme, contained in 0.1 ml of a 5% solution of the sample enzyme preparation, that will produce sufficient reducing sugars to reduce 5 ml of Fehling's solution when the sample is incubated

with 100 ml of the substrate for 1 h at 20° (NOTE: The definition of the unit does not correspond to the method of the determination.)

Calculate the diastase activity, expressed as DP°, of the sample by the formulas

$$\text{DP°, as is basis} = (B - S) \times 23,$$

and

$$\text{DP°, dry basis} = \text{DP°, as is basis} \times 100/(100 - M),$$

in which 23 is a factor, determined by collaborative study, required to convert to the units of the definition, and M is the percent moisture of the sample, determined by suitable means.

β-GLUCANASE ACTIVITY

Application and Principle This procedure is for the determination of β-glucanase activity of enzyme preparations derived from *Aspergillus niger*, var., and *Bacillus subtilis*, var. The assay is based on a 15-min hydrolysis of lichenin substrate at 40° and at pH 6.5. The increase in reducing power due to liberated reducing groups is measured by the neocuproine method.

Reagents and Solutions

Phosphate Buffer Dissolve 13.6 g of monobasic potassium phosphate in about 1900 ml of water, add 70% sodium hydroxide solution until the pH is 6.5 ± 0.05, then transfer the solution into a 2000-ml volumetric flask, dilute to volume with water, and mix.

Neocuproine Solution A Dissolve 40.0 g of anhydrous sodium carbonate, 16.0 g of glycine, and 450 mg of cupric sulfate pentahydrate in about 600 ml of water. Transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Neocuproine Solution B Dissolve 600 mg of neocuproine hydrochloride in about 400 ml of water, transfer the solution into a 500-ml volumetric flask, dilute to volume with water, and mix. Discard when a yellow color develops.

Lichenin Substrate Grind 150 mg of lichenin to a fine powder in a mortar, and dissolve it in about 50 ml of water at about 85°. After solution is complete (20 to 30 min), add 90 mg of sodium borohydride and continue heating below the boiling point for 1 h. Add 15 g of Amberlite MB-3, or equivalent ion-exchange resin, and stir continuously for 30 min. Filter with the aid of vacuum through Whatman No. 1 filter paper, or equivalent, in a Buchner funnel, and wash the paper with about 20 ml of water. Add 680 mg of monobasic potassium phosphate to the filtrate, and refilter through a 0.22-μm Millipore filter pad, or equivalent. Wash the pad with 10 ml of water, and adjust the pH of the filtrate to 6.5 ± 0.05 with 1 *N* sodium hydroxide or 1 *N* hydrochloric acid. Transfer the filtrate into a 100-ml volumetric flask, dilute to volume with water, and mix. Store at 2° to 4° for not more than 3 days.

Glucose Standard Solution Dissolve 36.0 mg of anhydrous dextrose in *Phosphate Buffer* in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Test Preparation Prepare a solution from the enzyme preparation sample so that 1 ml of the final dilution will contain between 0.01 and 0.02 β-glucanase units. Weigh the sample, transfer it into a volumetric flask of appropriate size, dilute to volume with *Phosphate Buffer*, and mix.

Procedure Pipet 2 ml of *Lichenin Substrate* into each of four separate test tubes graduated at 25 ml, and heat the tubes in a water bath at 40° for 10 to 15 min to equilibrate. After equilibration, add 1 ml of *Phosphate Buffer* to tube 1, 1 ml of *Glucose Standard Solution* to tube 2 (glucose standard), 4 ml of *Neocuproine Solution A* and 1 ml of the *Test Preparation* to tube 3 (enzyme blank), and 1 ml of the *Test Preparation* to tube 4 (sample). Prepare a fifth tube for the buffer blank, to which 3 ml of *Phosphate Buffer* is added.

Incubate the five tubes at 40° for exactly 15 min, and then add 4 ml of *Neocuproine Solution A* to tubes 1, 2, 4, and 5. Add 4 ml of *Neocuproine Solution B* to all five tubes, and cap them with a suitably sized glass marble. (*Caution:* Do not use rubber stoppers.) Heat the tubes in a vigorously boiling water bath for exactly 12 min to develop color, then cool to room temperature in cold water, and adjust the volume of each to 25 ml with water. Cap the tubes with Parafilm, or other suitable closure, and mix by inverting several times. Determine the absorbance of each solution at 450 nm in 1-cm cells, with a suitable spectrophotometer, against the buffer blank in tube 5.

Calculation One β-glucanase unit (BGU) is defined as that quantity of enzyme that will liberate 1 μmol of reducing sugar (as glucose equivalence) per min under the conditions of the assay.

Calculate the activity of the enzyme preparation taken for analysis as follows:

$$\text{BGU} = \frac{(A_4 - A_3) \times 36 \times 10^6}{(A_2 - A_1) \times 180 \times 15 \times \mu\text{g Sample}},$$

in which A_4 is the absorbance of the sample (tube 4), A_3 is the absorbance of the enzyme blank (tube 3), A_2 is the absorbance of the glucose standard (tube 2), A_1 is the absorbance of the substrate blank (tube 1), 36 is the μg of glucose in the *Glucose Standard Solution*, 10^6 is the factor converting μg to g, 180 is the weight of 1 μmol of glucose, and 15 is the reaction time.

GLUCOAMYLASE ACTIVITY (AMYLOGLUCOSIDASE ACTIVITY)

Application and Principle This procedure is designed for the determination of the glucoamylase activity of preparations derived from *Aspergillus niger*, var., but it may be modified for the determination of preparations derived from *Aspergillus oryzae*, var., and *Rhizopus oryzae*, var. (as indicated by the variations in the text below). The sample is allowed to convert a corn starch hydrolyzate solution, under carefully controlled conditions of time, temperature, pH, and concentration. The resulting reducing sugars are determined, and the activity is calculated as the weight, in g, of reducing sugars produced by a unit quantity of sample in 1 h under the specified conditions.

Reagents and Solutions

Starch Hydrolyzate Solution (4%) Weigh accurately an amount of 15 to 20 dextrose equivalent (DE) corn syrup solids

corresponding to 40.0 g of the dry substance. (If necessary, an equivalent amount of neutralized and filtered corn starch hydrolyzate having a DE of 15 to 20 may be substituted for the corn syrup solids. Suitable dried commercial products in the DE range may be obtained from A.E. Staley Manufacturing Co., Decatur, Ill. 62525; CPC International, Argo, Ill. 60501; and Grain Processing Corp., Muscatine, Iowa 52761.) Transfer quantitatively into a 1000-ml volumetric flask, dilute to volume with water, and mix thoroughly. Prepare this solution fresh daily.

Acetate Buffer Transfer 60 g of glacial acetic acid into a 1000-ml volumetric flask, dilute to volume with water, and mix. With the aid of a suitable pH meter, adjust the pH of this solution to 4.2 by the addition of a sodium acetate solution prepared by dissolving 136 g of $NaC_2H_3O_2.3H_2O$ in sufficient water to make 1000 ml. (NOTE: A pH of 5.0 should be used when testing preparations derived from *Aspergillus oryzae* or *Rhizopus oryzae*.)

Fehling's Solution A Prepare the *Copper Solution (A)* as directed under *Cupric Tartrate TS, Alkaline*, page 560. [This solution is quite commonly carefully standardized for use in determination of dextrose equivalent in the Lane and Eynon method. However, the concentration of the solution is not highly critical in the Schoorl method (specified later under *Determination of Reducing Sugars*), and adjustment of its concentration is usually unnecessary. The theoretical blank titer is 27.8 ml, but the solution may be considered to be satisfactory if the titer is between 27.5 and 29.5 ml.]

Fehling's Solution B Prepare the *Alkaline Tartrate Solution (B)* as directed under *Cupric Tartrate TS, Alkaline*, page 560.

Sample Preparation The *Procedure* below is based on the use of a sample containing 0.1 to 0.2 unit of glucoamylase activity. This sample size will produce 0.2 to 0.4 g of reducing sugars under the conditions specified, and maximum accuracy is obtained in this range. For slightly less accurate results, an enzyme dosage range of 0.05 to 3.0 units may be used if necessary.

Liquid samples, solid samples, and liquid concentrates should be prepared as directed in the following tables, and the aliquot size indicated should be used in the *Procedure* (*Production of Reducing Sugars*).

Liquid Samples

Enzyme in Sample (units/ml)	Dilute (ml)	Aliquot Size (ml)	Dilution Factor (*F*)
0.05 or less	—	5.0	0.2
0.06–0.1	—	2.0	0.5
0.11–0.25	—	0.80	1.25
0.3–0.5	—	0.40	2.5
0.6–1.0	—	0.20	5
1.1–2.0	—	0.10	10
2.1–4.0	5.0→100	1.00	20
4.1–5.0	4.0→100	1.00	25
5.1–7.0	3.0→100	1.00	33.3
7.1–10.0	2.0→100	1.00	50

Solid Samples and Liquid Concentrates

Enzyme in Sample (units/g)	Sample Weight[a] (g)	Dilute to (ml)	Aliquot Size (ml)
4 or less	10	1000	5.0
5–10	4	1000	5.0
11–25	1.6	1000	5.0
26–50	1.4	1000	3.0
51–75	1.25	1000	2.0
76–100	1.00	1000	2.0
101–150	1.25	1000	1.0
151–200	1.00	1000	1.0
201–250	1.50	2000	1.0
251–300	1.00	2000	1.0

[a] Accurately weigh the sample into a volumetric flask, fill the flask two-thirds full of water, and allow the stoppered flask to stand at room temperature for at least 30 min, shaking vigorously at least 5 times during that period. Dilute to volume with water, and mix well. Take the indicated aliquot from a portion of the sample solution that has been filtered through Whatman No. 12 or equivalent filter paper.

Procedure

Production of Reducing Sugars Pipet 50.0 ml of the *Starch Hydrolyzate Solution* and 5.0 ml of *Acetate Buffer* into a 100-ml volumetric flask. Prepare a second flask in the same manner for use as the control, and carry this flask through the same procedure concurrently, but use water in place of the *Sample Preparation*. Place the flask in a water bath maintained at 60°, and allow to stand for at least 10 min. (NOTE: Use 55° when testing preparations derived from *Aspergillus oryzae* and *Rhizopus oryzae*.) Pipet an appropriately sized aliquot of the *Sample Preparation* into the flask, and simultaneously begin timing the reaction. (NOTE: If a series of samples is being analyzed, pipet aliquots at timed intervals, so spaced as to permit neutralization of each after 120 min of reaction time.) Swirl the contents of the flask to mix thoroughly, and allow to stand in the water bath for 120 min. When 115 to 118 min of the reaction period has passed, add 3 drops of phenolphthalein TS, then when exactly 120 min has elapsed, remove the flask from the bath, and immediately neutralize the contents by the addition of 2% sodium hydroxide solution, preferably added with a fast-flowing buret (about 3 to 7 ml is usually required). Cool to room temperature in a running-water bath, dilute to volume with water, and mix thoroughly. Determine the reducing sugars content on a 10.0-ml aliquot of this solution, and on a 10.0-ml aliquot of the control, as directed below.

Determination of Reducing Sugars (Schoorl Method) (NOTE: This method is suitable for determining reducing sugars in soluble materials that are substantially free of protein. Samples containing significant amounts of protein can be analyzed, however, after treatment with a protein precipitant.) Pipet 10.0 ml each of *Fehling's Solution A* and *B* into a 250-ml Erlenmeyer flask, and then add 10.0 ml of the sample solution obtained under *Production of Reducing Sugars* above. Prepare a second flask in the same manner for use as the control, using 10.0 ml of the control solution, instead of the sample solution, obtained under *Production of Reducing Sugars*, and carry this

flask concurrently through the same procedure described for the sample. (NOTE: If large numbers of samples are to be analyzed, the sample solutions may be pipetted into a series of flasks first. Each sample may be diluted to 30 ml with water, and the *Fehling's Solution A* may be added at any time; however, *Fehling's Solution B* must not be added until just before heating begins, since the reaction is initiated at room temperature as soon as the solution is added.) Pipet water into the flask to make a total volume of 50 ml, and mix the contents of the flask by gentle swirling. Add two small glass beads, and close the mouth of the flask with a small funnel or glass bulb. Heat the solution, preferably with a hot plate, at such a rate that the solution is brought to boiling in just 3 min, and then continue boiling for exactly 2 min (total heating time, 5 min). Cool quickly to room temperature in an ice bath or in cold running water, and then rinse down the funnel (or bulb) and the walls of the flask with a few ml of water. Add 10 ml each of 30% potassium iodide solution and of 28% sulfuric acid, and titrate rapidly with 0.100 *N* sodium thiosulfate until the iodine color almost disappears. Add 1 ml of starch TS, and titrate dropwise, with continuous agitation, to the disappearance of the blue color. Record the volume, in ml, of 0.100 *N* sodium thiosulfate required for the sample solution as *S*, and that required for the control solution as *C*. Conduct two reagent blank determinations, substituting 30 ml of water for the sample, and record the average volume, in ml, of the blanks as *B*. Obtain the *Titer Difference*, expressed as ml of 0.100 *N* sodium thiosulfate, for the sample by subtracting *S* from *B*, recording the value thus obtained as T_S. Subtract *C* from *B* to obtain the *Titer Difference* for the control, and record this value as T_C. (See footnote to the table that follows.)

Conversion of Titer Difference to Reducing Sugars Content[a]

Titer Difference (ml)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Reducing Sugar (as Dextrose) (mg)									
0.0	0.0	0.3	0.7	1.0	1.3	1.6	1.9	2.2	2.5	2.8
1.0	3.2	3.5	3.8	4.1	4.4	4.7	5.0	5.3	5.6	5.9
2.0	6.4	6.6	6.9	7.2	7.5	7.8	8.1	8.5	8.8	9.1
3.0	9.4	9.8	10.1	10.4	10.7	11.0	11.4	11.7	12.0	12.3
4.0	12.6	13.0	13.3	13.6	14.0	14.3	14.6	15.0	15.3	15.6
5.0	15.9	16.3	16.6	16.9	17.2	17.6	17.9	18.2	18.5	18.9
6.0	19.2	19.5	19.8	20.1	20.5	20.8	21.1	21.4	21.8	22.1
7.0	22.4	22.7	23.0	23.3	23.7	24.0	24.3	24.6	24.9	25.2
8.0	25.6	25.9	26.2	26.6	26.9	27.3	27.6	28.0	28.3	28.6
9.0	28.9	29.3	29.6	30.0	30.3	30.6	31.0	31.3	31.6	31.9
10.0	32.3	32.7	33.0	33.3	33.7	34.0	34.3	34.6	35.0	35.3
11.0	35.7	36.0	36.3	36.7	37.0	37.3	37.6	38.0	38.3	38.7
12.0	39.0	39.3	39.6	40.0	40.3	40.6	41.0	41.3	41.7	42.0
13.0	42.4	42.8	43.1	43.4	43.7	44.1	44.4	44.8	45.2	45.5
14.0	45.8	46.2	46.5	46.9	47.2	47.6	47.9	48.3	48.6	48.9
15.0	49.3	49.6	49.9	50.3	50.7	51.1	51.4	51.7	52.1	52.4
16.0	52.8	53.2	53.5	53.9	54.2	54.5	54.9	55.3	55.6	56.0
17.0	56.3	56.7	57.0	57.3	57.7	58.1	58.4	58.8	59.1	59.5

Conversion of Titer Difference to Reducing Sugars Content[a] *(continued)*

Titer Difference (ml)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Reducing Sugar (as Dextrose) (mg)									
18.0	59.8	60.1	60.5	60.9	61.2	61.5	61.9	62.3	62.6	63.0
19.0	63.3	63.6	64.0	64.3	64.7	65.0	65.4	65.8	66.1	66.5
20.0	66.9	67.2	67.6	68.0	68.4	68.8	69.1	69.5	69.9	70.3
21.0	70.7	71.1	71.5	71.9	72.2	72.6	73.0	73.4	73.7	74.1
22.0	74.5	74.9	75.3	75.7	76.1	76.5	76.9	77.3	77.7	78.1
23.0	78.5	78.9	79.3	79.7	80.1	80.5	80.9	81.3	81.7	82.1
24.0	82.6	83.0	83.4	83.8	84.2	84.6	85.0	85.4	85.8	86.2
25.0	86.6	87.0	87.4	87.8	88.2	88.6	89.0	89.4	89.8	90.2
26.0	90.7	91.1	91.5	91.9	92.3	92.7	93.1	93.5	93.9	94.3
27.0	94.8									

[a] Use of this table presumes the ability of the analyst to duplicate exactly the conditions under which the data were developed. The risk of error can be avoided by careful duplicate standardization with known quantities of pure dextrose (5 samples, ranging from 10 to 70 mg). A plot of *Titer Difference* vs. mg of dextrose is slightly curvilinear, passing through the origin. If use of a standardization curve is adopted, the thiosulfate solution need not be standardized. Some additional increase in accuracy results from use of a 0.065 *N* sodium thiosulfate solution, which increases the blank titer to about 44–45 ml.

Calculation

Reducing Sugars Content By reference to the accompanying table, entitled *Conversion of Titer Difference to Reducing Sugars Content*, determine the weight, in mg, of reducing sugars equivalent to the volume T_S, and record the value thus obtained as W_S. In a similar manner, determine the weight of reducing sugars equivalent to the volume T_C, and record this value as W_C.

Calculate the total reducing sugars (as dextrose) produced by the aliquot of *Sample Preparation* taken by the formula

$$D_S,\ \text{g} = W_S \times 100/(1000 \times 10),$$

Calculate the total reducing sugars (as dextrose) produced by the control by the formula

$$D_C,\ \text{g} = W_C \times 100/(1000 \times 10).$$

Enzyme Activity, Liquid Samples Calculate the glucoamylase activity of the liquid enzyme preparation taken for analysis by the formula

$$\text{Glucoamylase, units/ml} = (D_S - D_C) \times (F/2\ \text{h}),$$

in which *F* is the dilution factor appropriate for the enzyme preparation analyzed (see table on *Liquid Samples* under *Sample Preparation*), or *F* is a factor appropriate to any adaptations used.

Enzyme Activity, Solid Samples, and Liquid Concentrates Calculate the glucoamylase activity of solid samples or liquid concentrates taken for analysis by the formula

$$\text{Glucoamylase, units/g} = \frac{(D_S - D_C) \times V}{(G \times A \times 2\text{ h})},$$

in which V is the dilution volume, in ml, and A is the aliquot size, in ml, appropriate for the enzyme preparation analyzed (see table on *Solid Samples and Liquid Concentrates* under *Sample Preparation*), and G is the weight, in g, of the enzyme preparation taken for analysis.

GLUCOSE ISOMERASE ACTIVITY

NOTE: Glucose isomerase activity of the commercial enzyme is usually determined on the enzyme that has been immobilized by binding with a polymer matrix or other suitable material. The following method is designed for use with such preparations.

Application and Principle This procedure is for the determination of glucose isomerase preparations derived from *Actinoplanes missouriensis*, *Bacillus coagulans*, *Streptomyces olivaceus*, *Streptomyces olivochromogenes*, and *Streptomyces rubiginosus*. It is based on measurement of the rate of conversion of glucose to fructose in a packed bed reactor. The procedure as outlined approximates an initial velocity assay method. Specific conditions are: glucose concentration, 45% w/w; pH (inlet), measured at room temperature in the 7.0 to 8.5 range, as specified; temperature, 60.0°; and magnesium concentration, 4×10^{-3} *M*. The optimum conditions for enzymes from different microbial sources and methods of preparation may vary; therefore, if different pH conditions, buffering systems, or methods of sample preparation are recommended by the manufacturer, such variations in the instructions given herein should be used.

Reagents and Solutions

Glucose Substrate Dissolve 539 g of anhydrous glucose and 1.0 g of magnesium sulfate, $MgSO_4.7H_2O$, in 700 ml of water or the manufacturer's recommended buffer, previously heated to 50° to 60°. Cool the solution to room temperature, and adjust the pH as specified by the enzyme manufacturer. Transfer the solution to a 1000-ml volumetric flask, dilute to volume with water or the specified buffer, and mix. Transfer to a vacuum flask, and deaerate for 30 min.

Magnesium Sulfate Solution Dissolve 1.0 g of magnesium sulfate, $MgSO_4.7H_2O$, in 700 ml of water. Adjust the pH to 7.5 to 8.0 as specified by the manufacturer, using 1 *N* sodium hydroxide, dilute to 1000 ml with water, and mix.

Column Assembly and Apparatus

NOTE: Make all connections with inert tubing, glass or plastic as appropriate.

The column assembly is shown in Fig. 7. Use a 2.5- × 40-cm glass column provided with a coarse sintered-glass bottom and a water jacket connected to a constant-temperature water bath, maintained at 60.0°, by means of a circulating pump. Connect the top of the column to a variable-speed peristaltic pump having a maximum flow rate of 800 ml per h. The diameter of the tubing with which the peristaltic pump is fitted should permit variation of the pumping volume from 60 to 150 ml per h. Connect the outlet of the column with a collecting vessel.

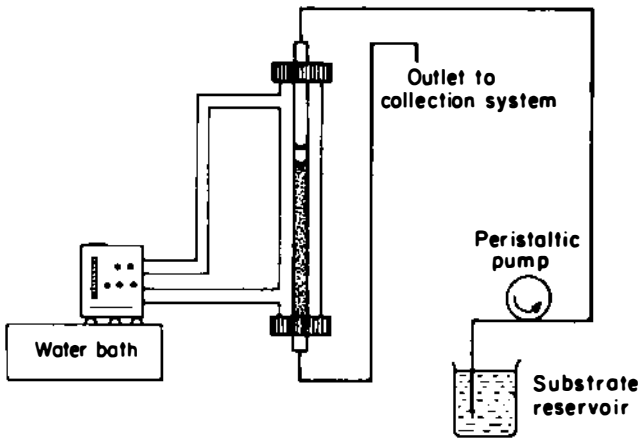


FIGURE 7 Column Assembly for Assay of Immobilized Glucose Isomerase

Sample Preparation Transfer to a 500-ml vacuum flask an amount of the sample, accurately weighed in g or measured in ml, as appropriate, sufficient to obtain 2000 to 8000 glucose isomerase units (GI_cU). Add 200 ml of *Glucose Substrate*, stir gently for 15 s, and repeat the stirring every 5 min for 40 min. Deaerate by vacuum for 30 min.

Column Preparation Quantitatively transfer the *Sample Preparation* to the column with the aid of *Magnesium Sulfate Solution* as necessary. Allow the enzyme granules to settle, and then place a porous disk so that it is even with, and in contact with, the top of the enzyme bed. All of the air should be displaced from the disk. Place a cotton plug about 1 or 2 cm above the disk. (This plug acts as a filter. It ensures proper heating of the solution and traps dissolved gases that may be present in the *Glucose Substrate*.) Connect the tubing from the peristaltic pump with the top of the column, and seal the connection by suitable means in order to protect the column contents from the atmosphere. Place the inlet tube of the peristaltic pump into the *Glucose Substrate* solution, and begin a downward flow of the *Glucose Substrate* into the column at a rate of at least 80 ml per h. Maintain the flow rate for 1 h at room temperature.

Assay Adjust the flow of the *Glucose Substrate* to such a rate that a fractional conversion of 0.2 to 0.3 will be produced, based on the estimated activity of the sample. The fractional conversion is calculated from optical rotation values obtained on the starting *Glucose Substrate* and the sample effluent, as specified under *Calculations* below. After the correct flow rate has been established, run the column overnight (16 h minimum), then check the pH of the *Glucose Substrate*, and readjust if necessary to the specified pH. Measure the flow rate, and collect a sample of the column effluent. Cover the effluent sample, allow it to stand for 30 min at room temperature, and then determine the fractional conversion of glucose to fructose (see *Calculations*

below). If the conversion is less than 0.2 or more than 0.3, adjust the flow rate to bring the conversion into this range. If a flow rate adjustment is required, collect an additional effluent sample after allowing the column to re-equilibrate for at least 2 h, and then determine the fractional conversion. Measure the flow rate, and collect an effluent sample. Cover the sample, let it stand at room temperature for 30 min, and determine the fractional conversion.

Calculations

Specific Rotation Measure the optical rotation of the effluent sample and of the starting *Glucose Substrate* at 25.0°, and calculate their specific rotations (see *Optical Rotation*, page 530) by the formula

$$[\alpha] = 100a/lpd,$$

in which a is the corrected observed rotation, in degrees; l is the length of the polarimeter tube, in dm; p is the concentration of the test solution, expressed as g of solute per 100 g of solution; and d is the specific gravity of the solution at 25°.

Fractional Conversion Calculate the fractional conversion, X, by the formula

$$X = (\alpha_E - \alpha_S)/(\alpha_F - \alpha_S),$$

in which α_E is the specific rotation of the column effluent, α_S is the specific rotation of the *Glucose Substrate*, and α_F is the specific rotation of fructose (which, in this case, has been calculated to be −94.54).

Activity The enzyme activity is expressed in glucose isomerase units (GI_cU, the subscript c signifying column process). One GI_cU is defined as the amount of enzyme that converts glucose to fructose at an initial rate of 1 μmol per min, under the conditions specified.

Calculate the glucose isomerase activity by the formula

$$GI_cU/g \text{ or } ml = (FS/W) \times X_E \times \ln[X_E/(X_E - X)],$$

in which F is the flow rate, in ml per min; S is the concentration of the *Glucose Substrate*, in μmol per ml; X is the fractional conversion, as determined above; X_E is the fractional conversion at equilibrium, or 0.51; and W is the weight or volume of the sample taken, in g or ml, respectively.

GLUCOSE OXIDASE ACTIVITY

Application and Principle This procedure is for the determination of glucose oxidase activity of preparations derived from *Aspergillus niger*, var. The assay is based upon oxygen uptake in the presence of excess substrate, excess air, and excess catalase.

Apparatus

Warburg Apparatus Use the apparatus, or its equivalent, supplied as Catalog No. 666900, Precision Scientific Co., 3737 W. Cortland Street, Chicago, Ill. 60647.

Manometer Use the manometer, or its equivalent, supplied as Catalog No. 66665, Precision Scientific Co.

Reaction Flasks Use the 15-ml flasks, or their equivalent, supplied as Catalog No. 66703, Precision Scientific Co.

Buffered Dextrose Substrate Dissolve 14.2 g of anhydrous dibasic sodium phosphate in about 750 ml of water. Dissove 4.0 g of sodium dehydroacetate (Ganes Chemical Works, or equivalent) in this solution, and adjust the pH to 5.9 ± 0.05 with 85% phosphoric acid. Finally, dissolve 33.0 g of dextrose monohydrate in the solution, dilute to 1000.0 ml with water, and mix. To ensure mutarotation to equilibrium, hold overnight at room temperature.

Sample Solution Weigh accurately a suitable amount of the enzyme preparation, and dilute it with water to a known volume to obtain a solution containing between 10 and 20 glucose oxidase units per ml. (NOTE: If the enzyme preparation contains less than one Baker Unit of catalase [see D. Scott and F. Hammer, *Enzymologia, 22*, 194 (1960)], catalase must be added to meet or exceed the minimum ratio.) Transfer a 1.0-ml aliquot into a 100-ml volumetric flask, dilute rapidly to volume with the *Buffered Dextrose Substrate* (previously adjusted to a temperature of 25°), and mix. This solution may be unstable and should be used as soon as possible.

Procedure Pipet 2.0-ml portions of the *Sample Solution* into four calibrated *Reaction Flasks*, taking care that none of the solution is pipetted into the wells of the flasks. Using rubber bands or springs, secure each flask to a calibrated *Manometer*, and place the assembly in the water bath, maintained at 30° ± 0.01°, of the *Warburg Apparatus*. Open the manometer stopcocks leading to the flasks, and allow the manometers to oscillate, using a mechanical shaker, at a rate of 120 times per min, with a stroke of 4 cm, for 10 min in order to equilibrate the temperature of the flasks. After the temperature equilibration has been reached, adjust the manometers to the initial volume for the respective reaction flasks. Close the stopcocks, and shake again for 30 min. Readjust the manometers to their original volumes, and note the change in pressure (P), in mm, for each flask.

Calculation One glucose oxidase unit (GOU) is defined as that quantity of enzyme that will cause the uptake of 10 mm^3 of oxygen per min in a Warburg manometer at 30° in the presence of excess air and excess catalase, and with a substrate containing 3.3% glucose monohydrate and 0.1 M phosphate buffer at pH 5.9, with 0.4% sodium dehydroacetate [see D. Scott, *J. Agric. Food Chem., 1*, 727 (1953)]. Calculate the activity of the enzyme preparation by the formula

$$GOU/g \text{ or } ml = P \times C \times D/(30 \text{ min} \times 10 \text{ mm}^3 \times V),$$

in which P is the pressure drop, in mm, observed in the reaction flask, corrected for thermobarometer change;* C is the reaction flask constant;† D is the dilution factor of the enzyme solution; and V is the volume, in ml, of *Sample Solution* used in the

*W.W. Umbreit, R.H. Burris, and J.F. Stauffer, *Manometric Techniques*, Third Edition, Burgess Publishing Co., Minneapolis, Minn., 1957, pp. 6–7.

†Umbreit *et al.*, *Ibid.*, pp. 61–63.

Procedure. Average the four values thus calculated to obtain the activity of the enzyme preparation taken for analysis.

HEMICELLULASE ACTIVITY

Application and Principle This procedure is for the determination of hemicellulase activity of preparations derived from *Aspergillus niger*, var. The test is based on the enzymatic hydrolysis of the interior glucosidic bonds of a defined locust (carob) bean gum substrate at pH 4.5 and 40°. The corresponding reduction in substrate viscosity is determined with a calibrated viscometer.

Apparatus

Viscometer Use a size 100 calibrated Cannon-Fenske Type Viscometer, or its equivalent. A suitable viscometer is supplied as Catalog No. 2885-100 by Scientific Products, 1210 Waukegan Road, McGaw Park, Ill. 60085.

Glass Water Bath Use a constant-temperature glass water bath, maintained at 40° ± 0.1°. A suitable bath is supplied as Catalog No. W3520-10 by Scientific Products.

Reagents and Solutions

Acetate Buffer (pH 4.5) Add 0.2 *N* sodium acetate, with continuous agitation, to 400 ml of 0.2 *N* acetic acid until the pH is 4.5 ± 0.05, as determined by a pH meter.

Locust Bean Gum Use Powdered Type D-200 locust bean gum, or its equivalent, supplied by Meer Corp., 9500 Railroad Avenue, North Bergen, N.J. 07047. Since the substrate may vary from lot to lot, each lot should be tested in parallel with a previous lot known to be satisfactory. Variations of more than ± 5% viscosity in the average of a series of parallel tests indicate an unsuitable lot.

Substrate Solution Place 12.5 ml of 0.2 *N* hydrochloric acid and 250 ml of warm water (72° to 75°) in the bowl of a power blender (Waring two-speed, or its equivalent, supplied as Catalog No. 58350-1 by Scientific Products), and set the blender on low speed. Slowly disperse 2.0 g of *Locust Bean Gum*, on a moisture-free basis, into the bowl, taking care not to splash out any of the liquid in the bowl. Wash down the sides of the bowl with warm water, using a rubber policeman, cover the bowl, and blend at high speed for 5 min. Quantitatively transfer the mixture to a 1000-ml beaker, and cool to room temperature. Using a pH meter, adjust the mixture to pH 6.0 with 0.2 *N* sodium hydroxide. Quantitatively transfer to a 1000-ml volumetric flask, dilute to volume with water, and mix. Filter the substrate through gauze before use.

Sample Preparation Prepare a solution of the sample in water so that 1 ml of the final dilution will produce a change in relative fluidity between 0.18 and 0.22 in 5 min under the conditions specified in the *Procedure*. Weigh the enzyme preparation, quantitatively transfer it to a glass mortar, and triturate with water. Quantitatively transfer the mixture to an appropriately sized volumetric flask, dilute to volume with water, and mix. Filter through Whatman No. 1 filter paper, or equivalent, before use.

Procedure Scrupulously clean the *Viscometer* by drawing a large volume of detergent solution, followed by water, through the instrument, and place the viscometer, previously calibrated, in the *Glass Water Bath* in an exactly vertical position. Pipet 20.0 ml of *Substrate Solution* and 4.0 ml of *Acetate Buffer* into a 50-ml Erlenmeyer flask, allowing at least two flasks for each enzyme sample and one flask for a substrate blank. Stopper the flasks, and equilibrate them in the water bath for 15 min. At zero time, pipet 1.0 ml of the *Sample Preparation* into the equilibrated substrate, start timing with a stopwatch (No. 1), and mix thoroughly. Immediately pipet 10.0 ml of this mixture into the wide arm of the *Viscometer*. After about 2 min, draw the reaction mixture above the upper mark into the driving fluid head by applying suction with a rubber tube connected to the narrow arm of the instrument. Measure the efflux time by allowing the reaction mixture to flow freely down past the upper mark. As the meniscus falls past the upper mark, start a second stopwatch (No. 2), and at the same time record the reaction time (T_R), in min, from stopwatch No. 1. As the meniscus of the reaction mixture falls past the lower mark, record the time (T_T), in seconds, from stopwatch No. 2. Immediately re-draw the reaction mixture above the upper mark and into the driving fluid head. As the meniscus falls freely past the upper mark, restart stopwatch No. 2, and at the same time record the reaction time (T_R), in min, from stopwatch No. 1. As the meniscus falls past the lower mark, record the time (T_T), in seconds, from stopwatch No. 2. Repeat the latter operation, beginning with "Immediately re-draw the reaction mixture, . . . " until a total of four determinations are obtained over a reaction time (T_R) of not more than 15 min.

Prepare a substrate blank by pipetting 1.0 ml of water into a mixture of 20.0 ml of *Substrate Solution* and 4.0 ml of *Acetate Buffer*, and then immediately pipet 10.0 ml of this mixture into the wide arm of the *Viscometer*. Determine the time (T_S), in seconds, required for the meniscus to fall between the two marks. Use an average of five determinations as T_S.

Prepare a water blank by pipetting 10.0 ml of water, previously equilibrated to 40° ± 0.1°, into the wide arm of the *Viscometer*. Determine the time (T_W), in seconds, required for the meniscus to fall between the two marks. Use an average of five determinations as T_W.

Calculation One hemicellulase unit (HCU) is that activity that will produce a relative fluidity change of 1 over a period of 5 min in a locust bean gum substrate under the conditions specified. Calculate the relative fluidities (F_R) and T_N values (see definition below) for each of the four efflux times (T_T) and reaction times (T_R) as follows:

$$F_R = (T_S - T_W)/(T_T - T_W),$$

and

$$T_N = 1/2(T_T/60 \text{ s/min}) + T_R = (T_T/120) + T_R,$$

in which F_R is the relative fluidity for each reaction time; T_S is the average efflux time for the substrate blank, in seconds; T_W is the average efflux time for the water blank, in seconds; T_T is the efflux time of the sample reaction mixture, in seconds; T_R is the elapsed time from zero time, in min, i.e., the time from addition of the enzyme solution to the buffered substrate until the beginning of the measurement of the efflux time (T_T); and T_N is

the reaction time (T_R), in min, plus one half of the efflux time (T_T) converted to min.

Plot the four relative fluidities (F_R) as the ordinate against the four reaction times (T_N) as the abscissa. A straight line should be obtained. The slope of the line corresponds to the relative fluidity change per min and is proportional to the enzyme concentration. The slope of the best line through a series of experimental points is a better criterion of enzyme activity than is a single relative fluidity value. From the curve determine the F_R values at 10 and 5 min. They should have a difference in fluidity of not more than 0.22 and not less than 0.18. Calculate the activity of the enzyme sample as follows:

$$\text{HCU/g} = 1000(F_{R10} - F_{R5})/W,$$

in which F_{R10} is the relative fluidity at 10 min reaction time; F_{R5} is the relative fluidity at 5 min reaction time; 1000 is mg per g; and W is the weight, in mg, of the enzyme sample contained in the 1.0-ml aliquot of *Sample Preparation* added to the equilibrated substrate in the *Procedure*.

INVERTASE ACTIVITY

Application and Principle This procedure is for the determination of invertase activity of yeast enzymes. The assay is based on a 30-min hydrolysis of sucrose at 20° and pH 4.5. The degree of hydrolysis is determined by measuring the optical rotation of the solution with a polarimeter.

Reagents and Solutions

Phosphate Buffer Solution Dissolve 115 g of monobasic sodium phosphate ($NaH_2PO_4.H_2O$) in sufficient water to make 500.0 ml.

Sucrose Substrate Solution Dissolve 100 g of sucrose (such as Domino Superfine cane sugar, available in supermarkets) in 300 ml of water, add 20.0 ml of *Phosphate Buffer Solution*, dilute to volume in a 1000-ml volumetric flask, and mix.

Neutral Lead Acetate Solution Dissolve 31 g of lead acetate ($C_4H_6PbO_4.3H_2O$) in 50 ml of water, adjust to pH 7.0 with sodium hydroxide TS, and dilute to 80 ml in a graduate. Filter through Whatman No. 1, or equivalent, filter paper, and store the filtrate in a glass-stoppered bottle.

Sample Preparation Prepare a solution of the sample so that 10 ml of the final dilution will give a polarimeter reading, in a 2-dm tube, between 0 and +20. For solid preparations, transfer an accurately weighed portion into a mortar, triturate with at least 5 times the sample weight of water, and dilute quantitatively and stepwise to the desired concentration. Liquid sample should be pipetted directly into a volumetric flask and diluted to volume with water.

Procedure Transfer 100.0 ml of the *Sucrose Substrate Solution* into a 100/110-ml sugar flask, and equilibrate to 20° for 15 min in a water bath maintained at 20° ± 0.1°. At zero time, rapidly pipet 10.0 ml of the *Sample Preparation* into the flask, and invert the flask five or six times to mix. Start the stopwatch at zero time. Return the flask to the water bath, and allow it to stand for 30.0 min. If a large amount of insoluble matter is present in the *Sample Preparation*, invert the incubation mixture in the flask every 10 min.

At the end of the incubation period, add about 2 g of sodium carbonate ($Na_2CO_3.H_2O$), and swirl to dissolve. Check the pH of the solution with a pH meter, and make the solution alkaline if necessary by the addition of sodium carbonate.

Pipet 50.0 ml of the enzyme digest into a 100-ml volumetric flask, add 6 drops of the *Neutral Lead Acetate Solution*, dilute to volume with water, and mix. To the solution add 3 g of a suitable filter aid, such as a cellulose-type flocculent, and filter through Whatman No. 1, or equivalent, filter paper, discarding the first 3 ml of filtrate. The subsequent filtrate must be perfectly clear in order to be read on the polarimeter.

Prepare enzyme blanks containing 10 ml of the *Sample Preparation* in 100 ml of water, and treat the blanks in the same manner as the enzyme digest.

Rinse a 2-dm polarimeter tube three times with the solution to be polarized, discard the washes, and fill the tube well up into the tubulature. Place the filled tube in the polarimeter, insert a thermometer (with a range of 10° to 30°, graduated in 0.1°), and allow the solution to achieve equilibration to 20°. For each solution, determine the reading five times, and then average the readings for each. Subtract the average of the blanks from the average of the sample to obtain the net reading for the sample.

Calculation One invertase unit is defined as that quantity of enzyme that will hydrolyze 77% of the sucrose applied under the conditions of the assay. Prepare a standard curve from the values for *Activity* and *Polarization Reading* (in degrees Ventzke) shown below. (NOTE: Angular rotation = °Ventzke × 0.346.)

Activity	Polarization Reading
0.960	0
0.735	+5
0.570	+10
0.420	+15
0.300	+20
0.190	+25
0.090	+30

By interpolation from the standard curve, determine the activity (A) of the *Sample Preparation*. For every degree above 20° at which the sample is read, subtract 0.004 from the activity, or add 0.004 per degree below 20°.

Calculate the invertase activity (IA) of the enzyme preparation as follows:

$$\text{IA, units/g} = A \times 2 \times 1000/W,$$

in which 2 is a dilution factor; 1000 converts mg to g; and W is the weight, in mg, of the enzyme sample added to the incubation mixture in the 10-ml aliquot of *Sample Preparation* used. (NOTE: The dilution factor, 2, is not exact, but it is satisfactory for this determination.)

LACTASE (β-GALACTOSIDASE) ACTIVITY

Application and Principle This procedure is for the determination of lactase activity of enzyme preparations derived from

Aspergillus niger, var., *Aspergillus oryzae*, var., and *Saccharomyces* sp. The assay is based on a 15-min hydrolysis of an *o*-nitrophenyl-β-D-galactopyranoside (ONPG) substrate at 37° and at the specified pH (4.5 for *Aspergillus niger*, var., and *Aspergillus oryzae*, var., and 6.5 for *Saccharomyces* sp.).

Reagents and Solutions

Acetate Buffer (for *Aspergillus niger*, var., and *Aspergillus oryzae*, var.) Pipet 50 ml of 2 *N* acetic acid into about 800 ml of water, and add 2 *N* sodium hydroxide until the pH is 4.5 ± 0.05, determined by pH meter. Transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

P-E-M Buffer (for *Saccharomyces* sp.) Dissolve 27.2 g of anhydrous monobasic potassium phosphate, 37.2 mg of disodium EDTA dihydrate, and 20.3 mg of magnesium chloride hexahydrate in about 800 ml of water, and add 2 *N* sodium hydroxide until the pH is 6.5 ± 0.05, determined by pH meter. Transfer the solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard o-Nitrophenol Solution Transfer 139.0 mg of *o*-nitrophenol into a 1000-ml volumetric flask, dissolve in 10 ml of 95% alcohol, dilute to volume with water, and mix. Pipet 2-, 4-, 6-, 8-, 10-, 12-, and 14-ml portions of this solution into a series of 100-ml volumetric flasks, dilute each to volume with 1% sodium bicarbonate solution, and mix. The dilutions contain, respectively, 0.02, 0.04, 0.06, 0.08, 0.10, 0.12, and 0.14 μmol of *o*-nitrophenol per ml. Determine the absorbance of each dilution at 420 nm in a 1-cm cell, with a suitable spectrophotometer, using water as the blank, and for each dilution plot absorbance against μmol of *o*-nitrophenol: a straight line through the origin must be obtained. Divide the absorbance of each dilution by μmol of *o*-nitrophenol to obtain the extinction coefficient (ε) at that dilution, and then average the seven values thus calculated: a value close to 4.65 should be obtained.

Aspergillus Substrate Transfer 370.0 mg of *o*-nitrophenyl-β-D-galactopyranoside into a 100-ml volumetric flask, dissolve in about 75 ml of *Acetate Buffer*, dilute to volume with this same solvent, and mix.

Saccharomyces Substrate Transfer 250.0 mg of *o*-nitrophenyl-β-D-galactopyranoside into a 100-ml volumetric flask, dissolve in about 75 ml of *P-E-M Buffer* , dilute to volume with this same solvent, and mix.

Test Preparation Prepare a solution from the enzyme preparation so that 1 ml of the final dilution will contain between 0.15 and 0.65 lactase unit. Weigh the enzyme, transfer it into a glass mortar, and triturate with the appropriate *Buffer* solution. Quantitatively transfer the mixture into a volumetric flask of appropriate size, dilute to volume with the *Buffer* solution, and mix.

Procedure Pipet 4-ml portions of the appropriate *Substrate* into a suitable series of 25- × 150-mm test tubes, stopper, and equilibrate them in a water bath maintained at 37° ± 0.1°. At zero time, rapidly pipet 1 ml of the *Test Preparation* into the equilibrated substrate, and mix by swirling, starting the stopwatch at zero time. After 15.0 min incubation time, pipet 1 ml from each incubation mixture into separate test tubes containing 1 ml of 10% sodium carbonate solution, then mix by swirling, dilute to 10 ml with water, and mix again. Determine the absorbance of each solution at 420 nm in a 1-cm cell, with a suitable spectrophotometer, using as the blank a solution prepared in the same manner as for the sample, substituting 1 ml of water for the *Test Preparation*.

Calculation One lactase unit (LacU) is defined as that quantity of enzyme that will liberate 1 μmol of *o*-nitrophenol per min under the conditions of the assay.

Calculate the activity of the enzyme preparation taken for analysis as follows:

$$\text{LacU/g} = A \times 5 \times 10/(\epsilon \times 15 \times W),$$

in which A is the average of the absorbance readings for the sample; 5 is the volume, in ml, of the incubation mixture; 10 is the final volume, in ml, of the diluted incubation mixture; ϵ is the extinction coefficient, determined as directed under *Standard o-Nitrophenol Solution*; 15 is the incubation time, in min; and W is the weight, in g, of original enzyme preparation contained in the 1-ml aliquot of *Test Preparation* used.

LIPASE ACTIVITY

Application and Principle This procedure is for the determination of lipase activity of preparations derived from *Aspergillus niger*, var., *Aspergillus oryzae*, var., and animal pancreatic tissues. The assay is based on a 5-min hydrolysis of an olive oil substrate at pH 6.5 and 30°. The fatty acids released on hydrolysis of the glycerol esters are determined by titration with sodium hydroxide.

Reagents and Solutions

Sterol Extract Use a suitable grade of multisterol extract containing sterols and higher alcohols in the free form only, such as Amerchol L-101, available from American Cholesterol Products, Edison, N.J. 08817.

Stock Buffer Solution Dissolve 9.7 g of sodium acetate ($NaC_2H_3O_2.3H_2O$) and 14.7 g of USP sodium barbital in sufficient water to make 500 ml. Store this solution, which has a pH of 9.9, in a refrigerator.

Diluted Buffer Solution Mix 40 ml of *Stock Buffer Solution* with 16 ml of 8.5% sodium chloride solution and 53 ml of 0.1 *N* hydrochloric acid, dilute to 200 ml with water, and mix. Adjust the pH of the final solution to 6.5, if necessary, with 0.1 *N* hydrochloric acid or 0.1 *N* sodium hydroxide. Store in a refrigerator until used, and discard if crystals appear during storage.

Substrate Emulsion Stir 30 g of gum arabic (acacia) and 270 ml of water with a magnetic stirrer for 30 min at room temperature, breaking any lumps with a glass rod, and then cool to 5°. Transfer 6 g of *Sterol Extract* into a 400-ml beaker, add 72 g of USP olive oil and 222 g of the chilled gum arabic solution, mix with a glass rod, then pour into a homogenizing blender, and blend for 5 min. Adjust the pH to 6.3 with 0.5 *N* sodium hydroxide, cool to 5°, and blend again for 7 min. The temperature may have increased to 47°, and the pH to 6.5; if necessary, adjust the pH to 6.5 with 0.5 *N* sodium hydroxide. The emulsion is stable for at least 8 days when stored in a refrigerator. (*Caution:* Do not allow the suspension to freeze.)

Sample Preparation Prepare a solution of the enzyme

preparation so that 2 ml of the final dilution will contain between 2 and 7 lipase units. Weigh or pipet the sample quantitatively, and use within 5 min of dilution.

Procedure Mix 3 parts of the *Substrate Emulsion* with 1 part of the *Diluted Buffer Solution* (w/w), and transfer 8 ml of the mixture into a 100-ml beaker. Equilibrate to 30° by heating for 10 min in a water bath maintained at 30° ± 0.1°. At zero time, rapidly pipet 2.0 ml of the *Sample Preparation* into the equilibrated substrate, starting the stopwatch at zero time. Mix with a glass rod, incubate for 5.0 min in the water bath, then add 40 ml of alcohol (23A denatured alcohol is suitable), and immediately mix with the glass rod. Remove from the water bath, and titrate with 0.02 *N* sodium hydroxide from a 10-ml microburet to a pH of 8.0, using a pH meter. Record the volume, in ml, of 0.02 *N* sodium hydroxide required as *S*.

Prepare a blank by mixing 8 ml of the *Substrate Emulsion* with 40 ml of alcohol and 2 ml of the *Sample Preparation*, and titrate to pH 8.0 as directed above, recording the volume of 0.02 *N* sodium hydroxide required as *B*.

Calculation One lipase unit (LU) is defined as that quantity of enzyme that will liberate the equivalent of 1 μmol of acid (H^+) per min from the substrate, under the conditions of the assay.

Calculate the lipase activity of the sample, as the number of LU per g of original preparation, as follows:

$$LU/g = K \times N \times 1000/(W \times 0.001 \times 5),$$

in which *K* is the net titration value ($S - B$); *N* is the exact normality of the sodium hydroxide solution; 1000 converts mmol of acid to μmol; *W* is the weight, in mg, of the enzyme sample added to the incubation mixture in the 2-ml aliquot of *Sample Preparation* used; 0.001 converts mg to g; and 5 is the reaction time, in min.

LIPASE/ESTERASE (FORESTOMACH) ACTIVITY

Application and Principle This procedure is primarily applicable to lipases from animal forestomach sources. The analysis is performed by potentiometric titration.

Apparatus Use a suitable automatic recording titrator equipped with thermostatic control (Thermostatic Recording pH Stat, Sargent-Welch, or equivalent).

Reagents and Solutions

Sodium Caseinate Use the hydrophile powder, soluble form, available from Sheffield Chemical (Div. Kraftco), 2400 Morris Avenue, Union, N.J. 07083.

Hydroxylated Lecithin Solution Use the material available from Food Technology, Inc., 5903 Northwest Highway, Chicago, Ill. 60600, and prepare a 10% solution in light mineral oil (FCC or USP grade).

Tri-n-butyrin Use the material available as #726, Eastman Organic Chemicals, or equivalent.

Substrate Preparation Disperse an amount of *Sodium Caseinate*, equivalent to 600 mg of casein, in 95 ml of water contained in a one-half pint freezer jar (Ball Mason, or equivalent) that fits the head of a suitable high-speed blender. Add 0.5 ml of *Hydroxylated Lecithin Solution* and 5.0 ml of *Tri-n-butyrin*, and mix for 60 s at low speed. Adjust the temperature of the mixture to 42°, and use within 4 h.

Sample Preparation Suspend or dissolve an accurately weighed amount of the enzyme preparation in water, and dilute to obtain an enzyme activity of 10 to 12 lipase (forestomach) units per ml.

Procedure Fill the buret of the titrator with 0.025 *N* sodium hydroxide, and calibrate the instrument following the manufacturer's instructions, setting the temperature at 42° and the pH at 6.20. Mix the *Substrate Preparation* for about 15 s with a magnetic stirrer, then pipet 10.0 ml into the reaction vessel of the titrator, and add a small stirring bar. Place the vessel on the titrator, add 1.0 ml of the *Sample Preparation*, and equilibrate the mixture for 15 min. Actuate the recorder, and record the titration curve for 15 min. (NOTE: The recorder trace reflecting delivery of the titrant must be linear.) Determine the rate, in ml per min, at which the titrant was delivered during the titration, and record this value as *R*.

Calculation One lipase (forestomach) unit (LFU) is the activity that releases 1.25 μmol of butyric acid per min under the conditions of the test.

Calculate the activity of the enzyme preparation by the formula

$$LFU/g = R \times 0.025 \times 10^3/(W \times 1.25),$$

in which *W* is the weight, in g, of the enzyme preparation contained in the 1.0 ml of *Sample Preparation* taken for analysis.

MILK-CLOTTING ACTIVITY

Application This procedure is to be applied to enzyme preparations derived from either animal or microbial sources.

Apparatus

Bottle-Rotating Apparatus Use a suitable assembly, designed to rotate at a rate of 16 to 18 rpm, such as that available from Dries-Jacques Associates, P.O. Box 17338, Milwaukee, Wis. 53217.

Sample Bottles Use 125-ml squat, round, wide-mouth bottles such as those available as Catalog No. B-7545-125 from Scientific Products, McGaw Park, Ill. 60085.

Substrate Solution Dissolve 60 g of low-heat, nonfat dry milk (such as Peake Grade A, available from Galloway West, Fond du Lac, Wis. 54935) in 500 ml of a solution, adjusted to pH 6.3 if necessary, containing in each ml 2.05 mg of sodium acetate ($NaC_2H_3O_2$) and 1.11 mg of calcium chloride ($CaCl_2$).

Standard Preparation Use a standard-strength rennet; bovine rennet; microbial rennet (*E. parasitica*); or microbial rennet (*Mucor* species), as appropriate for the preparation to be assayed. Such standards, which are available from commercial coagulant manufacturers, should be of known activity. Dilute

the standard-strength material 1 to 200 with water, and mix. Equilibrate to 30° before use, and prepare no more than 2 h prior to use.

Sample Preparation Prepare aqueous solutions or dilutions of the sample to produce a final concentration such that the clotting time, as determined in the *Procedure* below, will be within 1 min of that of the *Standard Preparation*. Prepare no more than 1 h prior to use.

Procedure Transfer 50.0 ml of the *Substrate Solution* into each of four 125-ml *Sample Bottles*. Place the bottles on the *Bottle-Rotating Apparatus*, and suspend the apparatus in a water bath, maintained at 30° ± 0.5°, so that the bottles are at an angle of approximately 20° to 30° to the horizontal. Immerse the bottles so that the water level in the bath is about equal to the substrate level in the bottles. Begin rotating the apparatus at 16 to 18 rpm, then add 1.0 ml of the *Sample Preparation* to each of two bottles, and record the exact time of addition. Add 1.0 ml of the *Standard Preparation* to each of the other two bottles, recording the exact time. Observe the rotating bottles, and record the exact time of the first evidence of clotting (i.e., when fine granules or flecks adhere to the sides of the bottle). Variations in the response of different lots of the substrate may cause variations in clotting time; therefore, the test samples and standards should be measured simultaneously on the same substrate. Average the clotting time, in seconds, of the duplicate samples, recording the time for the *Standard Preparation* as T_S and that for the *Sample Preparation* as T_U.

Calculation Calculate the activity of the enzyme preparation by the formula

$$\text{Milk-clotting units/ml} = 100 \times (T_S/T_U) \times (D_S/D_U),$$

in which 100 is the activity assigned to the *Standard Preparation*, D_S is the dilution factor for the *Standard Preparation*, and D_U is the dilution factor for the *Sample Preparation*. (NOTE: The dilution factors should be expressed as fractions; e.g., a dilution of 1 to 200 would be expressed as 1/200.)

PEPSIN ACTIVITY

Application This procedure is to be applied to preparations derived from porcine or other animal stomachs.

Measuring Vessels Use 100-ml conically shaped measuring vessels complying with the following descriptions: (1) diameters not exceeding 1 cm at the bottom; (2) comply in other respects with the water and sediment tube ASTM Standard Method D96-68; (3) graduated from 0 to 0.5 ml in 0.05-ml graduations, from 2 to 3 ml in 0.1-ml graduations, from 3 to 5 ml in 0.2-ml graduations, from 5 to 10 ml in 1-ml graduations, from 10 to 25 ml in 5-ml graduations, and with graduation marks at 50, 75, and 100 ml. (NOTE: Measuring vessels other than the type described herein may be used if they are of such design and graduation to permit measurement of the residue with equivalent accuracy.)

Reagents and Solutions

Hydrochloric Acid Solution Mix 35 ml of 1.0 *N* hydrochloric acid with 385 ml of water.

Substrate Boil one or more hen eggs for 15 min to provide coagulated albumen, and cool rapidly by immersion in cold water. Remove the shell and pellicle and all of the yolk, and at once rub the albumen through a clean, dry No. 40 sieve, rejecting the first portion that passes through the sieve.

Substrate Preparation Place 10 g of the *Substrate* in each of as many 100-ml wide-mouth bottles as needed for the test, and immediately add 35 ml of *Hydrochloric Acid Solution* (all at one time or in portions). By suitable means, thoroughly disintegrate the particles of albumen. Equilibrate to 52° before use in the *Procedure* below.

Standard Preparation Dissolve 100 mg of USP Pepsin Reference Standard in 150 ml of *Hydrochloric Acid Solution*. Use this solution within 1 h.

Sample Preparation Dissolve 100 mg of the sample pepsin, or an amount of the enzyme preparation that will provide a solution similar to or slightly stronger than the *Standard Preparation*, in 150 ml of *Hydrochloric Acid Solution*. Use this solution within 1 h.

Procedure Pipet 5.0 ml of the *Standard Preparation* into each of two bottles containing the *Substrate Preparation*. To two or more additional substrate bottles add graduated aliquots of the *Sample Preparation* so that one bottle will contain approximately the same amount, and the others will contain successively lesser amounts, of pepsin as is contained in the 5.0 ml of the *Standard Preparation*, using, for example, 5.0, 4.9, and 4.8 ml. When less than 5.0 ml of the *Sample Preparation* is used, add sufficient *Hydrochloric Acid Solution* to make 5.0 ml of combined *Sample Preparation* plus acid added. At once stopper the bottles securely, invert them three times, and heat in a water bath, maintained at 52° ± 0.5°, for 2.5 h, agitating the contents equally every 10 min by inverting the bottles once. Remove the bottles from the bath, and pour the contents of each into separate *Measuring Vessels*. Transfer the undigested albumen that adheres to the sides of the bottles into the respective *Measuring Vessel* with the aid of small portions of water until 50 ml has been used for each. Mix the contents of each vessel, allow them to stand for 30 min, and then read for each the volume of undigested albumen. Average the sediment volumes in the two standard vessels, and note which of the sample vessels contains undigested albumen closest to the average for the standards. Finally, record as v the volume, in ml, of *Sample Preparation* that produced the undigested albumen closest to the average produced by the *Standard Preparations*.

Calculation One pepsin unit is defined as that quantity of enzyme that digests 3000 times its weight of coagulated egg albumen under the conditions of the assay.

Calculate the activity of the enzyme preparation by the formula

$$\text{Pepsin units/mg} = 3000 \times (S/u) \times (5.0/v),$$

in which S is the weight, in mg, of USP Pepsin Reference Standard used to make the *Standard Preparation*; u is the

weight, in mg, of enzyme preparation taken for analysis; and v is as defined in the *Procedure*.

PLANT PROTEOLYTIC ACTIVITY

Application and Principle This procedure is for the determination of the proteolytic activity of papain, ficin, and bromelain. The assay is based on a 60-min proteolytic hydrolysis of a casein substrate at pH 6.0 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration; solubilized casein is then measured spectrophotometrically.

Reagents and Solutions

Sodium Phosphate Solution (0.05 *M*) Transfer 7.1 g of anhydrous dibasic sodium phosphate into a 1000-ml volumetric flask, dissolve in about 500 ml of water, dilute to volume with water, and mix. Add 1 drop of toluene as preservative.

Citric Acid Solution (0.05 *M*) Transfer 10.5 g of citric acid monohydrate into a 1000-ml volumetric flask, dissolve in about 500 ml of water, dilute to volume with water, and mix. Add 1 drop of toluene as preservative.

Phosphate-Cysteine-EDTA Buffer Solution Dissolve 7.1 g of anhydrous dibasic sodium phosphate in about 800 ml of water, and then dissolve in this solution 14.0 g of disodium EDTA dihydrate and 6.1 g of cysteine hydrochloride monohydrate. Adjust to pH 6.0 ± 0.1 with 1 *N* hydrochloric acid or 1 *N* sodium hydroxide, then transfer into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Trichloroacetic Acid Solution Dissolve 30 g of trichloroacetic acid in 100 ml of water.

Casein Substrate Solution Disperse 1 g (moisture-free basis) of Hammarsten casein in 50 ml of *Sodium Phosphate Solution*, and heat for 30 min in a boiling water bath, with occasional agitation. Cool to room temperature, and, with rapid and continuous agitation, adjust to pH 6.0 ± 0.1 by the addition of *Citric Acid Solution*. (NOTE: Rapid and continuous agitation during the addition prevents casein precipitation.) Quantitatively transfer the mixture into a 100-ml volumetric flask, dilute to volume with water, and mix.

Stock Standard Solution Transfer 100.0 mg of USP Papain Reference Standard into a 100-ml volumetric flask, dissolve and dilute to volume with *Phosphate-Cysteine-EDTA Buffer Solution*, and mix.

Diluted Standard Solutions Pipet 2, 3, 4, 5, 6, and 7 ml of *Stock Standard Solution* into a series of 100-ml volumetric flasks, dilute each to volume with *Phosphate-Cysteine-EDTA Buffer Solution*, and mix by inversion.

Test Solution Prepare a solution from the enzyme preparation so that 2 ml of the final dilution will give a ΔA in the *Procedure* between 0.2 and 0.5. Weigh the sample accurately, transfer it quantitatively to a glass mortar, and triturate with *Phosphate-Cysteine-EDTA Buffer Solution*. Transfer the mixture quantitatively into a volumetric flask of appropriate size, dilute to volume with *Phosphate-Cysteine-EDTA Buffer Solution*, and mix.

Procedure Pipet 5 ml of *Casein Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing three tubes for the enzyme unknown, six for a papain standard curve, and nine for enzyme blanks. Equilibrate the tubes for 15 min in a water bath maintained at 40° ± 0.1°. At zero time, rapidly pipet 2 ml of each of the *Diluted Standard Solutions*, and 2-ml portions of the *Test Solution*, into the equilibrated substrate, starting the stopwatch at zero time. Mix each by swirling, stopper, and place the tubes back in the water bath. After 60.0 min, add 3 ml of *Trichloroacetic Acid Solution* to each tube. (*Caution:* Do not use mouth suction.) Mix each tube immediately by swirling.

Prepare enzyme blanks containing 5.0 ml of *Casein Substrate Solution*, 3.0 ml of *Trichloroacetic Acid Solution*, and 2.0 ml of one of the appropriate *Diluted Standard Solutions* or the *Test Solution*.

Return all tubes to the water bath, and heat for 30.0 min, allowing the precipitated protein to coagulate completely. Filter each mixture through Whatman No. 42, or equivalent, filter paper, discarding the first 3 ml of filtrate. The subsequent filtrate must be perfectly clear. Determine the absorbance of each filtrate in a 1-cm cell at 280 nm, with a suitable spectrophotometer, against its respective blank.

Calculation One papain unit (PU) is defined in this assay as that quantity of enzyme that liberates the equivalent of 1 μg of tyrosine per hour under the conditions of the assay.

Prepare a standard curve by plotting the absorbances of filtrates from the *Diluted Standard Solutions* against the corresponding enzyme concentrations, in mg/ml. By interpolation from the standard curve, obtain the equivalent concentration of the filtrate from the *Test Solution*.

Calculate the activity of the enzyme preparation taken for analysis as follows:

$$\text{PU/mg} = A \times C \times 10/W,$$

in which A is the activity of USP Papain Reference Standard, in PU per mg; C is the concentration, in mg per ml, of Reference Standard from the standard curve, equivalent to the enzyme unknown; 10 is the total volume, in ml, of the final incubation mixture; and W is the weight, in mg, of original enzyme preparation in the 2-ml aliquot of *Test Solution* added to the incubation mixture.

PROTEOLYTIC ACTIVITY, BACTERIAL (PC)

Application and Principle This procedure is for the determination of protease activity, expressed as PC units, of preparations derived from *Bacillus subtilis*, var., and *Bacillus licheniformis*, var. The assay is based on a 30-min proteolytic hydrolysis of casein at 37° and pH 7.0. Unhydrolyzed casein is removed by filtration, and the solubilized casein is determined spectrophotometrically.

Reagents and Solutions

Casein Use Hammarsten-grade casein, available from Nutritional Biochemical Corp., 21010 Miles Avenue, Cleveland, Ohio 44128.

Tris Buffer (pH 7.0) Dissolve 12.1 g of enzyme-grade (or equivalent) tris(hydroxymethyl)aminomethane in 800 ml of water, and titrate with 1 *N* hydrochloric acid to pH 7.0. Transfer into a 1000-ml volumetric flask, dilute to volume with water, and mix.

TCA Solution Dissolve 18 g of trichloroacetic acid and 19 g of sodium acetate trihydrate in 800 ml of water in a 1000-ml volumetric flask, add 20 ml of glacial acetic acid, dilute to volume with water, and mix.

Substrate Solution Dissolve 6.05 g of enzyme-grade tris(hydroxymethyl)aminomethane in 500 ml of water, add 8 ml of 1 *N* hydrochloric acid, and mix. Dissolve 7 g of *Casein* in this solution, and heat for 30 min in a boiling water bath, stirring occasionally.

Cool to room temperature, and adjust to pH 7.0 with 0.2 *N* hydrochloric acid, adding the acid slowly, with vigorous stirring, to prevent precipitation of the casein. Transfer the mixture into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Sample Preparation Using *Tris Buffer*, prepare a solution of the sample enzyme preparation so that 2 ml of the final dilution will contain between 10 and 44 PC units.

Procedure Pipet 10.0 ml of the *Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing one tube for each enzyme test, one tube for each enzyme blank, and one tube for a substrate blank. Equilibrate the tubes for 15 min in a water bath maintained at 37° ± 0.1°.

At zero time, rapidly pipet 2.0 ml of the *Sample Preparation* into the equilibrated substrate, starting the stopwatch at zero time. Mix, and replace the tubes in the water bath. Add 2 ml of *Tris Buffer* (instead of the *Sample Preparation*) to the substrate blank. After exactly 30 min, add 10 ml of *TCA Solution* to each enzyme incubation and to the substrate blank to stop the reaction. (*Caution:* Do not use mouth suction for the *TCA Solution*.) Heat the tubes in the water bath for an additional 30 min to allow the protein to coagulate completely.

At the end of the second heating period, shake each tube vigorously, and filter through 11-cm Whatman No. 42, or equivalent, filter paper, discarding the first 3 ml of filtrate. (NOTE: The filtrate must be perfectly clear.) Determine the absorbance of each sample filtrate in a 1-cm cell, at 275 nm, with a suitable spectrophotometer, using the filtrate from the substrate blank to set the instrument at zero. Correct each reading by subtracting the appropriate enzyme blank reading, and record the value so obtained as A_U.

Standard Curve Transfer 100.0 mg of L-tyrosine, chromatographic-grade or equivalent (Calbiochem, La Jolla, Calif. 92037), previously dried to constant weight, to a 1000-ml volumetric flask. Dissolve in 60 ml of 0.1 *N* hydrochloric acid. When completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 100 μg of tyrosine in 1.0 ml. Prepare three more dilutions from this stock solution to contain 75.0, 50.0, and 25.0 μg of tyrosine per ml. Determine the absorbance of the four solutions at 275 nm in a 1-cm cell on a suitable spectrophotometer versus 0.006 *N* hydrochloric acid. Prepare a plot of absorbance versus tyrosine concentration.

Calculation One bacterial protease unit (PC) is defined as that quantity of enzyme that produces the equivalent of 1.5 μg per ml of L-tyrosine per min under the conditions of the assay.

From the *Standard Curve*, and by interpolation, determine the absorbance of a solution having a tyrosine concentration of 60 μg per ml. A figure close to 0.0115 should be obtained. Divide the interpolated value by 40 to obtain the absorbance equivalent to that of a solution having a tyrosine concentration of 1.5 μg per ml, and record the value thus derived as A_S.

Calculate the activity of the sample enzyme preparation by the formula

$$PC/g = (A_U/A_S) \times (22/30W),$$

in which 22 is the final volume, in ml, of the reaction mixture; 30 is the time of the reaction, in min; and W is the weight of the original sample taken, in g.

PROTEOLYTIC ACTIVITY, FUNGAL (HUT)

Application and Principle This procedure is for the determination of the proteolytic activity, expressed as hemoglobin units on the tyrosine basis (HUT), of preparations derived from *Aspergillus oryzae*, var., and *Aspergillus niger*, var., and it may be used to determine the activity of other proteases at pH 4.7. The test is based on the 30-min enzymatic hydrolysis of a hemoglobin substrate at pH 4.7 and 40°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized hemoglobin in the filtrate is determined spectrophotometrically.

Reagents and Solutions

Hemoglobin Use Hemoglobin Substrate Powder (Worthington Biochemical Corp., Freehold, N.J. 07728) or a similar high-grade material that is completely soluble in water.

Acetate Buffer Solution Dissolve 136 g of sodium acetate ($NaC_2H_3O_2 3H_2O$) in sufficient water to make 500 ml. Mix 25.0 ml of this solution with 50.0 ml of 1 *M* acetic acid, dilute to 1000 ml with water, and mix. The pH of this solution should be 4.7 ± 0.02.

Substrate Solution Transfer 4.0 g of the *Hemoglobin* into a 250-ml beaker, add 100 ml of water, and stir for 10 min to dissolve. Immerse the electrodes of a pH meter in the solution, and adjust the pH to 1.7, stirring continuously, by the addition of 0.3 *N* hydrochloric acid. After 10 min, adjust the pH to 4.7 by the addition of 0.5 *M* sodium acetate. Transfer the solution into a 200-ml volumetric flask, dilute to volume with water, and mix. This solution is stable for about 5 days when refrigerated.

Trichloroacetic Acid Solution Dissolve 140 g of trichloroacetic acid in about 75 ml of water. Transfer the solution to a 100-ml volumetric flask, dilute to volume with water, and mix thoroughly.

Sample Preparation Dissolve an amount of the sample in the *Acetate Buffer Solution* to produce a solution containing, in each ml, between 9 and 22 HUT. (Such a concentration will produce an absorbance reading, in the procedure below, within the preferred range of 0.2 to 0.5.)

Procedure Pipet 10.0 ml of the *Substrate Solution* into each of a series of 25- × 155-mm test tubes: one for each enzyme test and one for the substrate blank. Heat the tubes in a water bath at 40° for about 5 min. To each tube except the substrate blank add 2.0 ml of the *Sample Preparation*, and begin timing the reaction at the moment the solution is added; add 2.0 ml of the

Acetate Buffer Solution to the substrate blank tube. Close the tubes with No. 4 rubber stoppers, and tap each tube gently for 30 s against the palm of the hand to mix. Heat each tube in a water bath at 40° for exactly 30 min, and then pipet rapidly 10.0 ml of the *Trichloroacetic Acid Solution* into each tube. (*Caution:* Do not use mouth suction on the pipet.) Shake each tube vigorously against the stopper for about 40 s, and then allow to cool to room temperature for 1 h, shaking each tube against the stopper at 10- to 12-min intervals during this period. Prepare enzyme blanks as follows: Heat, in separate tubes, 10.0 ml of the *Substrate Solution* and about 5 ml of the *Sample Preparation* in the water bath for 30 min, then add 10.0 ml of the *Trichloroacetic Acid Solution* to the *Substrate Solution*, shake well for 40 s, and to this mixture add 2.0 ml of the preheated *Sample Preparation*. Shake again, and cool at room temperature for 1 h, shaking at 10- to 12-min intervals.

At the end of 1 h, shake each tube vigorously, and filter through 11-cm Whatman No. 42, or equivalent, filter paper, refiltering the first half of the filtrate through the same paper. Determine the absorbance of each filtrate in a 1-cm cell, at 275 nm, with a suitable spectrophotometer, using the filtrate from the substrate blank to set the instrument to zero. Correct each reading by subtracting the appropriate enzyme blank reading, and record the value so obtained as A_U. (NOTE: If a corrected absorbance reading between 0.2 and 0.5 is not obtained, repeat the test using more or less of the enzyme preparation as necessary.)

Standard Curve Transfer 100.0 mg of L-tyrosine, chromatographic-grade or equivalent (Calbiochem, La Jolla, Calif. 92037), previously dried to constant weight, to a 1000-ml volumetric flask. Dissolve in 60 ml of 0.1 *N* hydrochloric acid. When completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 100 μg of tyrosine in 1.0 ml. Prepare three more dilutions from this stock solution to contain 75.0, 50.0, and 25.0 μg of tyrosine per ml. Determine the absorbance of the four solutions at 275 nm in a 1-cm cell on a suitable spectrophotometer versus 0.006 *N* hydrochloric acid. Prepare a plot of absorbance versus tyrosine concentration. Determine the slope of the curve in terms of absorbance per μg of tyrosine. Multiply this value by 1.10, and record it as A_S. A value of approximately 0.0084 should be obtained.

Calculation One HUT unit of proteolytic (protease) activity is defined as that amount of enzyme that produces, in 1 min under the specified conditions, a hydrolysate whose absorbance at 275 nm is the same as that of a solution containing 1.10 μg per ml of tyrosine in 0.006 *N* hydrochloric acid.

Calculate the HUT per g of the original enzyme preparation by the formula

$$\text{HUT/g} = (A_U/A_S) \times (22/30W),$$

in which 22 is the final volume of the test solution, 30 is the reaction time in min, and W is the weight of the original sample taken, in g. (NOTE: The value for A_S, under carefully controlled and standardized conditions, is 0.0084; this value may be used for routine work in lieu of the value obtained from the standard curve, but the exact value calculated from the standard curve should be used for more accurate results and in cases of doubt.)

PROTEOLYTIC ACTIVITY, FUNGAL (SAP)

Application and Principle This procedure is for the determination of proteolytic activity, expressed in spectrophotometric acid protease units (SAPU), of preparations derived from *Aspergillus niger*, var., and *Aspergillus oryzae*, var. The test is based on a 30-min enzymatic hydrolysis of a Hammarsten Casein Substrate at pH 3.0 and 37°. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized casein in the filtrate is determined spectrophotometrically.

Reagents and Solutions

Casein Use Hammarsten-grade casein, available from Nutritional Biochemical Corp., 21010 Miles Avenue, Cleveland, Ohio 44128.

Glycine–Hydrochloric Acid Buffer (0.05 *M*) Dissolve 3.75 g of glycine in about 800 ml of water. Add 1 *N* hydrochloric acid until the solution is pH 3.0, determined with a pH meter. Quantitatively transfer the solution to a 1000-ml volumetric flask, dilute to volume with water, and mix.

TCA Solution Dissolve 18.0 g of trichloroacetic acid and 11.45 g of anhydrous sodium acetate in about 800 ml of water, and add 21.0 ml of glacial acetic acid. Quantitatively transfer the solution to a 1000-ml volumetric flask, dilute to volume with water, and mix.

Substrate Solution Pipet 8 ml of 1 *N* hydrochloric acid into about 500 ml of water, and disperse 7.0 g (moisture-free basis) of *Casein* into this solution, using continuous agitation. Heat for 30 min in a boiling water bath, stirring occasionally, and cool to room temperature. Dissolve 3.75 g of glycine in the solution, and adjust to pH 3.0 with 0.1 *N* hydrochloric acid, using a pH meter. Quantitatively transfer the solution to a 1000-ml volumetric flask, dilute to volume with water, and mix.

Sample Preparation Using *Glycine–Hydrochloric Acid Buffer*, prepare a solution of the sample enzyme preparation so that 2 ml of the final dilution will give a corrected absorbance of enzyme incubation filtrate at 275 nm (ΔA, as defined in the *Procedure*) between 0.200 and 0.500. Weigh the enzyme preparation, quantitatively transfer it to a glass mortar, and triturate with *Glycine–Hydrochloric Acid Buffer*. Quantitatively transfer the mixture to an appropriately sized volumetric flask, dilute to volume with *Glycine–Hydrochloric Acid Buffer*, and mix.

Procedure Pipet 10.0 ml of *Substrate Solution* into each of a series of 25- × 150-mm test tubes, allowing at least two tubes for each sample, one for each enzyme blank, and one for a substrate blank. Stopper the tubes, and equilibrate them for 15 min in a water bath maintained at 37° ± 0.1°.

At zero time, start the stopwatch, and rapidly pipet 2.0 ml of the *Sample Preparation* into the equilibrated substrate. Mix by swirling, and replace the tubes in the water bath. (NOTE: The tubes must be stoppered during incubation.) Add 2 ml of *Glycine–Hydrochloric Acid Buffer* (instead of the *Sample Preparation*) to the substrate blank. After exactly 30 min, add 10 ml

of *TCA Solution* to each enzyme incubation and to the substrate blank to stop the reaction. (*Caution:* Do not use mouth suction for the *TCA Solution*.) In the following order, prepare an enzyme blank containing 10 ml of *Substrate Solution*, 10 ml of *TCA Solution*, and 2 ml of the *Sample Preparation*. Heat all tubes in the water bath for 30 min, allowing the precipitated protein to coagulate completely.

At the end of the second heating period, cool the tubes in an ice bath for 5 min, and filter through Whatman No. 42 filter paper, or equivalent. The filtrates must be perfectly clear. Determine the absorbance of each filtrate in a 1-cm cell at 275 nm with a suitable spectrophotometer, against the substrate blank. Correct each absorbance by subtracting the absorbance of the respective enzyme blank.

Standard Curve Transfer 181.2 mg of L-tyrosine, chromatographic-grade or equivalent (Calbiochem, La Jolla, Calif. 92037), previously dried to constant weight, to a 1000-ml volumetric flask. Dissolve in 60 ml of 0.1 *N* hydrochloric acid. When completely dissolved, dilute the solution to volume with water, and mix thoroughly. This solution contains 1.00 μmol of tyrosine in 1.0 ml. Prepare dilutions from this stock solution to contain 0.10, 0.20, 0.30, 0.40, and 0.50 μmol per ml. Determine the absorbance of each dilution in a 1-cm cell at 275 nm, against a water blank. Prepare a plot of absorbance versus μmol of tyrosine per ml. A straight line must be obtained. Determine the slope and intercept for use in the *Calculation* below. A value close to 1.38 should be obtained. The slope and intercept may be calculated by the least squares method as follows:

$$\text{Slope} = \frac{n\Sigma(MA) - \Sigma(M)\Sigma(A)}{n\Sigma(M^2) - (\Sigma M)^2},$$

$$\text{Intercept} = \frac{\Sigma(A)\Sigma(M^2) - \Sigma(M)\Sigma(MA)}{n\Sigma(M^2) - (\Sigma M)^2},$$

in which n is the number of points on the standard curve, M is the μmol of tyrosine per ml for each point on the standard curve, and A is the absorbance of the sample.

Calculation One spectrophotometric acid protease unit is that activity that will liberate 1 μmol of tyrosine per min under the conditions specified. The activity is expressed as follows:

$$\text{SAPU/g} = (\Delta A - I) \times 22/(S \times 30 \times W),$$

in which ΔA is the corrected absorbance of the enzyme incubation filtrate; I is the intercept of the *Standard Curve*; 22 is the final volume of the incubation mixture, in ml; S is the slope of the *Standard Curve*; 30 is the incubation time, in min; and W is the weight, in g, of the enzyme sample contained in the 2.0-ml aliquot of *Sample Preparation* added to the incubation mixture in the *Procedure*.

TRYPSIN ACTIVITY

Application This procedure is for the determination of the trypsin activity of trypsin preparations derived from purified extracts of porcine or bovine pancreas.

Reagents and Solutions

Fifteenth Molar Phosphate Buffer, pH 7.6 Dissolve 4.54 g of monobasic potassium phosphate in sufficient water to make 500 ml of solution. Dissolve 4.73 g of anhydrous dibasic sodium phosphate in sufficient water to make 500 ml of solution. Mix 13 ml of the monobasic potassium phosphate solution with 87 ml of the anhydrous dibasic sodium phosphate solution.

Substrate Solution Dissolve 85.7 mg of *N*-benzoyl-L-arginine ethyl ester hydrochloride, suitable for use in assaying trypsin, in sufficient water to make 100 ml. (NOTE: Determine the suitability of the substrate and check the adjustment of the spectrophotometer by performing the assay using USP Trypsin Reference Standard.) Dilute 10.0 ml of this solution to 100.0 ml with *Fifteenth Molar Phosphate Buffer, pH 7.6*. Determine the absorbance of this solution at 253 nm in a 1-cm cell, with a suitable spectrophotometer, using water as the blank and maintaining the cell temperature at 25° ± 0.1°. Adjust the absorbance of the solution, if necessary, by the addition of *Fifteenth Molar Phosphate Buffer, pH 7.6* so that it measures not less than 0.575 and not more than 0.585. Use this solution within a period of 2 h.

Sample Preparations Dissolve a sufficient amount of sample, accurately weighed, in 0.001 *N* hydrochloric acid to produce a solution containing about 3000 USP trypsin units in each ml. Prepare three dilutions using 0.001 *N* hydrochloric acid so that the final solutions will contain 12, 18 and 24 USP trypsin units in each 0.2 ml. Use these concentrations in the *Procedure* below.

Procedure Conduct the test in a spectrophotometer equipped to maintain a temperature of 25° ± 0.1° in the cell compartment. The temperature in the reaction cell should be determined before and after the measurement of absorbance to assure that the temperature does not change by more than 0.5°.

Pipet 0.2 ml of 0.001 *N* hydrochloric acid and 3.0 ml of *Substrate Solution* into a 1-cm cell. Place this cell in the spectrophotometer, and adjust the instrument so that the absorbance will read 0.050 at 253 nm. Pipet 0.2 ml of the *Sample Preparation* containing 12 USP units into another 1-cm cell. Add 3.0 ml of *Substrate Solution*, and place the cell in the spectrophotometer. At the same time the *Substrate Solution* is added, start a stopwatch, and read the absorbance at 30-s intervals for 5 min. Repeat the procedure with each of the *Sample Preparations* containing 18 and 24 USP units. Plot curves of absorbance versus time for each concentration, and use only those values that form a straight line to determine the activity of the trypsin. Discard the values on the plateau, and take the average of the results from the three concentration levels as the actual activity of the trypsin.

Calculations One USP trypsin unit is the activity causing a change in the absorbance of 0.003 per min under the conditions specified in this assay.

Calculate the number of USP trypsin units per mg at each level by the formula

$$\text{USP trypsin units} = (A_1 - A_2)/(T \times W \times 0.003),$$

in which A_1 is the absorbance straight-line final reading; A_2 is the absorbance straight-line initial reading; T is the elapsed time, in min, between the initial and final readings; and W is the

weight, in mg, of trypsin in the volume of solution used in determining the absorbance.

Essential Oils and Flavors

ACETALS

Hydroxylamine Hydrochloride Solution Prepare as directed under *Aldehydes*, this page.

Procedure Weigh accurately the quantity of the sample specified in the monograph, and transfer it into a 125-ml Erlenmeyer flask. Add 30 ml of *Hydroxylamine Hydrochloride Solution*, and reflux on a steam bath for exactly 60 min. Allow the condenser to drain into the flask for 5 min after removing the flask from the steam bath. Detach and rapidly cool the flask to room temperature. Add bromophenol blue TS as indicator, and titrate with 0.5 *N* alcoholic potassium hydroxide to pH 3.4, or to the same light color as produced in the original hydroxylamine hydrochloride solution on adding the indicator. Calculate the ml of 0.5 *N* alcoholic potassium hydroxide consumed per g of sample (*A*).

Using a separate portion of the sample, proceed as directed under *Aldehydes*, this page. Calculate the ml of 0.5 *N* alcoholic potassium hydroxide consumed per g of sample (*B*).

Calculate the percentage of acetals by the formula

$$(A - B) \times f,$$

in which f is the equivalence factor given in the monograph.

ACID VALUE

Dissolve about 10 g of the sample, accurately weighed, in 50 ml of alcohol, previously neutralized to phenolphthalein with 0.1 *N* sodium hydroxide. (Add 50 g of ice when testing cinnamyl formate, citronellyl formate, geranyl formate, isoamyl formate, and linalyl formate.) Add 1 ml of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide until the solution remains faintly pink after shaking for 10 s, unless otherwise directed in the individual monograph. Calculate the acid value (*AV*) by the formula

$$AV = (5.61 \times S)/W,$$

in which S is the number of ml of 0.1 *N* sodium hydroxide consumed in the titration of the sample, and W is the weight of the sample, in g.

TOTAL ALCOHOLS

Unless otherwise stated in the monograph, transfer 10 g of a solid sample, or 10 ml of a liquid sample, accurately weighed, into a 100-ml flask having a standard-taper neck. Add 10 ml of acetic anhydride and 1 g of anhydrous sodium acetate, mix these materials, attach a reflux condenser to the flask, and reflux the mixture for 1 h. Cool, and add 50 ml of water at a temperature between 50° and 60° through the condenser. Shake intermittently during a period of 15 min, cool to room temperature, transfer the mixture completely to a separator, allow the layers to separate, and then remove and reject the lower, aqueous layer. Wash the oil layer successively with 50 ml of a saturated sodium chloride solution, 50 ml of a 10% sodium carbonate solution, and 50 ml of saturated sodium chloride solution. If the oil is still acid to moistened litmus paper, wash it with additional portions of sodium chloride solution until it is free from acid. Drain off the oil, dry it with anhydrous sodium sulfate, then filter it.

Weigh the quantity of acetylized oil specified in the monograph into a tared 125-ml Erlenmeyer flask, add 10 ml of neutral alcohol, 10 drops of phenolphthalein TS, and 0.1 *N* alcoholic potassium hydroxide, dropwise, until a pink endpoint is obtained. If more than 0.20 ml is needed, reject the sample, and wash and test the remaining acetylized oil until its acid content is below this level. Prepare a blank for residual titration (see page 2), using the same volume of alcohol and indicator, and add 1 drop of 0.1 *N* alkali to produce a pink endpoint. Measure 25.0 ml of 0.5 *N* alcoholic potassium hydroxide into each of the flasks, reflux them simultaneously for 1 h, cool, and titrate the contents of each flask with 0.5 *N* hydrochloric acid to the disappearance of the pink color. Calculate the percentage of *Total Alcohols* by the formula

$$A = \frac{(b-S)(100e)}{W-21(b-S)},$$

in which b is the number of ml of 0.5 *N* hydrochloric acid consumed in the residual blank titration, S is the number of ml of 0.5 *N* hydrochloric acid consumed in the titration of the sample, e is the equivalence factor given in the monograph, and W is the weight of the sample of the acetylized oil in mg.

ALDEHYDES

Hydroxylamine Hydrochloride Solution Dissolve 50 g of hydroxylamine hydrochloride, preferably reagent grade or freshly recrystallized before using, in 90 ml of water and dilute to 1000 ml with aldehyde-free alcohol. Adjust the solution to a pH of 3.4 with 0.5 *N* alcoholic potassium hydroxide.

Procedure Weigh accurately the quantity of sample specified in the monograph, and transfer it into a 125-ml Erlenmeyer flask. Add 30 ml of *Hydroxylamine Hydrochloride Solution*, mix thoroughly, and allow to stand at room temperature for 10 min, unless otherwise specified in the monograph. Titrate with 0.5 *N* alcoholic potassium hydroxide to a greenish yellow endpoint that matches the color of 30 ml of *Hydroxylamine Hydrochloride Solution* in a 125-ml flask when the same volume of bromophenol blue TS has been added to each flask, or preferably titrate to a pH of 3.4 using a suitable pH meter. Calculate the percentage of aldehyde (*A*) by the formula

$$A = (S - b)(100e)/W,$$

in which S is the number of ml of 0.5 *N* alcoholic potassium hydroxide consumed in the titration of the sample, b is the number of ml of 0.5 *N* alcoholic potassium hydroxide consumed

in the titration of the blank, *e* is the equivalence factor given in the monograph, and *W* is the weight of the sample in mg.

ALDEHYDES AND KETONES—HYDROXYLAMINE METHOD

Hydroxylamine Solution Dissolve 20 g of hydroxylamine hydrochloride (reagent grade or preferably freshly crystallized) in 40 ml of water and dilute to 400 ml with alcohol. Add, with stirring, 300 ml of 0.5 *N* alcoholic potassium hydroxide, and filter. Use this solution within 2 days.

Procedure Weigh accurately the quantity of the sample specified in the individual monograph, and transfer it into a 250-ml glass-stoppered flask. Add 75.0 ml of *Hydroxylamine Solution* to this flask and to a similar flask for a residual blank titration (see page 2). If the component to be determined is an *aldehyde*, stopper the flasks and allow them to stand at room temperature for 1 h unless otherwise stated in the monograph. If the component to be determined is a *ketone*, attach the flask to a suitable condenser, and reflux the mixture for 1 h unless otherwise stated in the monograph, and then cool to room temperature. Titrate both flasks to the same greenish yellow endpoint using bromophenol blue TS as indicator, or preferably to a pH of 3.4 using a pH meter. (If the indicator is used, the endpoint color must be the same as that produced when the blank is titrated to a pH of 3.4.) Calculate the percentage of aldehyde or ketone by the formula

$$AK = (b - S)(100e)/W,$$

in which *AK* is the percentage of aldehyde or ketone, *b* is the number of ml of 0.5 *N* hydrochloric acid consumed in the residual blank titration, *S* is the number of ml of 0.5 *N* hydrochloric acid consumed in the titration of the sample, *e* is the equivalence factor given in the monograph, and *W* is the weight of the sample, in mg.

ALDEHYDES AND KETONES—HYDROXYLAMINE/TERT-BUTYL ALCOHOL METHOD

Hydroxylamine Solution Dissolve 45 g of reagent-grade hydroxylamine hydrochloride in 130 ml of water, add 850 ml of *tert*-butyl alcohol, mix, and neutralize to a pH of 3.0 to 3.5 with sodium hydroxide, using a pH meter.

Caution: Do not heat the solution.

Procedure Weigh accurately the quantity of the sample specified in the individual monograph, and transfer it into a 250-ml glass-stoppered flask. Add 50 ml of the *Hydroxylamine Solution*, or the volume specified in the monograph, mix thoroughly, and allow to stand at room temperature for the time specified in the monograph. Titrate with 0.5 *N* sodium hydroxide to the same pH as the *Hydroxylamine Solution* used. Calculate the percentage of aldehyde or ketone by the formula

$$AK = (S)(100e)/W,$$

in which *AK* is the percentage of aldehyde or ketone, *S* is the number of ml of 0.5 *N* sodium hydroxide consumed in the titration of the sample, *e* is the equivalence factor given in the monograph, and *W* is the weight of the sample, in mg.

ALDEHYDES AND KETONES—NEUTRAL SULFITE METHOD

Pipet a 10-ml sample into a 100-ml cassia flask fitted with a stopper, and add 50 ml of a freshly prepared 30 in 100 solution of sodium sulfite. Add 2 drops of phenolphthalein TS and neutralize with 50% (by volume) acetic acid solution. Heat the mixture in a boiling water bath, and shake the flask repeatedly, neutralizing the mixture from time to time by the addition of a few drops of the 50% acetic acid solution, stoppering the flask to prevent loss of volatile material. After no coloration appears upon the addition of a few more drops of phenolphthalein TS and heating for 15 min, cool to room temperature. When the liquids have separated completely, add sufficient sodium sulfite solution to raise the lower level of the oily layer within the graduated portion of the neck of the flask. Calculate the percentage, by volume, of the aldehyde or ketone by the formula

$$AK = 100 - (V \times 10),$$

in which *AK* is the percentage, by volume, of the aldehyde or ketone in the sample and *V* is the number of ml of separated oil in the graduated neck of the flask.

CHLORINATED COMPOUNDS

Wind a 1.5- × 5-cm strip of 20-mesh copper gauze around the end of a copper wire. Heat the gauze in a nonluminous flame of a Bunsen burner until it glows without coloring the flame green. Permit the gauze to cool and re-ignite it several times until a good coat of oxide has formed. With a medicine dropper, apply 2 drops of the sample to the cooled gauze, ignite, and permit it to burn freely in the air. Again cool the gauze, add 2 more drops, and burn as before. Continue this process until a total of 6 drops has been added and ignited. Then hold the gauze in the outer edge of a Bunsen flame adjusted to a height of 4 cm. Not even a transient green color is imparted to the flame. If at any of the additions the sample appears to be instantly vaporized, the test must be repeated from the beginning.

ESTERS

Ester Determination Weigh accurately the quantity of the sample specified in the monograph, and transfer it into a 125-ml Erlenmeyer flask containing a few boiling stones. Add to this flask, and, simultaneously, to a similar flask for a residual blank titration (see page 2), 25.0 ml of 0.5 *N* alcoholic potassium hydroxide. Connect each flask to a reflux condenser, and reflux the mixtures on a steam bath for exactly 1 h, unless otherwise directed in the monograph. Allow the mixtures to cool, add 10 drops of phenolphthalein TS to each flask, and titrate the excess alkali in each flask with 0.5 *N* hydrochloric acid. Calculate the percentage of esters (*E*) in the sample by the formula

$$E = (b - S)(100e)/W,$$

in which b is the number of ml of 0.5 N hydrochloric acid consumed in the residual blank titration, S is the number of ml of 0.5 N hydrochloric acid consumed in the titration of the sample, e is the equivalence factor given in the monograph, and W is the weight of the sample, in mg.

Ester Determination (High-Boiling Solvent)

0.5 N Potassium Hydroxide Solution Dissolve about 35 g of potassium hydroxide in 75 ml of water, add 1000 ml of a suitable grade of monoethyl ether of diethylene glycol, and mix.

Procedure Weigh accurately the quantity of the sample specified in the monograph, and transfer it into a 200-ml Erlenmeyer flask having a standard-taper joint. To this flask and to a similar flask for a residual blank titration (see page 2) add two glass beads and 25.0 ml of *0.5 N Potassium Hydroxide Solution*, allowing exactly 1 min for drainage from the buret or pipet. Attach an air condenser to each flask, reflux gently for 1 h, and cool. Rinse down the condensers with about 50 ml of water, then add phenolphthalein TS to each flask, and titrate the excess alkali with 0.5 N sulfuric acid to the disappearance of the pink color. Calculate the percentage of esters (E) in the sample by the formula

$$E = (b - S)(100e)/W,$$

in which b is the number of ml of 0.5 N sulfuric acid consumed in the blank determination, S is the number of ml of 0.5 N sulfuric acid required in the titration of the sample, e is the equivalence factor given in the monograph, and W is the weight of the sample, in mg.

Saponification Value Proceed as directed for *Ester Determination* or *Ester Determination (High-Boiling Solvent)*, as specified in the monograph. Calculate the saponification value (SV) by the formula

$$SV = (b - S)(28.05)/W,$$

in which b and S are as defined under *Ester Determination*, and W is the weight of the sample, in g.

Ester Value If the sample contains no free acids, the saponification value and the ester value are identical. If a determination of the *Acid Value* (AV) is specified in the monograph, calculate the ester value (EV) by the formula

$$EV = SV - AV.$$

LINALOOL DETERMINATION

Transfer a 10-ml sample, previously dried with sodium sulfate, into a 125-ml glass-stoppered Erlenmeyer flask previously cooled in an ice bath. Add to the cooled oil 20 ml of dimethyl aniline (monomethyl-free) and mix thoroughly. To the mixture add 8 ml of acetyl chloride and 5 ml of acetic anhydride, cool for several min, permit to stand at room temperature for another 30 min, then immerse the flask in a water bath maintained at 40° ± 1° for 16 h. Wash the acetylated oil with three 75-ml portions of ice water, followed by successive washes with 25-ml portions of 5% sulfuric acid, until the separated acid layer no longer becomes cloudy or emits an odor of dimethyl aniline when made alkaline. After removal of the dimethyl aniline, wash the acetylated oil first with 10 ml of sodium carbonate TS and then with successive portions of water until the washings are neutral to litmus. Finally, dry the acetylated oil with anhydrous sodium sulfate and proceed as directed for *Ester Determination* under *Esters*, page 500. Calculate the percentage of linalool ($C_{10}H_{18}O$) by the formula

$$L = \frac{7.707(b-S)}{W-0.021(b-S)},$$

in which L is the percentage of linalool, b is the the number of ml of 0.5 N hydrochloric acid consumed in the residual blank titration, S is the number of ml of 0.5 N hydrochloric acid consumed in the titration of the sample, and W is the weight of the sample, in g.

NOTE: When this method is applied to essential oils containing appreciable amounts of esters, perform an *Ester Determination*, page 500, on a sample of the *original oil* and calculate the percentage of total linalool by the formula

$$L = \frac{7.707(b-S)(1-0.0021E)}{W-0.21(b-S)},$$

in which L is the percentage of linalool, E is the percentage of esters, calculated as linalyl acetate ($C_{12}H_{20}O_2$) in the sample of the original oil, and b, S, and W are as defined in the preceding paragraph.

NOTE: This entire procedure is applicable only to linalool and linalool-containing oils. It is not intended for the determination of other tertiary alcohols.

PERCENTAGE OF CINEOLE

Temperature	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
24	45.6	45.7	45.9	46.0	46.1	46.3	46.4	46.5	46.6	46.8
25	46.9	47.0	47.2	47.3	47.4	47.6	47.7	47.8	47.9	48.1
26	48.2	48.3	48.5	48.6	48.7	48.9	49.0	49.1	49.2	49.4
27	49.5	49.6	49.8	49.9	50.0	50.2	50.3	50.4	50.5	50.7
28	50.8	50.9	51.1	51.2	51.3	51.5	51.6	51.7	51.8	52.0
29	52.1	52.2	52.4	52.5	52.6	52.8	52.9	53.0	53.1	53.3
30	53.4	53.5	53.7	53.8	53.9	54.1	54.2	54.3	54.4	54.6
31	54.7	54.8	55.0	55.1	55.2	55.4	55.5	55.6	55.7	55.9
32	56.0	56.1	56.3	56.4	56.5	56.7	56.8	56.9	57.0	57.2
33	57.3	57.4	57.6	57.7	57.8	58.0	58.1	58.2	58.3	58.5
34	58.6	58.7	58.9	59.0	59.1	59.3	59.4	59.5	59.6	59.8
35	59.9	60.0	60.2	60.3	60.4	60.6	60.7	60.8	60.9	61.1
36	61.2	61.3	61.5	61.6	61.7	61.9	62.0	62.1	62.2	62.4
37	62.5	62.6	62.8	62.9	63.0	63.2	63.3	63.4	63.5	63.7
38	63.8	63.9	64.1	64.2	64.4	64.5	64.6	64.8	64.9	65.1
39	65.2	65.4	65.5	65.7	65.8	66.0	66.2	66.3	66.5	66.6
40	66.8	67.0	67.2	67.3	67.5	67.7	67.9	68.1	68.2	68.4
41	68.6	68.8	69.0	69.2	69.4	69.6	69.7	69.9	70.1	70.3
42	70.5	70.7	70.9	71.0	71.2	71.4	71.6	71.8	71.9	72.1
43	72.3	72.5	72.7	72.9	73.1	73.3	73.4	73.6	73.8	74.0
44	74.2	74.4	74.6	74.8	75.0	75.2	75.3	75.5	75.7	75.9

PERCENTAGE OF CINEOLE (*continued*)

Temperature	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
45	76.1	76.3	76.5	76.7	76.9	77.1	77.2	77.4	77.6	77.8
46	78.0	78.2	78.4	78.6	78.8	79.0	79.2	79.4	79.6	79.8
47	80.0	80.2	80.4	80.6	80.8	81.1	81.3	81.5	81.7	81.9
48	82.1	82.3	82.5	82.7	82.9	83.2	83.4	83.6	83.8	84.0
49	84.2	84.4	84.6	84.8	85.0	85.3	85.5	85.7	85.9	86.1
50	86.3	86.6	86.8	87.1	87.3	87.6	87.8	88.1	88.3	88.6
51	88.8	89.1	89.3	89.6	89.8	90.1	90.3	90.6	90.8	91.1
52	91.3	91.6	91.8	92.1	92.3	92.6	92.8	93.1	93.3	93.6
53	93.8	94.1	94.3	94.6	94.8	95.1	95.3	95.6	95.8	96.1
54	96.3	96.6	96.9	97.2	97.5	97.8	98.1	98.4	98.7	99.0
55	99.3	99.7	100.0							

PHENOLS

Pipet 10 ml of the oil, which has been subjected to any treatment specified in the monograph, into a 100-ml cassia flask, add 75 ml of potassium hydroxide TS, and shake vigorously for 5 min to ensure complete extraction of the phenol by the alkali solution. Allow the mixture to stand for about 30 min, then add sufficient potassium hydroxide TS to raise the oily layer into the graduated portion of the flask, stopper the flask, and allow it to stand overnight. Read the volume of insoluble oil to 0.05 ml. Calculate the percentage, by volume, of phenols by the formula

$$P = (10 - V) \times 10,$$

in which P is the percentage of phenols, by volume, and V is the observed volume of insoluble oil, in ml.

FREE PHENOLS

Transfer about 5 g of the sample, accurately weighed, into a 150-ml flask having a standard-taper neck. Pipet exactly 10 ml of a 1 in 10 solution of acetic anhydride in anhydrous pyridine into the flask, and pipet exactly 10 ml of this solution, preferably measured with the same pipet, into a second 150-ml flask for the residual blank titration (see page 2). Connect the flasks to condensers, reflux for 1 h, and cool to a temperature below 100°. Add 25 ml of water to each flask through the condensers, and reflux again for 10 min. Cool the flasks, add phenolphthalein TS, and titrate with 0.5 N potassium hydroxide. Calculate the percentage of free phenols by the formula

$$\text{Percentage of Free Phenols} = (b - S) \times 100f/W,$$

in which b is the number of ml of 0.5 N potassium hydroxide consumed in the residual blank titration, s is the number of ml of 0.5 N potassium hydroxide consumed in the titration of the sample, f is the equivalence factor given in the monograph, and W is the weight of the sample, in mg.

RESIDUE ON EVAPORATION

Weigh accurately the quantity of sample specified in the monograph, and transfer it into a suitable evaporating dish that has previously been heated on a steam bath, cooled to room temperature in a desiccator, and accurately weighed. Weigh the sample in the dish. Heat the evaporating dish containing the sample on the steam bath for the period of time specified in the monograph. Cool the dish and its contents to room temperature in a desiccator, and weigh accurately. Calculate the residue as percentage of the sample used.

SOLUBILITY IN ALCOHOL

Transfer a 1.0-ml sample into a calibrated 10-ml glass-stoppered cylinder graduated in 0.1-ml subdivisions, and add slowly, in small portions, alcohol of the concentration specified in the monograph. Maintain the temperature at 25°, and shake the cylinder thoroughly after each addition of alcohol. When a clear solution is first obtained, record the number of ml of alcohol required. Continue the addition of the alcohol until a total of 10 ml has been added. If opalescence or cloudiness occurs during these subsequent additions of alcohol, record the number of ml of alcohol at which the phenomenon occurs.

ULTRAVIOLET ABSORBANCE OF CITRUS OILS

Transfer the quantity of the sample specified in the monograph into a 100-ml volumetric flask, add alcohol to volume, and mix. Determine the ultraviolet absorption spectrum of the solution in the range of 260 to 400 nm in a 1-cm cell with a suitable recording or manual spectrophotometer, using alcohol as the blank. If a manual instrument is used, read absorbances at 5-nm intervals from 260 nm to a point about 12 nm from the expected maximum absorbance, then at 3-nm intervals for three readings, and at 1-nm intervals to a point about 5 nm beyond the maximum, and then at 10-nm intervals to 400 nm. From these data, plot the absorbances as ordinates against wavelength on the abscissa, and draw the spectrogram. Draw a baseline tangent to the areas of minimum absorbance, as shown Fig. 8 (which is typical of lemon oil), joining point A in the region of

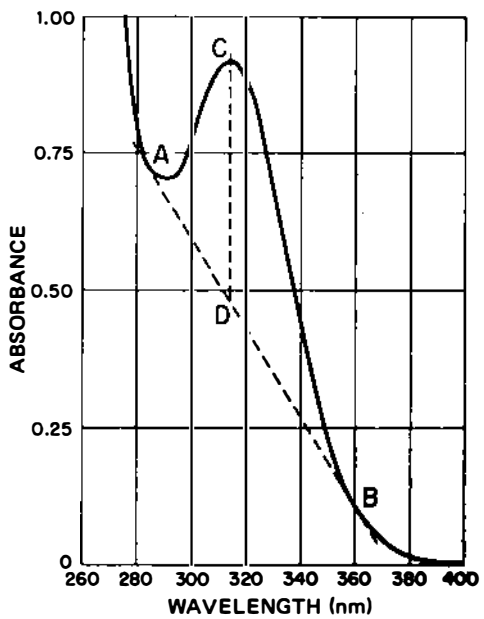


FIGURE 8 Typical Spectrogram of Lemon Oil

280 to 300 nm and a second point, *B*, in the region of 355 to 380 nm. Locate the point of maximum absorbance, *C*, and from it drop a vertical line, perpendicular to the abscissa, that intersects line *AB* at *D*. Read from the ordinate the absorbances corresponding to points *C* and *D*, subtract the latter from the former, and correct the difference for the actual weight of oil taken, calculating to the basis of the sample weight specified in the monograph.

VOLATILE OIL CONTENT

This procedure is used, when specified in the individual monograph, for determining the volatile oil content of gums, resins, and essential oils.

Apparatus The apparatus* is shown in Fig. 9. It consists of a 1000-ml boiling flask, *A*, attached through trap *D* to a Liebig condenser, *C*, which is connected to a 25-ml collector tube, *B*, graduated in 0.10-ml units.

Procedure Place 750 ml of water in the boiling flask, boil for 10 min, and cool to 50°. Transfer the specified volume of the sample, prepared as directed in the monograph, into the flask, then immediately attach the remainder of the apparatus to the flask, and boil until the volume of distilled oil collected in the graduated collector tube remains constant. Avoid splashing the contents of the flask in order to prevent contamination of the distillate with nonvolatile material, and do not continue distillation for an extended time after the volume of distillate becomes constant. If the distilled oil is heavier than water, set the stopcock in the closed position to prevent return of the heavy distillate to the flask.

When distillation is complete, allow the contents of the collection tube to settle until the oil and water layers are separated completely. Allow the distillate to cool to room temperature, read its volume, and calculate therefrom the percentage of volatile oil. (NOTE: When the volatile oil thus collected is to be used in additional tests, as may be specified in the monograph, the oil should be drained off, dried, and filtered before use.)

*Available as Catalog No. JD1135 from Scientific Glass Apparatus Company, 735 Broad St., Bloomfield, N.J. 07003.

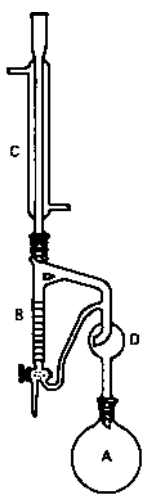


FIGURE 9 Apparatus for Determination of Volatile Oil Content

Fats and Related Substances

ACETYL VALUE
(Based on AOCS Method Cd 4-40)

The acetyl value is defined as the number of mg of potassium hydroxide required to neutralize the acetic acid obtained by saponifying 1 g of the acetylated sample.

Acetylation Boil 50 ml of the oil or melted fat with 50 ml of freshly distilled acetic anhydride for 2 h under a reflux condenser. Pour the mixture into a beaker containing 500 ml of water, and boil for 15 min, bubbling a stream of nitrogen or carbon dioxide through the mixture to prevent bumping. Cool slightly, remove the water, add another 500 ml of water, and boil again. Repeat for a third time with another 500-ml portion of water, and remove the wash water, which should be neutral to litmus. Transfer the acetylated fat to a separator, and wash with two 200-ml portions of warm water, separating as much as possible of the wash water each time. Transfer the washed sample to a beaker, add 5 g of anhydrous sodium sulfate, and let stand for 1 h, agitating occasionally to assist drying. Filter the oil through a dry filter paper, preferably in an oven at 100° to 110°, and keep the filtered oil in the oven until it is completely dry. The acetylated product should be a clear, brilliant oil.

Saponification Weigh accurately from 2 to 2.5 g each of the acetylated oil and of the original, untreated sample into separate 250-ml Erlenmeyer flasks. Add to each flash 25.0 ml of 0.5 *N* alcoholic potassium hydroxide, and continue as directed in the *Procedure* under *Saponification Value*, page 509, beginning with "Connect an air condenser. . . . " Record the saponification value of the untreated sample as *S*, and that of the acetylized oil as *S'*, then calculate the acetyl value of the sample by the formula

$$(S' - S)/(1.000 - 0.00075S).$$

ACID VALUE
(Based on AOCS Methods Te 1a-64 and Cd 3a-63)

The acid value is defined as the number of mg of potassium hydroxide required to neutralize the fatty acids in 1 g of the test substance.

Method I

Unless otherwise directed, weigh accurately about 5 g of the sample into a 500-ml Erlenmeyer flask, and dissolve it in from 75 to 100 ml of hot alcohol, previously boiled and neutralized to

phenolphthalein TS with sodium hydroxide. Agitation and further heating may be necessary to effect complete solution of the sample. Add 0.5 ml of phenolphthalein TS, and titrate immediately, while shaking, with 0.5 *N* sodium hydroxide to the first pink color that persists for at least 30 s. Calculate the acid value by the formula $56.1V \times N/W$, in which V is the volume, in ml, and N is the normality, respectively, of the sodium hydroxide solution, and W is the weight, in g, of the sample taken.

Method II

Prepare a solvent mixture consisting of equal parts, by volume, of isopropyl alcohol and toluene. Add 2 ml of a 1% solution of phenolphthalein in isopropyl alcohol to 125 ml of the mixture, and neutralize with alkali to a faint but permanent pink color. Weigh accurately the appropriate amount of well-mixed liquid sample indicated in the table below, dissolve it in the neutralized solvent mixture, warming if necessary, and shake vigorously while titrating with 0.1 *N* potassium hydroxide to the first permanent pink color of the same intensity as that of the neutralized solvent before mixing with the sample. Calculate the acid value by the formula $56.1V \times N/W$, in which V is the volume, in ml, and N is the normality, respectively, of the potassium hydroxide solution, and W is the weight, in g, of the sample taken.

Acid Value	Sample Weight (g)
0–1	20
1–4	10
4–15	2.5
15–75	0.5
75 and over	0.1

FREE FATTY ACIDS
(Based on AOCS Method Ca 5a-40)

Unless otherwise directed, weigh accurately the appropriate amount of the sample, indicated in the table below, into a 250-ml Erlenmeyer flask or other suitable container. Add 2 ml of phenolphthalein TS to the specified amount of hot alcohol, neutralize with alkali to the first faint but permanent pink color, and then add the hot neutralized alcohol to the sample container. Titrate with the appropriate normality of sodium hydroxide, shaking vigorously, to the first permanent pink color of the same intensity as that of the neutralized alcohol. The color must persist for at least 30 s. Calculate the percentage of free fatty acids (FFA) in the sample by the formula VNe/W, in which V is the volume and N is the normality, respectively, of the sodium hydroxide used, W is the weight of the sample, in g, and e is the equivalence factor given in the monograph.

FFA Range (%)	Grams of Sample	Milliliters of Alcohol	Strength of NaOH
0.00–0.2	56.4 ± 0.2	50	0.1 *N*
0.2–1.0	28.2 ± 0.2	50	0.1 *N*
1.0–30.0	7.05 ± 0.05	75	0.25 *N*
30.0–50.0	7.05 ± 0.05	100	0.25–1.0 *N*
50.0–100	3.525 ± 0.001	100	1.0 *N*

FREE GLYCERIN OR PROPYLENE GLYCOL
(Based on AOCS Method Ca 14-56)

Reagents and Solutions Use the *Periodic Acid Solution*, *Potassium Iodide Solution*, and the *Chloroform* as described under *1-Monoglycerides*, page 506.

Procedure To the combined aqueous extracts obtained as directed under *1-Monoglycerides* add 50.0 ml of *Periodic Acid Solution*. Run two blanks by adding 50.0 ml of this reagent solution to two 500-ml glass-stoppered Erlenmeyer flasks, each containing 75 ml of water. Continue as directed in the *Procedure* under *1-Monoglycerides*, beginning with " . . . and allow to stand for at least 30 min but no longer than 90 min."

Calculation Calculate the percentage of free glycerin in the original sample by the formula

$$(b - S) \times N \times 2.30/W,$$

or calculate the percentage of free propylene glycol by the formula

$$(b - S) \times N \times 3.81/W,$$

in which b is the number of ml of sodium thiosulfate consumed in the blank determination, S is the number of ml required in the titration of the aqueous extracts from the sample, N is the exact normality of the sodium thiosulfate, W is the weight, in g, of the original sample taken, 2.30 is the molecular weight of glycerin divided by 40, and 3.81 is the molecular weight of propylene glycol divided by 20.

NOTE: If the aqueous extract contains more than 20 mg of glycerin or more than 30 mg of propylene glycol, dilute the extract in a volumetric flask and transfer a suitable aliquot into a 500-ml glass-stoppered Erlenmeyer flask before proceeding with the test. The weight of the sample should be corrected in the calculation.

HYDROXYL VALUE
(Based on AOCS Methods Cd 4-40 and Cd 13-60)

The hydroxyl value is defined as the number of mg of potassium hydroxide equivalent to the hydroxyl content of 1 g of the unacetylated sample.

Method I

Proceed as directed under *Acetyl Value*, page 503, but calculate the hydroxyl value by the formula

$$(S' - S)/(1.000 - 0.00075S').$$

Method II

Unless otherwise directed, weigh accurately the appropriate amount of the sample indicated in the table below, transfer it into a 250-ml glass-stoppered Erlenmeyer flask, and add 5.0 ml of pyridine–acetic anhydride reagent (mix 3 volumes of freshly distilled pyridine with 1 volume of freshly distilled acetic anhydride).

Hydroxyl Value	Sample Weight (g)
0–20	10
20–50	5
50–100	3
100–150	2
150–200	1.5
200–250	1.25
250–300	1.0
300–350	0.75

Pipet 5 ml of the pyridine–acetic anhydride reagent into a second 250-ml flask for the reagent blank. Heat the flasks for 1 h on a steam bath under reflux condensers, then add 10 ml of water through each condenser, heat for 10 min longer, and allow the flasks to cool to room temperature. Add 15 ml of *n*-butyl alcohol, previously neutralized to phenolphthalein TS with 0.5 *N* alcoholic potassium hydroxide, through the condenser, then remove the condensers and wash the sides of the flasks with 10 ml of *n*-butyl alcohol. To each flask add 1 ml of phenolphthalein TS, and titrate to a faint pink endpoint with 0.5 *N* alcoholic potassium hydroxide, recording the ml required for the sample as *S* and that for the blank as *B*. To correct for free acid, mix about 10 g of the sample, accurately weighed, with 10 ml of freshly distilled pyridine, previously neutralized to phenolphthalein, add 1 ml of phenolphthalein TS, and titrate to a faint endpoint with 0.5 *N* alcoholic potassium hydroxide, recording the ml required as *A*. Calculate the hydroxyl value by the formula

$$[B + (WA/C) - S] \times 56.1N/W,$$

in which *W* and *C* are the weights, in g, of the samples taken for acetylation and for the free acid determination, respectively, and *N* is the exact normality of the alcoholic potassium hydroxide.

IODINE VALUE

The iodine value is a measure of unsaturation and is expressed as the number of g of iodine absorbed, under the prescribed conditions, by 100 g of the test substance.

Hanus Method

Iodobromide TS Dissolve 13.615 g of iodine, with the aid of heat, in 825 ml of glacial acetic acid that shows no reduction with dichromate and sulfuric acid. Cool, and titrate 25 ml of the solution with 0.1 *N* sodium thiosulfate, recording the volume required as *B*. Prepare another solution containing 3 ml (about 9 g) of bromine in 200 ml of glacial acetic acid. To 5 ml of this solution add 10 ml of potassium iodide TS, and titrate with 0.1 *N* sodium thiosulfate, recording the volume required as *C*. Calculate the quantity, *A*, of the bromine solution required to double the halogen content of the remaining 800 ml of iodine solution by the formula $800B/5C$. Mix the calculated volume, *A*, of the bromine solution with the iodine solution, and store in glass containers protected from light.

Procedure Weigh accurately the quantity of the sample specified in the monograph, transfer it into a 250-ml iodine flask, and dissolve it in 10 ml of chloroform. Add 25.0 ml of iodobromide TS, stopper the flask securely, and allow it to stand for exactly 30 min protected from light. Add 30 ml of potassium iodide TS followed by 100 ml of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, shaking thoroughly after each addition of the titrant. When the iodine color becomes quite pale, add 1 ml of starch TS and continue the titration until the blue color is discharged. Perform a blank determination (see page 2), and calculate the iodine value by the formula

$$(B - S) \times 12.69N/W,$$

in which $B - S$ represents the difference between the volumes of 0.1 *N* sodium thiosulfate required for the blank and the sample, respectively, *N* is the exact normality of the sodium thiosulfate, and *W* is the weight, in g, of the sample taken.

Wijs Method

Wijs Solution Dissolve 13 g of resublimed iodine in 1000 ml of glacial acetic acid. Pipet 10.0 ml of this solution into a 250-ml flask, add 20 ml of potassium iodide TS and 100 ml of water, and titrate with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Record the volume required as *A*. Set aside about 100 ml of the iodine–acetic acid solution for future use. Pass chlorine gas, washed and dried with sulfuric acid, through the remainder of the solution until a 10.0-ml portion requires not quite twice the volume of 0.1 *N* sodium thiosulfate consumed in the titration of the original iodine solution. A characteristic color change occurs when the desired amount of chlorine has been added. Alternatively, Wijs solution may be prepared by dissolving 16.5 g of iodine monochloride, ICl, in 100 ml of glacial acetic acid. Store the solution in amber bottles sealed with paraffin until ready for use, and use within 30 days.

Total Halogen Content Pipet 10.0 ml of *Wijs Solution* into a 500-ml Erlenmeyer flask containing 150 ml of recently boiled and cooled water and 15 ml of potassium iodide TS. Titrate immediately with 0.1 *N* sodium thiosulfate, recording the volume required as *B*.

Halogen Ratio Calculate the I/Cl ratio by the formula $A/(B - A)$. The halogen ratio must be between 1.0 and 1.2. If the ratio is not within this range, the halogen content can be adjusted by the addition of the original solution or by passing more chlorine through the solution.

Procedure The appropriate weight of the sample, in g, is calculated by dividing the number 25 by the expected iodine value. Melt the sample, if necessary, and filter it through a dry filter paper. Transfer the accurately weighed quantity of the sample into a clean, dry, 500-ml glass-stoppered bottle or flask containing 20 ml of carbon tetrachloride, and pipet 25.0 ml of *Wijs Solution* into the flask. The excess of iodine should be between 50% and 60% of the quantity added, that is, between 100% and 150% of the quantity absorbed. Swirl, and let stand in the dark for 30 min. Add 20 ml of potassium iodide TS and 100 ml of recently boiled and cooled water, and titrate the excess iodine with 0.1 *N* sodium thiosulfate, adding the titrant gradually and shaking constantly until the yellow color of the

solution almost disappears. Add starch TS, and continue the titration until the blue color disappears entirely. Toward the end of the titration, stopper the container and shake it violently so that any iodine remaining in solution in the carbon tetrachloride may be taken up by the potassium iodide solution. Concomitantly, conduct two determinations on blanks in the same manner and at the same temperature (see page 2). Calculate the iodine value by the formula

$$(B - S) \times 12.69N/W,$$

in which $B - S$ represents the difference between the volumes of sodium thiosulfate required for the blank and for the sample, respectively, N is the normality of the sodium thiosulfate, and W is the weight, in g, of the sample taken.

1-MONOGLYCERIDES
(Based on AOCS Method Cd 11-57)

Reagents and Solutions

Periodic Acid Solution Dissolve 5.4 g of periodic acid, H_5IO_6, in 100 ml of water, add 1900 ml of glacial acetic acid, and mix. Store in a light-resistant, glass-stoppered bottle, or in a clear, glass-stoppered bottle protected from light.

Chloroform Use chloroform meeting the following test: To each of three 500-ml flasks add 50.0 ml of *Periodic Acid Solution*, then add 50 ml of chloroform and 10 ml of water to two of the flasks and 50 ml of water to the third. To each flask add 20 ml of potassium iodide TS, mix gently, and continue as directed in the *Procedure*, beginning with " . . . allow to stand at least 1 min. . . . " The difference between the volume of 0.1 N sodium thiosulfate required in the titrations with and without the chloroform is not greater than 0.5 ml.

Procedure Melt the sample, if not liquid, at a temperature not higher than 10° above its melting point, and mix thoroughly. Transfer an accurately weighed portion of the sample, equivalent to about 150 mg of 1-monoglycerides, into a 100-ml beaker (or weigh a sample equivalent to 20 mg of glycerin or 30 mg of propylene glycol if only *Free Glycerin or Propylene Glycol* is to be determined), and dissolve in 25 ml of chloroform. Transfer the solution, with the aid of an additional 25 ml of chloroform, into a separator, wash the beaker with 25 ml of water, and add the washing to the separator. Stopper the separator tightly, shake vigorously for 30 to 60 s, and allow the layers to separate. (Add 1 to 2 ml of glacial acetic acid to break emulsions formed due to the presence of soap.) Collect the aqueous layer in a 500-ml glass-stoppered Erlenmeyer flask, and extract the chloroform solution again using two 25-ml portions of water. Retain the combined aqueous extracts for the determination of *Free Glycerin or Propylene Glycol*, page 504. Transfer the chloroform to a 500-ml glass-stoppered Erlenmeyer flask, and add 50.0 ml of *Periodic Acid Solution* to this flask and to each of two blank flasks containing 50 ml of chloroform and 10 ml of water. Swirl the flasks during the addition of the reagent, and allow to stand for at least 30 min but no longer than 90 min. To each flask add 20 ml of potassium iodide TS, and allow to stand at least 1 min but no longer than 5 min before titrating. Add 100 ml of water, and titrate with 0.1 N sodium thiosulfate, using a magnetic stirrer to keep the solution thoroughly mixed, to the disappearance of the brown iodine color, then add 2 ml of starch TS and continue the titration to the disappearance of the blue color. Calculate the percentage of 1-monoglycerides* in the sample by the formula

$$(B - S) \times N \times 17.927/W,$$

in which B is the number of ml of sodium thiosulfate consumed in the blank determination, S is the number of ml required in the titration of the sample, N is the exact normality of the sodium thiosulfate, W is the weight, in g, of the sample taken, and 17.927 is the molecular weight of glyceryl monostearate divided by 20.

TOTAL MONOGLYCERIDES

Preparation of Silica Gel Place about 10 g of 100- to 200-mesh silica gel of a grade suitable for chromatographic work in a tared weighing bottle, cap immediately, and weigh accurately. Remove the cap, dry at 200° for 2 h, cap immediately, and cool for 30 min. Raise the cap momentarily to equalize the pressure, then weigh again, reheat for 5 min at 200°, cool, and reweigh. Repeat this 5-min drying cycle until two consecutive weights agree within 10 mg. Calculate the percentage of water in the original silica gel (A) by the formula (loss in wt/sample wt) × 100, then calculate the amount of water required to adjust the water content to 5% by the formula

$$W \times (5 - A)/95,$$

in which W is the weight, in g, of the undried sample to be used.

Weigh accurately the appropriate amount of the undried silica gel to be used in the determination, transfer to a suitable blender or mixer, and add the calculated amount of water to give a final water content of 5% ± 0.1%. Blend for 1 h to ensure complete water distribution, and store in a sealed container. Determine the water content of the adjusted silica gel as directed above, and readjust if necessary. (NOTE: Each new lot of silica gel should be checked for suitability by the analysis of a monoglyceride of known composition.)

Sample Preparation

Caution: To avoid rearrangement of partial glycerides, use extreme caution in applying heat to samples, and do not heat above 50°.

Samples Melting below 50° Melt the sample, if necessary, by warming for short periods below 50°, not exceeding a total of 30 min.

Samples Melting above 50° Grind about 10 g in a mortar and pestle, chilling solid samples, if necessary, in carbon dioxide.

Weigh accurately about 1 g of the prepared sample into a 100-ml beaker, add 15 ml of chloroform, and warm, if necessary, to effect solution. Use only minimum heat, and do not heat above 40°.

*The monoglyceride may be calculated to some monoester other than glyceryl monostearate by dividing the molecular weight of the monoglyceride by 20 and substituting the value so obtained for 17.927 in the formula, using 17.80, for example, in calculating to the monooleate.

Preparation of Chromatographic Column Connect a 19- × 290-mm chromatographic tube, equipped with an outer 19/22 standard-taper joint at the top and a coarse fritted-glass disk and inner 19/22 standard-taper joint at the bottom, with an adapter consisting of an outer 19/22 joint connected to a Teflon stopcock. Do not grease the joints. Weigh 30 g of the prepared silica gel into a 150-ml beaker, add 50 to 60 ml of petroleum ether, and stir slowly with a glass rod until all air bubbles are expelled. Transfer the slurry to the column through a powder funnel, and open the stopcock, allowing the liquid level to drop to about 2 cm above the silica gel. Transfer any silica gel slurry remaining in the beaker into the column with a minimum amount of petroleum ether, then rinse the funnel and sides of the column. Drain the solvent through the stopcock until the level drops to 2 cm above the silica gel, and remove the powder funnel.

Procedure Carefully add the *Sample Preparation* to the prepared column. Open the stopcock, and adjust the flow rate to about 2 ml per min, discarding the eluate. Rinse the sample beaker with 5 ml of chloroform, and add the rinsing to the column when the level drops to 2 cm above the silica gel. Never allow the column to become dry on top, and maintain a flow rate of 2 ml per min throughout the elution. Avoid interruptions during elution as they may cause pressure buildup and result in leakage through the stopcock or cracks in the silica gel packing.

Attach a 250-ml reservoir separator, provided with a Teflon stopcock and a 19/22 standard-taper drip tip inner joint, to the column. Add 200 ml of benzene, elute, and discard the eluate, which contains the triglycerides fraction. When the level of benzene drops to 2 cm above the silica gel, add 200 ml of a 1 in 10 mixture of ether in benzene, elute, and discard the eluate, which contains the diglycerides and the free fatty acid fraction. When all of the ether-benzene solvent has been added from the separator and the level in the column drops to 2 cm above the silica gel, add from 250 to 300 ml of ether, and collect the monoglyceride fraction in a tared flask. Rinse the tip of the column into the flask with a few ml of ether, and evaporate to dryness on a steam bath under a stream of nitrogen or dry air. Cool for at least 15 min, weigh, then reheat on the steam bath for 5 min in the same manner. Cool, reweigh, and repeat the 5-min evaporation, cooling, and reweighing procedures until two consecutive weights agree within 2 mg. The weight of the residue represents the total monoglycerides in the sample taken.

OXYETHYLENE DETERMINATION

Apparatus The apparatus for oxyethylene group determination is shown in Fig. 10. It consists of a boiling flask, *A*, fitted with a capillary side tube to provide an inlet for carbon dioxide and connected by a condenser with trap *B* , which contains an aqueous suspension of red phosphorus. The first absorption tube, *C*, contains a silver nitrate solution to absorb ethyl iodide. Absorption tube *D* is fitted with a 1.75-mm spiral rod (23 turns, 8.5-mm rise per turn), which is required to provide a longer contact of the evolved ethylene with the bromine solution. A standard-taper adapter and stopcock are connected to tube *D* to permit the transfer of the bromine solution into a titration flask without loss. A final trap, *E*, containing a potassium iodide solution, collects any bromine swept out by the flow of carbon dioxide.

Dimensions of the apparatus not readily determined from Fig. 10 are as follows: carbon dioxide inlet capillary, 1-mm id; flask *A*, 28-mm diameter, 12/18 standard-taper joint; condenser, 9-mm id; inlet to trap *B*, 2-mm id; inlet to trap *C*, 7/15 standard-taper joint, 2-mm id; trap *C*, 14-mm id; trap *D*, inner tube, 8-mm od, 2-mm opening at bottom of spiral; outer tube, approximately 12.5-mm id; side arm 7 cm from top of inserted spiral, 3.5-mm id, 2-mm opening at bottom.

Reagents

Hydriodic Acid Use special-grade hydriodic acid suitable for alkoxyl determinations, or purify reagent-grade as follows: Distil over red phosphorus in an all-glass apparatus, passing a slow stream of carbon dioxide through the apparatus until the distillation is terminated and the receiving flask has completely cooled.

Caution: Use a safety shield and conduct the distillation in a hood.

Silver Nitrate Solution Dissolve 15 g of silver nitrate in 50 ml of water, mix with 400 ml of alcohol, and add a few drops of nitric acid.

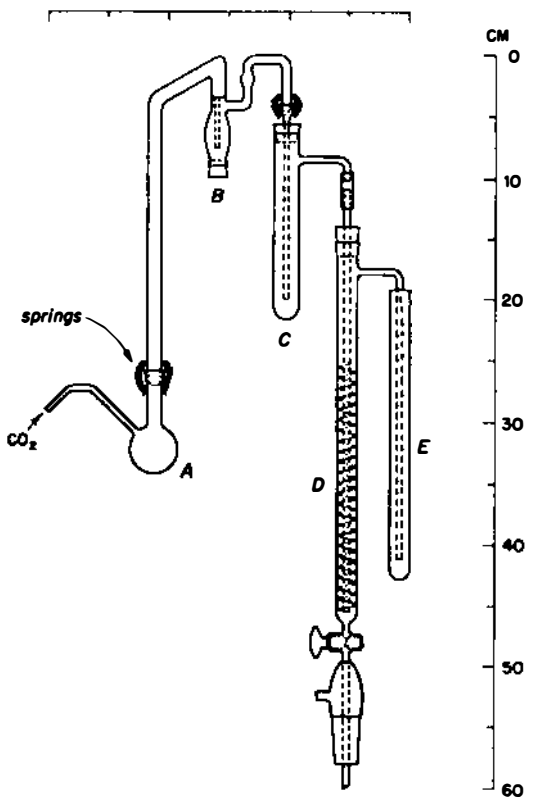


FIGURE 10 Apparatus for Oxyethylene Determination

Bromine-Bromide Solution Add 1 ml of bromine to 300 ml of glacial acetic acid saturated with dry potassium iodide (about 5 g). Fifteen ml of this solution requires about 40 ml of 0.05 *N* sodium thiosulfate. Store in a brown bottle in a dark place, and standardize at least once a day during use.

Procedure Fill trap *B* with enough of a suspension of 60 mg of red phosphorus in 100 ml of water to cover the inlet tube. Pipet 10 ml of the *Silver Nitrate Solution* into tube *C* and 15 ml of the *Bromine-Bromide Solution* into tube *D*, and place 10 ml of a 1 in 10 solution of potassium iodide in trap *E*. Transfer an accurately weighed quantity of the sample specified in the monograph into the reaction flask, *A*, and add 10 ml of *Hydriodic Acid* along with a few glass beads or boiling stones. Connect the flask to the condenser, and begin passing carbon dioxide through the apparatus at the rate of about one bubble per second. Heat the flask in an oil bath at 140° to 145°, and continue the reaction at this temperature for at least 40 min. Heating should be continued until the cloudy reflux in the condenser becomes clear and until the supernatant liquid in the silver nitrate tube, *C*, is almost completely clarified. Five min before the reaction is terminated, heat the *Silver Nitrate Solution* in tube *C* in a hot water bath at 50° to 60° to expel any dissolved olefin. At the completion of the decomposition, disconnect cautiously tubes *D* and *C* in the order named, then disconnect the carbon dioxide source and remove the oil bath. Connect tube *D* to a 500-ml iodine flask containing 150 ml of water and 10 ml of a 1 in 10 solution of potassium iodide, run the *Bromine-Bromide Solution* into the flask, and rinse the tube and spiral with water. Add the potassium iodide solution from trap *E* to the flask, rinsing the side arm and tube with a few ml of water, stopper the flask, and allow to stand for 5 min. Add 5 ml of diluted sulfuric acid TS, and titrate immediately with 0.05 *N* sodium thiosulfate, using 2 ml of starch TS for the endpoint. Transfer the silver nitrate solution from tube *C* into a flask, rinsing the tube with water, dilute to 150 ml with water, and heat to boiling. Cool, and titrate with 0.05 *N* ammonium thiocyanate, using 3 ml of ferric ammonium sulfate TS as the indicator. Perform a blank determination (see page 2). Calculate the percentage of oxyethylene groups (—CH_2CH_2O—), as ethylene, by the formula

$$(B - S) \times N \times 2.203/W,$$

in which $B - S$ represents the difference between the volumes of sodium thiosulfate required for the blank and the sample solution, respectively, N is the normality of the sodium thiosulfate, W is the weight, in g, of the sample taken, and 2.203 is an equivalence factor for oxyethylene. Calculate the percentage of oxyethylene groups, as ethyl iodide, by the formula

$$(B' - S') \times N' \times 4.405/W,$$

in which $B' - S'$ represents the difference between the volumes of ammonium thiocyanate required for the blank and the sample solution, respectively, N' is the normality of the ammonium thiocyanate, and 4.405 is an equivalence factor for oxyethylene. The sum of the values so obtained represents the percentage of oxyethylene groups in the sample taken.

REICHERT-MEISSL VALUE
(Based on AOCS Method Cd 5-40)

The Reichert-Meissl value is a measure of soluble volatile fatty acids (chiefly butyric and caproic). It is expressed in terms of the number of ml of 0.1 *N* sodium hydroxide required to neutralize the fatty acids obtained from a 5-g sample under the specified conditions of the method.

Apparatus Use a glass distillation apparatus of the same dimensions and construction as that shown in Fig. 11.

Reagents

Sodium Hydroxide Solution Prepare a solution containing 50.0% by weight of NaOH, and protect from contact with carbon dioxide. Allow the solution to settle and use only the clear liquid.

Glycerin–Sodium Hydroxide Mixture Add 20 ml of the *Sodium Hydroxide Solution* to 180 ml of glycerin.

Procedure Unless otherwise directed, weigh accurately about 5 g of the sample, previously melted if necessary, into the 300-ml distillation flask. Add 20.0 ml of the *Glycerin–Sodium Hydroxide Mixture*, and heat until the sample is completely saponified, as indicated by the mixture becoming perfectly clear. Shake the flask gently if any foaming occurs. Add 135 ml of recently boiled and cooled water, dropwise at first to prevent foaming, then add 6 ml of dilute sulfuric acid (1 in 5) and a few pieces of pumice stone or silicon carbide. Rest the flask on a

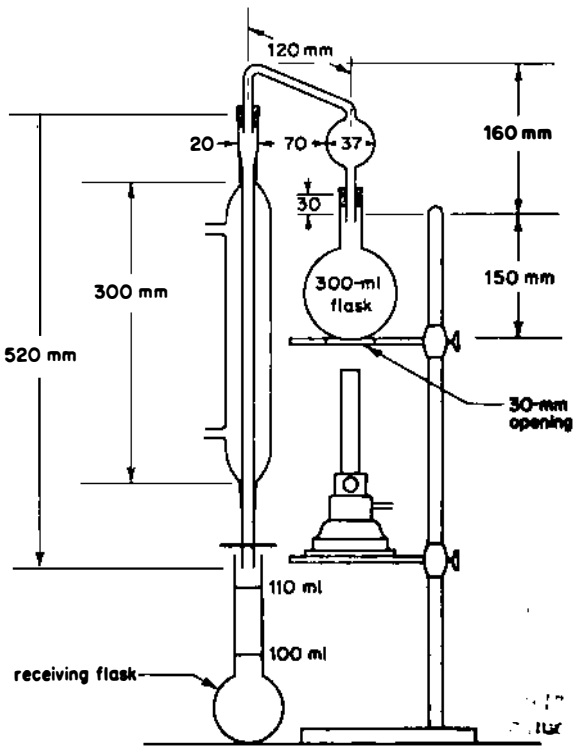


FIGURE 11 Reichert-Meissl Distillation Apparatus

piece of asbestos board having a center hole 5 cm in diameter, and begin the distillation, regulating the flame so as to collect 110 ml of distillate in 30 ± 2 min (measure time from the passage of the first drop of distillate from the condenser to the receiving flask), letting the distillate drip into the flask at a temperature not higher than 20°.

When 110 ml has distilled, disconnect the receiving flask, and remove the flame. Mix the contents of the flask with gentle shaking, and immerse almost completely for 15 min in water cooled to 15°. Filter the distillate through dry 9-cm moderately retentive paper (S & S No. 589 White Ribbon or equivalent), add phenolphthalein TS, and titrate 100 ml of the filtrate with 0.1 *N* sodium hydroxide to the first pink color that remains unchanged for 2 to 3 min. Perform a blank determination (see page 2) using the same quantities of the same reagents, and calculate the Reichert-Meissl value by the formula

$$1.1 \times (S - B),$$

in which S is the volume of 0.1 *N* sodium hydroxide required for the sample, and B is the volume required for the blank.

SAPONIFICATION VALUE
(Based on AOCS Methods Tl 1a-64 and Cd 3-25)

The saponification value is defined as the number of mg of potassium hydroxide required to neutralize the free acids and saponify the esters in 1 g of the test substance.

Procedure Melt the sample, if necessary, and filter it through a dry filter paper to remove any traces of moisture. Unless otherwise directed, weigh accurately into a 250-ml flask a sample of such size that the titration of the sample solution after saponification will require between 45% and 55% of the volume of 0.5 *N* hydrochloric acid required for the blank, and add to the flask 50.0 ml of 0.5 *N* alcoholic potassium hydroxide. Connect an air condenser, at least 65 cm in length, to the flask, and reflux gently until the sample is completely saponified (usually 30 min to 1 h). Cool slightly, wash the condenser with a few ml of water, add 1 ml of phenolphthalein TS, and titrate the excess potassium hydroxide with 0.5 *N* hydrochloric acid. Heat the contents of the flask to boiling, again titrate to the disappearance of any pink color that may have developed, and record the total volume of acid required. Perform a blank determination using the same amount of 0.5 *N* alcoholic potassium hydroxide (see page 2). Calculate the saponification value by the formula

$$56.1(B - S) \times N/W,$$

in which $B - S$ represents the difference between the volumes of 0.5 *N* hydrochloric acid required for the blank and the sample, respectively, N is the normality of the hydrochloric acid, and W is the weight, in g, of the sample taken. (NOTE: A "masked phenolphthalein indicator" may be used with off-color materials. Prepare the indicator by dissolving 1.6 g of phenolphthalein and 2.7 g of methylene blue in 500 ml of alcohol, and adjust the pH with alcoholic alkali solution so that the greenish blue color is faintly tinged with purple. The color change, when going from acid to alkali, is from green to purple.)

SOAP

Prepare a solvent mixture consisting of equal parts, by volume, of benzene and methanol, add bromophenol blue TS, and neutralize with 0.5 *N* hydrochloric acid, or use neutralized acetone as the solvent. Weigh accurately the amount of sample specified in the individual monograph, dissolve it in 100 ml of the neutralized solvent mixture, and titrate with 0.5 *N* hydrochloric acid to a definite yellow endpoint. Calculate the percentage of soap in the sample by the formula VNe/W, in which V and N are the volume and normality, respectively, of the hydrochloric acid, W is the weight of the sample, in g, and e is the equivalence factor given in the monograph.

SPECIFIC GRAVITY

The specific gravity of a fat or oil is determined at 25°, except when the substance is a solid at that temperature, in which case the specific gravity is determined at the temperature specified in the monograph, and is referred to water at 25°.

Clean a suitable pycnometer by filling it with a saturated solution of chromic acid (CrO_3) in sulfuric acid and allowing it to stand for at least 4 h. Empty the pycnometer, rinse it thoroughly, then fill it with recently boiled water, previously cooled to about 20°, and place in a constant-temperature bath at 25°. After 30 min, adjust the level of water to the proper point on the pycnometer, and stopper. Remove the pycnometer from the bath, wipe dry with a clean cloth free from lint, and weigh. Empty the pycnometer, rinse several times with alcohol and then with ether, allow to dry completely, remove any ether vapor, and weigh. Determine the weight of the contained water at 25° by subtracting the weight of the pycnometer from its weight when full.

Filter the oil or melted sample through filter paper to remove any impurities and the last traces of moisture, and cool to a few degrees below the temperature at which the determination is to be made. Fill the clean, dry pycnometer with the sample, and place it in the constant-temperature bath at the specified temperature. After 30 min, adjust the level of the oil to the mark on the pycnometer, insert the stopper, wipe dry, and weigh. Subtract the weight of the empty pycnometer from its weight when filled with the sample, and divide the difference by the weight of the water contained at 25°. The quotient is the specific gravity at the temperature of observation, referred to water at 25°.

UNSAPONIFIABLE MATTER
(Based on AOCS Method Ca 6a-40)

This procedure determines those substances frequently found dissolved in fatty materials that cannot be saponified by alkali hydroxides but that are soluble in the ordinary fat solvents.

Procedure Weigh accurately 5.0 g of the sample into a 250-ml flask, add a solution of 2 g of potassium hydroxide in 40 ml of alcohol, and boil gently under a reflux condenser for 1 h or until saponification is complete. Transfer the contents of the flask to a glass-stoppered extraction cylinder (approximately 30 cm in

length, 3.5 cm in diameter, and graduated at 40, 80, and 130 ml). Wash the flask with sufficient alcohol to make a volume of 40 ml in the cylinder, and complete the transfer with warm and then cold water until the total volume is 80 ml. Finally, wash the flask with a few ml of petroleum ether, add the washings to the cylinder, cool the contents of the cylinder to room temperature, and add 50 ml of petroleum ether.

Insert the stopper, shake the cylinder vigorously for at least 1 min, and allow both layers to become clear. Siphon the upper layer as completely as possible without removing any of the lower layer, collecting the ether fraction in a 500-ml separator. Repeat the extraction and siphoning at least six times with 50-ml portions of petroleum ether, shaking vigorously each time. Wash the combined extracts, with vigorous shaking, with 25-ml portions of 10% alcohol until the wash water is neutral to phenolphthalein, and discard the washings. Transfer the ether extract to a tared beaker, and rinse the separator with 10 ml of ether, adding the rinsings to the beaker. Evaporate the ether on a steam bath just to dryness, and dry the residue to constant weight, preferably at 75° to 80° under a vacuum of not more than 200 mm of Hg, or at 100° for 30 min. Cool in a desiccator, and weigh to obtain the uncorrected weight of unsaponifiable matter.

Determine the quantity of fatty acids in the residue as follows: Dissolve the residue in 50 ml of warm alcohol (containing phenolphthalein TS and previously neutralized with sodium hydroxide to a faint pink color), and titrate with 0.02 *N* sodium hydroxide to the same color. Each ml of 0.02 *N* sodium hydroxide is equivalent to 5.659 mg of fatty acids, calculated as oleic acid.

Subtract the calculated weight of fatty acids from the weight of the residue to obtain the corrected weight of unsaponifiable matter in the sample.

VOLATILE ACIDITY

Modified Hortvet-Sellier Method

Apparatus Assemble a modified Hortvet-Sellier distillation apparatus as shown in Fig. 12, using a sufficiently large (approximately 38- × 203-mm) inner Sellier tube and large distillation trap.

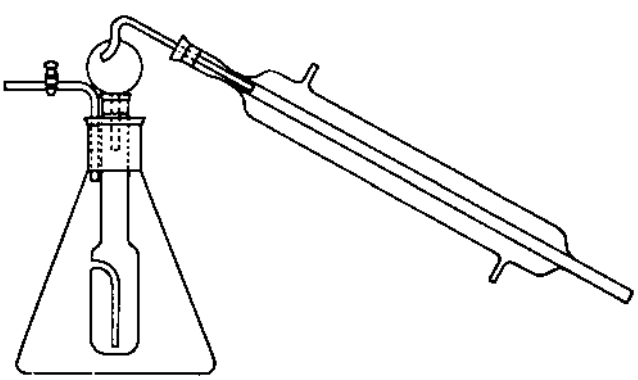


FIGURE 12 Modified Hortvet-Sellier Distillation Apparatus

Procedure Transfer the amount of sample, accurately weighed, specified in the monograph, into the inner tube of the assembly, and insert the tube in the outer flask containing about 300 ml of recently boiled hot water. To the sample add 10 ml of approximately 4 *N* perchloric acid [35 ml (60 g) of 70% perchloric acid in 100 ml of water], and connect the inner tube to a water-cooled condenser through the distillation trap. Distil by heating the outer flask so that 100 ml of distillate is collected within 20 to 25 min. Collect the distillate in 100-ml portions, add phenolphthalein TS to each portion, and titrate with 0.5 *N* sodium hydroxide. Continue the distillation until a 100-ml portion of the distillate requires no more than 0.5 ml of 0.5 *N* sodium hydroxide for neutralization. (*Caution:* Do not distil to dryness.) Calculate the weight, in mg, of volatile acids in the sample taken by the formula $V \times e$, in which V is the total volume, in ml, of 0.5 *N* sodium hydroxide consumed in the series of titrations and e is the equivalence factor given in the monograph.

Fluoride Limit Test

Method I (Thorium Nitrate Colorimetric Method)

This method should be used unless otherwise directed in the individual monograph.

Caution: When applying this test to organic compounds, the temperature at which the distillation is conducted must be rigidly controlled at all times to the recommended range of 135° to 140° to avoid the possibility of explosion.

NOTE: To minimize the distillation blank resulting from fluoride leached from the glassware, the distillation apparatus should be treated as follows: Treat the glassware with hot 10% sodium hydroxide solution, followed by flushing with tap water and rinsing with distilled water. At least once daily, treat in addition by boiling down 15 to 20 ml of dilute sulfuric acid (1 in 2) until the still is filled with fumes; cool, pour off the acid, treat again with 10% sodium hydroxide solution, and rinse thoroughly. For further details, see sections 25.050 and 25.054 in *Official Methods of Analysis of the A.O.A.C.*, Thirteenth Edition, 1980.

Unless otherwise directed, place a 5.0-g sample and 30 ml of water in a 125-ml distillation flask having a side arm and trap. The flask is connected with a condenser and carries a thermometer and a capillary tube, both of which must extend into the liquid. Slowly add, with continuous stirring, 10 ml of perchloric acid, and then add 2 or 3 drops of silver nitrate solution (1 in 2) and a few glass beads. Connect a small dropping funnel or a steam generator to the capillary tube. Support the flask on an asbestos mat with a hole that exposes about one third of the flask to the flame. Distil until the temperature reaches 135°. Add water from the funnel or introduce steam through the capillary, maintaining the temperature between 135° and 140° at

all times. Continue the distillation until 100 ml of distillate has been collected. After the 100-ml portion (*Distillate A*) is collected, collect an additional 50-ml portion of distillate (*Distillate B*) to ensure that all of the fluorine has been volatilized.

Place 50 ml of *Distillate A* in a 50-ml Nessler tube. In another similar Nessler tube place 50 ml of water distilled through the apparatus as a control. Add to each tube 0.1 ml of a filtered solution of sodium alizarinsulfonate (1 in 1000) and 1 ml of freshly prepared hydroxylamine hydrochloride solution (1 in 4000), and mix well. Add, dropwise and with stirring, either 1 *N* or 0.05 *N* sodium hydroxide, depending upon the expected volume of volatile acid distilling over, to the tube containing the distillate until its color just matches that of the control, which is faintly pink. Then add to each tube 1.0 ml of 0.1 *N* hydrochloric acid, and mix well. From a buret, graduated in 0.05 ml, add slowly to the tube containing the distillate enough thorium nitrate solution (1 in 4000) so that, after mixing, the color of the liquid just changes to a faint pink. Note the volume of the solution added, then add exactly the same volume to the control, and mix. Now add to the control solution sodium fluoride TS (10 μg F per ml) from a buret to make the colors of the two tubes match after dilution to the same volume. Mix well, and allow all air bubbles to escape before making the final color comparison. Check the endpoint by adding 1 or 2 drops of sodium fluoride TS to the control. A distinct change in color should take place. Note the volume of sodium fluoride TS added.

Dilute *Distillate B* to 100 ml, and mix well. Place 50 ml of this solution in a 50-ml Nessler tube, and follow the procedure used for *Distillate A*. The total volume of sodium fluoride TS required for the solutions from both *Distillate A* and *Distillate B* should not exceed 2.5 ml.

Method II (Ion-Selective Electrode Method A)

Buffer Solution Dissolve 36 g of cyclohexylenedinitrilotetraacetic acid (CDTA) in sufficient 1 *M* sodium hydroxide to make 200 ml. Transfer 20 ml of this solution (equivalent to 4 g of disodium CDTA) into a 1000-ml beaker containing 500 ml of water, 57 ml of glacial acetic acid, and 58 g of sodium chloride, and stir to dissolve. Adjust the pH of the solution to between 5.0 and 5.5 by the addition of 5 *M* sodium hydroxide, then cool to room temperature, dilute to 1000 ml with water, and mix.

Procedure Unless otherwise directed in the individual monograph, place an 8.0-g sample and 20 ml of water in a 250-ml distilling flask, cautiously add 20 ml of perchloric acid, and then add 2 or 3 drops of silver nitrate solution (1 in 2) and a few glass beads. Following the directions, and observing the *Caution* and *Note*, as given under *Method I*, distil the solution until 200 ml of distillate has been collected.

Transfer a 25.0-ml aliquot of the distillate into a 250-ml plastic beaker, and dilute to 100 ml with the *Buffer Solution*. Place the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selective electrode apparatus (such as the Orion Model 407) in the solution, and adjust the calibration control until the indicator needle points to the center on the logarithmic concentration scale, allowing sufficient time for equilibration (about 20 min) and stirring constantly during the equilibration period and throughout the remainder of the procedure. Pipet 1.0 ml of a solution containing 100 μg of fluoride ion (F) per ml (prepared by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 ml) into the beaker, allow the electrode to come to equilibrium, and record the final reading on the logarithmic concentration scale. (NOTE: Follow the instrument manufacturer's instructions regarding precautions and interferences, electrode filling and check, temperature compensation, and calibration.)

Calculations Calculate the fluoride content, in ppm, of the sample taken by the formula

$$[IA/(R - I)] \times 100 \times [200/25W],$$

in which I is the initial scale reading before the addition of the sodium fluoride solution; A is the concentration, in μg per ml, of fluoride in the sodium fluoride solution added to the sample solution; R is the final scale reading, after addition of the sodium fluoride solution; and W is the original weight of the sample, in g.

Method III (Ion-Selective Electrode Method B)

Sodium Fluoride Solution (5 μg F per ml) Transfer 2.210 g of sodium fluoride, previously dried at 110° for 2 h and accurately weighed, into a 400-ml plastic beaker, add 200 ml of water, and stir until dissolved. Quantitatively transfer this solution into a 1000-ml volumetric flask with the aid of water, dilute to volume with water, and mix. Store this stock solution in a plastic bottle. On the day of use, transfer 5.0 ml of the stock solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Calibration Curve Transfer into separate 250-ml plastic beakers 1.0, 2.0, 3.0, 5.0, 10.0, and 15.0 ml of the *Sodium Fluoride Solution*, add 50 ml of water, 5 ml of 1 *N* hydrochloric acid, 10 ml of 1 *M* sodium citrate, and 10 ml of 0.2 *M* disodium EDTA to each beaker, and mix. Transfer each solution into a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer a 50-ml portion of each solution into a 125-ml plastic beaker, and measure the potential of each solution with a suitable ion-selective electrode apparatus (such as the Orion Model No. 94-09, with solid-state membrane), using a suitable reference electrode (such as the Orion Model No. 90-01, with single junction). Plot the calibration curve on two-cycle semilogarithmic paper (such as K & E No. 465130), with μg F per 100 ml solution on the logarithmic scale.

Procedure Transfer 1.00 g of the sample into a 150-ml glass beaker, add 10 ml of water, and, while stirring continuously, add 20 ml of 1 *N* hydrochloric acid slowly to dissolve the sample. Boil rapidly for 1 min, then transfer into a 250-ml plastic beaker, and cool rapidly in ice water. Add 15 ml of 1 *M* sodium citrate and 10 ml of 0.2 *M* disodium EDTA, and mix. Adjust the pH to 5.5 ±0.1 with 1 *N* hydrochloric acid or 1 *M* sodium hydroxide, if necessary, then transfer into a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer a 50-ml portion of this solution into a 125-ml plastic beaker,

and measure the potential of the solution with the apparatus described under *Calibration Curve*. Determine the fluoride content, in µg, of the sample from the *Calibration Curve*.

Method IV (Ion-Selective Electrode Method C)

Buffer Solution A Add 2 volumes of 6 *N* acetic acid to 1 volume of water, and adjust the pH to 5.0 with 50% potassium hydroxide solution.

Buffer Solution B Dissolve 150 g of sodium citrate dihydrate and 10.3 g of disodium EDTA dihydrate in 800 ml of water, adjust the pH to 8.0 with 50% sodium hydroxide solution, and dilute to 1000 ml with water.

Buffer Solution C Dissolve 36 g of cyclohexylene dinitrilotetraacetic acid (CDTA) in sufficient 1 *N* sodium hydroxide to make 200 ml by boiling, then cool, and filter through glass-fiber filter paper. Pipet 30 ml of this solution into a mixture consisting of 750 ml of water, 87 g of sodium chloride, and 85.5 ml of glacial acetic acid. Adjust the pH to between 5.0 and 5.5 by the addition of 50% sodium hydroxide solution, then cool, and dilute to 3000 ml with water.

Fluoride Standard Solution Use a solution containing 100 µg of fluoride ion (F) per ml (100 ppm), obtained commercially or prepared by dissolving 22.2 mg of sodium fluoride, previously dried at 200° for 4 h, in sufficient water to make 100.0 ml.

Sample Preparation Weigh accurately the amount of sample specified in the monograph, transfer it into a 100-ml volumetric flask, and dissolve it in a minimum amount of water or in the volume of hydrochloric acid solution specified in the monograph. Add 50.0 ml of the appropriate buffer solution, *A*, *B*, or *C*, as specified in the monograph, dilute to volume with water, and mix.

Procedure Pipet a 50-ml aliquot of the *Sample Preparation* into a plastic beaker, and place in the solution the fluoride ion and reference electrodes (or a combination fluoride electrode) of a suitable ion-selective electrode apparatus with magnetic stirrer (Orion Model 407 or equivalent). Begin stirring slowly, and set the slope of the meter to 100% and the temperature control to room temperature, which should be the temperature of the solution. Adjust the calibration control to read infinity on the increment logarithmic scale, and allow the instrument to equilibrate. (NOTE: The ion-selective electrode responds much slower than does a pH electrode, and a stable reading may not be obtained until 2 or 3 min. The reading should not change for 30 to 60 s.) Add the volume, accurately measured, of *Fluoride Standard Solution* specified in the monograph, allow the electrode to equilibrate with continued stirring, and take the final reading on the increment logarithmic scale, recording the value obtained as *S*. Perform a blank determination using 50 ml of the same buffer solution as used for the sample under analysis, and record the value obtained as *B*.

Calculation Determine the Δ value by the formula $(V \times C)/50$, in which *V* is the volume of *Fluoride Standard Solution* added, in ml; *C* is the exact concentration of the *Fluoride Standard Solution*, in ppm; and 50 is the ml of *Sample Preparation* used. Calculate the ppm of fluoride (F) in the sample by the formula

$$[(S \times \Delta) - B] \times (100/W),$$

in which *W* is the weight of sample taken, in g.

Heavy Metals Test

This test is designed to limit the content of common metallic impurities that are colored by sulfide ion (Ag, As, Bi, Cd, Cu, Hg, Pb, Sb, Sn) under the specified test conditions. It demonstrates that the test substance is not grossly contaminated by such heavy metals and, within the precision of the test, that it does not exceed the *Heavy Metals* limit given in the individual monograph, as determined by concomitant visual comparison with a control solution. It has been found that, in the specified pH range, the optimum concentration of lead ion (Pb) for matching purposes by this method is 20 µg in 50 ml of solution.

Determine the amount of heavy metals by *Method I*, unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless solutions prior to addition of sulfide ion. *Method II* is used for those substances that do not yield clear, colorless solutions under the test conditions specified for *Method I*, or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion. *Method III*, a wet digestion method, is used only in those cases where neither *Method I* nor *Method II* can be used.

Special Reagents

Ammonia TS Dilute 400 ml of ACS reagent-grade ammonium hydroxide to 1000 ml with water.

Hydrochloric Acid, Sulfuric Acid, Nitric Acid, 30% Hydrogen Peroxide Use ACS reagent-grade chemicals.

Lead Nitrate Stock Solution Dissolve 159.8 mg of ACS reagent-grade lead nitrate, $Pb(NO_3)_2$, in 100 ml of water containing 1 ml of nitric acid, dilute with water to 1000.0 ml, and mix. Prepare and store this solution in glass containers that are free from lead salts.

Standard Lead Solution On the day of use, dilute 10.0 ml of *Lead Nitrate Stock Solution* with water to 100.0 ml. Each ml of *Standard Lead Solution* contains the equivalent of 10 µg of lead ion (Pb).

Procedure

NOTE: In the following procedures, failure to adjust accurately the pH of the solution within the specified limits may result in a significant loss of test sensitivity.

Method I

Solution A Pipet 2.0 ml of *Standard Lead Solution* (20 µg of Pb) into a 50-ml color-comparison tube, and add water to make 25 ml. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by addition of diluted acetic acid TS or ammonia TS, dilute with water to 40 ml, and mix.

Solution B Place 25 ml of the solution prepared as directed in the individual monograph in a 50-ml color-comparison tube that matches the one used for *Solution A*, adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by addition of diluted acetic acid TS or ammonia TS, dilute with water to 40 ml, and mix.

Solution C Into a third color-comparison tube that matches those used for *Solutions A* and *B*, place 25 ml of the solution prepared as directed in the individual monograph, and add 2.0 ml of *Standard Lead Solution*. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by addition of diluted acetic acid TS or ammonia TS, dilute with water to 40 ml, and mix.

To each tube add 10 ml of freshly prepared hydrogen sulfide TS, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*, and the intensity of the color of *Solution C* is equal to or greater than that of *Solution A*. If the color of *Solution C* is lighter than that of *Solution A*, the test substance is providing an interference with the test procedure and *Method II* must be used for the substance under examination.

Method II

Solution A Prepare as directed under *Method I*.

Solution B Place the quantity, accurately weighed, of sample specified in the individual monograph in a suitable crucible, add sufficient sulfuric acid to wet the sample, and carefully ignite at a low temperature until thoroughly charred, covering the crucible loosely with a suitable lid during the ignition. After the substance is thoroughly carbonized, add 2 ml of nitric acid and 5 drops of sulfuric acid, cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace, at 500° to 600° until all of the carbon is burned off. Cool, add 4 ml of dilute hydrochloric acid (1 in 2), cover, and digest on a steam bath for 10 to 15 min. Uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Add ammonia TS dropwise until the solution is just alkaline to litmus paper, dilute with water to 25 ml, and adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) by addition of diluted acetic acid TS. Filter if necessary, rinse the crucible and the filter with 10 ml of water, transfer the solution and rinsings to a 50-ml color-comparison tube, dilute with water to 40 ml, and mix.

To each tube add 10 ml of freshly prepared hydrogen sulfide TS, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Method III

Solution A Transfer a mixture of 8 ml of sulfuric acid and 10 ml of nitric acid into a 100-ml Kjeldahl flask, clamp the flask at an angle of 45°, and then add, in small increments, an additional volume of nitric acid equal to that added in the preparation of *Solution B*, below. Heat the solution to dense, white fumes, cool, and cautiously add 10 ml of water. Add a volume of 30% hydrogen peroxide equal to that added in the preparation of *Solution B*, below, then boil gently to dense, white fumes, and cool. Cautiously add 5 ml of water, mix, and boil gently to dense, white fumes. Continue boiling until the volume is reduced to about 2 or 3 ml, then cool, and dilute cautiously with a few ml of water. Into this solution pipet 2.0 ml of *Standard Lead Solution*, and mix. Transfer into a 50-ml color-comparison tube, rinse the flask with water, adding the rinsings to the tube until the volume is 25 ml, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) with stronger ammonia TS initially, and then with ammonia TS as the desired range is neared, dilute with water to 40 ml, and mix.

Solution B Transfer into a 100-ml Kjeldahl flask (or into a 300-ml flask if the reaction foams excessively) the quantity, accurately weighed, of sample specified in the individual monograph, clamp the flask at an angle of 45°, and then add sufficient of a mixture of 8 ml of sulfuric acid and 10 ml of nitric acid to moisten the sample thoroughly. (NOTE: For liquid samples use 3 ml of the acid mixture.) Warm gently until the reaction commences, allow the reaction to subside, and then add additional portions of the acid mixture, heating after each addition, until all of the 18 ml of acid mixture has been added. Increase the heat, and boil gently until the reaction mixture darkens. Remove the flask from the heat, add 2 ml of nitric acid, and heat to boiling again. Continue the intermittent heating and addition of 2-ml portions of nitric acid until no further darkening occurs, then heat strongly to dense, white fumes, and cool. Cautiously add 5 ml of water, mix, boil gently to dense, white fumes, and continue heating until the volume is reduced to about 2 or 3 ml. Cool, cautiously add 5 ml of water, and examine. If the solution is yellow-colored, cautiously add 1 ml of 30% hydrogen peroxide, and again evaporate to dense, white fumes and to a volume of about 2 or 3 ml. Cool, dilute cautiously with a few ml of water, and mix. Transfer into a 50-ml color-comparison tube, rinse the flask with water, adding the rinsings to the tube until the volume is 25 ml, and mix. Adjust the pH to between 3.0 and 4.0 (using short-range pH indicator paper) with stronger ammonia TS initially, and then with ammonia TS as the desired range is neared, dilute with water to 40 ml, and mix.

To each tube add 10 ml of freshly prepared hydrogen sulfide TS, mix, allow to stand for 5 min, and view downward over a white surface. The color of *Solution B* is not darker than that of *Solution A*.

Hydrochloric Acid Table

Be°	Sp. Gr.	Percent HCl	Be°	Sp. Gr.	Percent HCl
1.00	1.0069	1.40	18.4	1.1453	28.61
2.00	1.0140	2.82	18.5	1.1462	28.78
3.00	1.0211	4.25	18.6	1.1471	28.95
4.00	1.0284	5.69	18.7	1.1480	29.13
5.00	1.0357	7.15	18.8	1.1489	29.30
5.25	1.0375	7.52	18.9	1.1498	29.48
5.50	1.0394	7.89	19.0	1.1508	29.65
5.75	1.0413	8.26	19.1	1.1517	29.83
6.00	1.0432	8.64	19.2	1.1526	30.00
6.25	1.0450	9.02	19.3	1.1535	30.18
6.50	1.0469	9.40	19.4	1.1544	30.35
6.75	1.0488	9.78	19.5	1.1554	30.53
7.00	1.0507	10.17	19.6	1.1563	30.71
7.25	1.0526	10.55	19.7	1.1572	30.90
7.50	1.0545	10.94	19.8	1.1581	31.08
7.75	1.0564	11.32	19.9	1.1590	31.27
8.00	1.0584	11.71	20.0	1.1600	31.45
8.25	1.0603	12.09	20.1	1.1609	31.64
8.50	1.0623	12.48	20.2	1.1619	31.82
8.75	1.0642	12.87	20.3	1.1628	32.01
9.00	1.0662	13.26	20.4	1.1637	32.19
9.25	1.0681	13.65	20.5	1.1647	32.38
9.50	1.0701	14.04	20.6	1.1656	32.56
9.75	1.0721	14.43	20.7	1.1666	32.75
10.00	1.0741	14.83	20.8	1.1675	32.93
10.25	1.0761	15.22	20.9	1.1684	33.12
10.50	1.0781	15.62	21.0	1.1694	33.31
10.75	1.0801	16.01	21.1	1.1703	33.50
11.00	1.0821	16.41	21.2	1.1713	33.69
11.25	1.0841	16.81	21.3	1.1722	33.88
11.50	1.0861	17.21	21.4	1.1732	34.07
11.75	1.0881	17.61	21.5	1.1741	34.26
12.00	1.0902	18.01	21.6	1.1751	34.45
12.25	1.0922	18.41	21.7	1.1760	34.64
12.50	1.0943	18.82	21.8	1.1770	34.83
12.75	1.0964	19.22	21.9	1.1779	35.02
13.00	1.0985	19.63	22.0	1.1789	35.21
13.25	1.1006	20.04	22.1	1.1798	35.40
13.50	1.1027	20.45	22.2	1.1808	35.59
13.75	1.1048	20.86	22.3	1.1817	35.78
14.00	1.1069	21.27	22.4	1.1827	35.97
14.25	1.1090	21.68	22.5	1.1836	36.16
14.50	1.1111	22.09	22.6	1.1846	36.35
14.75	1.1132	22.50	22.7	1.1856	36.54
15.00	1.1154	22.92	22.8	1.1866	36.73
15.25	1.1176	23.33	22.9	1.1875	36.93
15.50	1.1197	23.75	23.0	1.1885	37.14
15.75	1.1219	24.16	23.1	1.1895	37.36
16.0	1.1240	24.57	23.2	1.1904	37.58
16.1	1.1248	24.73	23.3	1.1914	37.80
16.2	1.1256	24.90	23.4	1.1924	38.03
16.3	1.1265	25.06	23.5	1.1934	38.26
16.4	1.1274	25.23	23.6	1.1944	38.49
16.5	1.1283	25.39	23.7	1.1953	38.72
16.6	1.1292	25.56	23.8	1.1963	38.95
16.7	1.1301	25.72	23.9	1.1973	39.18
16.8	1.1310	25.89	24.0	1.1983	39.41
16.9	1.1319	26.05	24.1	1.1993	39.64
17.0	1.1328	26.22	24.2	1.2003	39.86

Hydrochloric Acid Table (*continued*)

Be°	Sp. Gr.	Percent HCl	Be°	Sp. Gr.	Percent HCl
17.1	1.1336	26.39	24.3	1.2013	40.09
17.2	1.1345	26.56	24.4	1.2023	40.32
17.3	1.1354	26.73	24.5	1.2033	40.55
17.4	1.1363	26.90	24.6	1.2043	40.78
17.5	1.1372	27.07	24.7	1.2053	41.01
17.6	1.1381	27.24	24.8	1.2063	41.24
17.7	1.1390	27.41	24.9	1.2073	41.48
17.8	1.1399	27.58	25.0	1.2083	41.72
17.9	1.1408	27.75	25.1	1.2093	41.99
18.0	1.1417	27.92	25.2	1.2103	42.30
18.1	1.1426	28.09	25.3	1.2114	42.64
18.2	1.1435	28.26	25.4	1.2124	43.01
18.3	1.1444	28.44	25.5	1.2134	43.40

Source: Courtesy of the Manufacturing Chemists Association.

Specific gravity determinations were made at 60°F, compared with water at 60°F.

From the specific gravities, the corresponding degrees Baumé were calculated by the following formula:

$$\text{Baumé} = 145 - (145/\text{sp. gr.})$$

Baumé hydrometers for use with this table must be graduated by the above formula, which should always be printed on the scale.

ALLOWANCE FOR TEMPERATURE

10°–15°Be: 1/40°Be or 0.0002 sp. gr. for 1°F
15°–22°Be: 1/30°Be or 0.0003 sp. gr. for 1°F
22°–25°Be: 1/28°Be or 0.00035 sp. gr. for 1°F

Hydroxypropoxyl Determination

Apparatus The apparatus for hydroxypropoxyl group determination is shown in Fig. 13. The boiling flask, *D*, is fitted with an aluminum foil–covered Vigreaux column, *E*, on the side arm and with a bleeder tube through the neck and to the bottom of the flask for the introduction of steam and nitrogen. A steam generator, *B*, is attached to the bleeder tube through tube *C*, and a condenser, *F*, is attached to the Vigreaux column. The boiling flask and steam generator are immersed in an oil bath, *A*, equipped with a thermoregulator such that a temperature of 155° and the desired heating rate may be maintained. The distillate is collected in a 150-ml beaker, *G*, or other suitable container.

Procedure Unless otherwise directed, transfer about 100 mg of the sample, previously dried at 105° for 2 h and accurately weighed, into the boiling flask, and add 10 ml of chromium trioxide solution (60 g in 140 ml of water). Immerse the steam generator and the boiling flask in the oil bath (at room temperature) to the level of the top of the chromium trioxide

solution. Start cooling water through the condenser, and pass nitrogen gas through the boiling flask at the rate of one bubble per second. Starting at room temperature, raise the temperature of the oil bath to 155° over a period of not less than 30 min, and maintain this temperature until the end of the determination. Distil until 50 ml of distillate is collected. Detach the condenser from the Vigreaux column, and wash it with water, collecting the washings in the distillate container. Titrate the combined washings and distillate with 0.02 *N* sodium hydroxide to a pH of 7.0, using a pH meter set at the expanded scale. (NOTE: Phenolphthalein TS may be used for this titration if it is also used for all standards and blanks.) Record the volume, V_a, of the 0.02 *N* sodium hydroxide used. Add 500 mg of sodium bicarbonate and 10 ml of dilute sulfuric acid TS, and then after evolution of carbon dioxide has ceased, add 1 g of potassium iodide. Stopper the flask, shake the mixture, and allow it to stand in the dark for 5 min. Titrate the liberated iodine with 0.02 *N* sodium thiosulfate to the sharp disappearance of the yellow color, confirming the endpoint by the addition of a few drops of starch TS. Record the volume of 0.02 *N* sodium thiosulfate required as Y_a.

Make several reagent blank determinations, using only the chromium trioxide solution in the above procedure. The ratio of the sodium hydroxide titration (V_b) to the sodium thiosulfate titration (Y_b), corrected for variation in normalities, will give the acidity-to-oxidizing ratio, $V_b/Y_b = K$, for the chromium trioxide carried over in the distillation. The factor K should be constant for all determinations.

Make a series of blank determinations using 100 mg of methylcellulose (containing no foreign material) in place of the sample, recording the average volume of 0.02 *N* sodium hydroxide required as V_m and the average volume of 0.02 *N* sodium thiosulfate required as Y_m.

Calculate the hydroxypropoxyl content of the sample, in mg, by the formula

$$75.0 \times [N_1(V_a - V_m) - kN_2(Y_a - Y_m)],$$

in which N_1 is the exact normality of the 0.02 *N* sodium hydroxide solution, N_2 is the exact normality of the 0.02 *N* sodium thiosulfate solution, and $k = V_bN_1/Y_bN_2$.

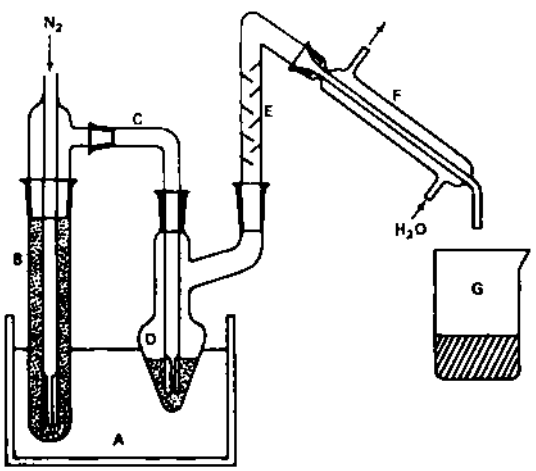


FIGURE 13 Apparatus for Hydroxypropoxyl Determination

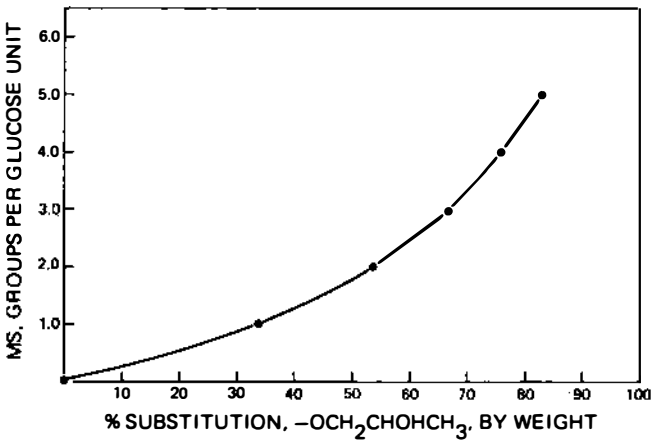


FIGURE 14 Chart for Converting Percentage of Substitution, by Weight, of Hydroxypropoxyl Groups to Molecular Substitution per Glucose Unit

The percentage of substitution, by weight, of hydroxypropoxyl groups, determined as directed above, may be converted to molecular substitution, per glucose unit, by reference to Fig. 14.

Identification Tests

The tests under this heading are frequently referred to in the Codex for the presumptive identification of FCC chemicals taken from labeled containers. These tests are not intended to be applied to mixtures unless so specified (see *Identification*, page 2).

Acetate

Acetic acid or acetates, when warmed with sulfuric acid and alcohol, form ethyl acetate, recognizable by its characteristic odor. With neutral solutions of acetates, ferric chloride TS produces a deep red color that is destroyed by the addition of a mineral acid.

Aluminum

Solutions of aluminum salts yield with ammonia TS a white, gelatinous precipitate that is insoluble in an excess of ammonia TS. The same precipitate is produced by sodium hydroxide TS or sodium sulfide TS, but it dissolves in an excess of either reagent.

Ammonium

Sodium hydroxide TS decomposes ammonium salts with the evolution of ammonia, recognizable by its odor and its alkaline

effect upon moistened red litmus paper. The decomposition is accelerated by warming.

Benzoate

Neutral solutions of benzoates yield a salmon-colored precipitate with ferric chloride TS. From moderately concentrated solutions of benzoate, diluted sulfuric acid TS precipitates free benzoic acid, which is readily soluble in ether.

Bicarbonate

See *Carbonate*.

Bisulfite

See *Sulfite*.

Bromide

Free bromine is liberated from solutions of bromides upon the addition of chlorine TS, dropwise. When shaken with chloroform, the bromine dissolves, coloring the chloroform red to reddish brown. A yellowish white precipitate, which is insoluble in nitric acid and slightly soluble in ammonia TS, is produced when solutions of bromides are treated with silver nitrate TS.

Calcium

Insoluble oxalate salts are formed when solutions of calcium salts are treated in the following manner: Using 2 drops of methyl red TS as indicator, neutralize a solution of a calcium salt (1 in 20) with ammonia TS, then add diluted hydrochloric acid TS, dropwise, until the solution is acid. A white precipitate of calcium oxalate forms upon the addition of ammonium oxalate TS. This precipitate is insoluble in acetic acid but dissolves in hydrochloric acid.

Calcium salts moistened with hydrochloric acid impart a transient yellowish red color to a nonluminous flame.

Carbonate

Carbonates and bicarbonates effervesce with acids, yielding a colorless gas that produces a white precipitate immediately when passed into calcium hydroxide TS. Cold solutions of soluble carbonates are colored red by phenolphthalein TS, whereas solutions of bicarbonates remain unchanged or are slightly changed.

Chloride

Solutions of chlorides yield with silver nitrate TS a white, curdy precipitate that is insoluble in nitric acid but soluble in a slight excess of ammonia TS.

Citrate

When a few mg of a citrate are added to a mixture of 15 ml of pyridine and 5 ml of acetic anhydride, a carmine red color is produced.

Cobalt

Solutions of cobalt salts (1 in 20) in 3 *N* hydrochloric acid yield a red precipitate when heated on a steam bath with an equal volume of a hot, freshly prepared 1 in 10 solution of 1-nitroso-2-naphthol in 9 *N* acetic acid. Solutions of cobalt salts yield a yellow precipitate when saturated with potassium chloride and treated with potassium nitrite and acetic acid.

Copper

When solutions of cupric compounds are acidified with hydrochloric acid, a red film of metallic copper is deposited upon a bright untarnished surface of metallic iron. An excess of ammonia TS, added to a solution of a cupric salt, produces first a bluish precipitate and then a deep blue-colored solution. Solutions of cupric salts yield with potassium ferrocyanide TS a reddish brown precipitate, insoluble in diluted acids.

Hypophosphite

Hypophosphites evolve spontaneously flammable phosphine when strongly heated. Solutions of hypophosphites yield a white precipitate with mercuric chloride TS. This precipitate becomes gray when an excess of hypophosphite is present. Hypophosphite solutions, acidified with sulfuric acid and warmed with copper sulfate TS, yield a red precipitate.

Iodide

Solutions of iodides, upon the addition of chlorine TS, dropwise, liberate iodine, which colors the solution yellow to red. Chloroform is colored violet when shaken with this solution. The iodine thus liberated gives a blue color with starch TS. Silver nitrate TS produces in solutions of iodides a yellow, curdy precipitate that is insoluble in nitric acid and in ammonia TS.

Iron

Solutions of ferrous and ferric compounds yield a black precipitate with ammonium sulfide TS. This precipitate is dissolved by cold diluted hydrochloric acid TS with the evolution of hydrogen sulfide.

Ferric Salts Potassium ferrocyanide TS produces a dark blue precipitate in acid solutions of ferric salts. With an excess of sodium hydroxide TS, a reddish brown precipitate is formed. Solutions of ferric salts produce with ammonium thiocyanate TS a deep red color that is not destroyed by diluted mineral acids.

Ferrous Salts Potassium ferricyanide TS produces a dark blue precipitate in solutions of ferrous salts. This precipitate, which is insoluble in dilute hydrochloric acid, is decomposed by sodium hydroxide TS. Solutions of ferrous salts yield with sodium hydroxide TS a greenish white precipitate, the color rapidly changing to green and then to brown when shaken.

Lactate

When solutions of lactates are acidified with sulfuric acid, and potassium permanganate TS is added and the mixture heated, acetaldehyde, recognizable by its distinctive odor, is evolved.

Magnesium

Solutions of magnesium salts in the presence of ammonium chloride yield no precipitate with ammonium carbonate TS, but a white crystalline precipitate, which is insoluble in ammonia TS, is formed upon the subsequent addition of sodium phosphate TS.

Manganese

Solutions of manganous salts yield with ammonium sulfide TS a salmon-colored precipitate that dissolves in acetic acid.

Nitrate

When a solution of a nitrate is mixed with an equal volume of sulfuric acid, the mixture cooled, and a solution of ferrous sulfate superimposed, a brown color is produced at the junction of the two liquids. Brownish red fumes are evolved when a nitrate is heated with sulfuric acid and metallic copper. Nitrates do not decolorize acidified potassium permanganate TS (distinction from nitrites).

Nitrite

Nitrites yield brownish red fumes when treated with diluted mineral acids or acetic acid. A few drops of potassium iodide TS and a few drops of diluted sulfuric acid TS added to a solution of nitrite liberate iodine, which colors starch TS blue.

Peroxide

Solutions of peroxides slightly acidified with sulfuric acid yield a deep blue color upon the addition of potassium dichromate TS. On shaking the mixture with an equal volume of ether and allowing the liquids to separate, the blue color is transferred to the ether layer.

Phosphate

Neutral solutions of orthophosphates yield with silver nitrate TS a yellow precipitate, which is soluble in diluted nitric acid TS or in ammonia TS. With ammonium molybdate TS, a yellow precipitate, which is soluble in ammonia TS, is formed.

Potassium

Potassium compounds impart a violet color to a nonluminous flame if not masked by the presence of small quantities of sodium. In neutral, concentrated or moderately concentrated solutions of potassium salts, sodium bitartrate TS slowly produces a white, crystalline precipitate that is soluble in ammonia TS and in solutions of alkali hydroxides or carbonates. The precipitation may be accelerated by stirring or rubbing the inside of the test tube with a glass rod or by the addition of a small amount of glacial acetic acid or alcohol.

Sodium

A solution of a sodium compound, previously converted to chloride or nitrate, yields, when mixed with five times its volume of cobalt-uranyl acetate TS, a golden yellow precipitate, which forms upon shaking. Sodium compounds impart an intense yellow color to a nonluminous flame.

Sulfate

Solutions of sulfates yield with barium chloride TS a white precipitate that is insoluble in hydrochloric and nitric acids. Sulfates yield with lead acetate TS a white precipitate that is soluble in ammonium acetate solution. Hydrochloric acid produces no precipitate when added to solutions of sulfates (distinction from thiosulfates).

Sulfite

When treated with diluted hydrochloric acid TS, sulfites and bisulfites yield sulfur dioxide, recognizable by its characteristic odor. This gas blackens filter paper moistened with mercurous nitrate TS.

Tartrate

When a few mg of a tartrate are added to a mixture of 15 ml of pyridine and 5 ml of acetic anhydride, an emerald green color is produced.

Thiosulfate

Solutions of thiosulfates yield with hydrochloric acid a white precipitate that soon turns yellow, liberating sulfur dioxide, recognizable by its odor. The addition of ferric chloride TS to solutions of thiosulfates produces a dark violet color that quickly disappears.

Zinc

Zinc salts, in the presence of sodium acetate, yield a white precipitate with hydrogen sulfide. This precipitate, which is insoluble in acetic acid, is dissolved by diluted hydrochloric acid TS. A similar precipitate is produced by ammonium sulfide TS in neutral or alkaline solutions. Solutions of zinc salts yield with potassium ferrocyanide TS a white precipitate that is insoluble in diluted hydrochloric acid TS.

Lead Limit Test

Special Reagents Select reagents having as low a lead content as practicable, and store all solutions in containers of borosilicate glass. Rinse all glassware thoroughly with warm dilute nitric acid (1 in 2) followed by water.

Ammonia-Cyanide Solution Dissolve 2 g of potassium cyanide in 15 ml of stronger ammonia TS, and dilute with water to 100 ml.

Ammonium Citrate Solution Dissolve 40 g of citric acid in 90 ml of water, add 2 or 3 drops of phenol red TS, then cautiously add stronger ammonia TS until the solution acquires a reddish color. Extract it with 20-ml portions of *Dithizone Extraction Solution* until the dithizone solution retains its green color or remains unchanged.

Diluted Standard Lead Solution (1 µg Pb in 1 ml) Immediately before use, transfer 10.0 ml of *Standard Lead Solution*, page 512, containing 10 µg of lead per ml, to a 100-ml volumetric flask, dilute to volume with dilute nitric acid (1 in 100), and mix.

Dithizone Extraction Solution Dissolve 30 mg of dithizone in 1000 ml of chloroform, add 5 ml of alcohol, and mix. Store in a refrigerator. Before use, shake a suitable volume of the solution with about half its volume of dilute nitric acid (1 in 100), discarding the nitric acid. Do not use if more than 1 month old.

Hydroxylamine Hydrochloride Solution Dissolve 20 g of hydroxylamine hydrochloride in sufficient water to make about 65 ml, transfer the solution to a separator, add a few drops of thymol blue TS, then add stronger ammonia TS until the solution assumes a yellow color. Add 10 ml of sodium diethyldithiocarbamate solution (1 in 25), mix, and allow to stand for 5 min. Extract the solution with successive 10- to 15-ml portions of chloroform until a 5-ml test portion of the chloroform extract does not assume a yellow color when shaken with a dilute cupric sulfate solution. Add diluted hydrochloric acid TS until the extracted solution is pink, adding 1 or 2 drops more of thymol blue TS if necessary, then dilute with water to 100 ml, and mix.

Potassium Cyanide Solution Dissolve 50 g of potassium cyanide in sufficient water to make 100 ml. Remove the lead from the solution by extraction with successive portions of *Dithizone Extraction Solution* as described under *Ammonium Citrate Solution*, then extract any dithizone remaining in the cyanide solution by shaking with chloroform. Finally, dilute the cyanide solution with sufficient water so that each 100 ml contains 10 g of potassium cyanide.

Standard Dithizone Solution Dissolve 10 mg of dithizone in 1000 ml of chloroform, keeping the solution in a glass-stoppered lead-free bottle suitably wrapped to protect it from light and stored in a refrigerator.

Sample Solution The solution obtained by treating the sample as directed in an individual monograph is used directly as the *Sample Solution* in the *Procedure*. Sample solutions of organic compounds are prepared, unless otherwise directed, according to the following general method:

Caution: Some substances may react unexpectedly with explosive violence when digested with hydrogen peroxide. Appropriate safety precautions must be employed at all times.

Transfer 1.0 g of the sample into a suitable flask, add 5 ml of sulfuric acid and a few glass beads, and digest at a temperature not exceeding 120° until charring begins, using preferably a hot plate in a fume hood. (Additional sulfuric acid may be necessary to completely wet some samples, but the total volume added should not exceed about 10 ml.) After the sample has been initially decomposed by the acid, add with caution, dropwise, 30% hydrogen peroxide, allowing the reaction to subside and reheating between drops. The first few drops must be added very slowly with sufficient mixing to prevent a rapid reaction, and heating should be discontinued if foaming becomes excessive. Swirl the solution in the flask to prevent unreacted substance from caking on the walls or bottom of the flask during the digestion. *Add small quantities of the peroxide when the solution begins to darken*, and continue the digestion until the organic matter is destroyed, gradually raising the temperature of the hot plate to 250°–300° until fumes of sulfur trioxide are copiously evolved and the solution becomes colorless or retains only a light straw color. Cool, add cautiously 10 ml of water, again evaporate to strong fuming, and cool. Quantitatively transfer the solution into a separator with the aid of small quantities of water.

Procedure Transfer the *Sample Solution*, prepared as directed in the individual monograph, into a separator, and, unless otherwise directed, add 6 ml of *Ammonium Citrate Solution* and 2 ml of *Hydroxylamine Hydrochloride Solution*. (Use 10 ml of the citrate solution when determining lead in iron salts.) To the separator add 2 drops of phenol red TS, and make the solution just alkaline (red in color) by the addition of stronger ammonia TS. Cool the solution, if necessary, under a stream of tap water, then add 2 ml of *Potassium Cyanide Solution*. Immediately extract the solution with 5-ml portions of *Dithizone Extraction Solution*, draining each extract into another separator, until the dithizone solution retains its green color. Shake the combined dithizone solutions for 30 s with 20 ml of dilute nitric acid (1 in 100), discard the chloroform layer, add to the acid solution 5.0 ml of *Standard Dithizone Solution* and 4 ml of *Ammonia-Cyanide Solution*, and shake for 30 s. The purplish hue in the chloroform solution of the sample due to any lead dithizonate present does not exceed that in a control, containing the volume of *Diluted Standard Lead Solution* equivalent to the amount of lead specified in the monograph, when treated in the same manner as the sample.

Loss on Drying

This procedure is used to determine the amount of volatile matter expelled under the conditions specified in the monograph. Since the volatile matter may include material other than adsorbed moisture, this test is designed for compounds in which the loss on drying may not definitely be attributable to water

alone. For substances appearing to contain water as the only volatile constituent, the *Karl Fischer Titrimetric Method* provided under *Water*, page 552, is usually appropriate.

Procedure Unless otherwise directed in the monograph, conduct the determination on 1 to 2 g of the substance, previously mixed and accurately weighed. If the sample is in the form of large crystals, reduce the particle size to about 2 mm, quickly crushing to avoid absorption or loss of moisture. Tare a glass-stoppered shallow weighing bottle that has been dried for 30 min under the same conditions to be observed in the determination. Transfer the sample to the bottle, replace the cover, and weigh the bottle and its contents. By gentle sidewise shaking distribute the sample as evenly as possible to a depth of about 5 mm for most substances and not over 10 mm in the case of bulky materials. Place the loaded bottle in the drying chamber, removing the stopper and leaving it also in the chamber, and dry at the temperature and for the length of time specified. Upon opening the chamber, close the bottle promptly and allow it to come to room temperature, preferably in a desiccator, before weighing.

If the test substance melts at a temperature lower than that specified for the determination, preheat the bottle and its contents for 1 to 2 h at a temperature 5° to 10° below the melting range, then continue drying at the specified temperature for the determination. When drying in a desiccator, exercise particular care to ensure that the desiccant is kept fully effective by frequent replacement.

Melting Range or Temperature

For purposes of this Codex, the melting range or temperature of a solid is defined as those points of temperature within which or the point at which the solid coalesces and is completely melted, when determined as directed below. Any apparatus or method capable of equal accuracy may be used. The accuracy should be checked frequently by the use of one or more of the six USP Melting Point Reference Standards, preferably the one that melts nearest the melting temperature of the compound to be tested.

Five procedures for the determination of melting range or temperature are given herein, varying in accordance with the nature of the substance. When no class is designated in the monograph, use the procedure for *Class I*.

The procedure known as the mixed melting point determination, whereby the melting range of a solid under test is compared with that of an intimate mixture of equal parts of the solid and an authentic specimen of it, may be used as a confirmatory identification test. Agreement of the observations on the original and the mixture usually constitutes reliable evidence of chemical identity.

Apparatus The melting range apparatus consists of a glass container for a bath of colorless fluid, a suitable stirring device, an accurate thermometer (see page 547), and a controlled source of heat. The bath fluid is selected with a view to the temperature required, but light paraffin is used generally, and certain liquid silicones are well adapted to the higher temperature ranges. The fluid is deep enough to permit immersion of the thermometer to its specified immersion depth so that the bulb is still about 2 cm above the bottom of the bath. The heat may be supplied by an open flame or electrically. The capillary tube is about 10 cm long and 0.8 to 1.2 mm in internal diameter with walls 0.2 to 0.3 mm in thickness.

The thermometer is preferably one that conforms to the specifications provided under *Thermometers*, page 547, selected for the desired accuracy and range of temperature.

Procedure for Class I Reduce the sample to a very fine powder, and, unless otherwise directed, render it anhydrous when it contains water of hydration by drying it at the temperature specified in the monograph, or, when the substance contains no water of hydration, dry it over a suitable desiccant for 16 to 24 h.

Charge a capillary glass tube, one end of which is sealed, with sufficient of the dry powder to form a column in the bottom of the tube 2.5 to 3.5 mm high when packed down as closely as possible by moderate tapping on a solid surface.

Heat the bath until a temperature approximately 30° below the expected melting point is reached, attach the capillary tube to the thermometer, and adjust its height so that the material in the capillary is level with the thermometer bulb. Return the thermometer to the bath, continue the heating, with constant stirring, at a rate of rise of approximately 3° per min until a temperature 3° below the expected melting point is attained, then carefully regulate the rate to about 1° to 2° per min until melting is complete.

The temperature at which the column of the sample is observed to collapse definitely against the side of the tube at any point is defined as the beginning of melting, and the temperature at which the sample becomes liquid throughout is defined as the end of melting. The two temperatures fall within the limits of the melting range.

Procedure for Class Ia Prepare the sample and charge the capillary glass tube as directed for *Class I*. Heat the bath until a temperature 10° ± 1° below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3° ± 0.5° per min until melting is complete. Record the melting range as for *Class I*.

Procedure for Class Ib Place the sample in a closed container, and cool to 10°, or lower, for at least 2 h. Without previous powdering, charge the cooled material into the capillary tube as directed for *Class I*, then immediately place the charged tube in a vacuum desiccator and dry at a pressure not exceeding 20 mm of Hg for 3 h. Immediately upon removal from the desiccator, fire-seal the open end of the tube, and as soon as practicable proceed with the determination of the melting range as follows: Heat the bath until a temperature of 10° ± 1° below the expected melting range is reached, then introduce the charged tube, and heat at a rate of rise of 3° ± 0.5° per min until melting is complete. Record the melting range as directed in *Class I*.

If the particle size of the material is too large for the capillary, precool the sample as directed above, then with as

little pressure as possible gently crush the particles to fit the capillary, and immediately charge the tube.

Procedure for Class II Carefully melt the material to be tested at as low a temperature as possible, and draw it into a capillary tube that is left open at both ends, to a depth of about 10 mm. Cool the charged tube at 10°, or lower, for 24 h, or in contact with ice for at least 2 h. Then attach the tube to the thermometer by means of a rubber band, adjust it in a water bath so that the upper edge of the material is 10 mm below the water level, and heat as directed for *Class I* except, within 5° of the expected melting temperature, regulate the rate of rise of temperature to 0.5° to 1.0° per min. The temperature at which the material is observed to rise in the capillary tube is the melting temperature.

Procedure for Class III Melt a quantity of the substance slowly, while stirring, until it reaches a temperature of 90° to 92°. Remove the source of heat, and allow the molten substance to cool to a temperature of 8° to 10° above the expected melting point. Chill the bulb of an ASTM 14C thermometer (see page 547) to 5°, wipe it dry, and while it is still cold dip it into the molten substance so that approximately the lower half of the bulb is submerged. Withdraw it immediately, and hold it vertically away from the heat until the wax surface dulls, then dip it for 5 min into a water bath having a temperature not higher than 16°.

Fix the thermometer securely in a test tube so that the lower point is 15 mm above the bottom of the test tube. Suspend the test tube in a water bath adjusted to about 16°, and raise the temperature of the bath at the rate of 2° per min to 30°, then change to a rate of 1° per min, and note the temperature at which the first drop of melted substance leaves the thermometer. Repeat the determination twice on a freshly melted portion of the sample. If the variation of three determinations is less than 1°, take the average of the three as the melting point. If the variation of three determinations is greater than 1°, make two additional determinations and take the average of the five.

Mercury Limit Test

Mercury Detection Instrument Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder and capable of measuring the radiation absorbed by mercury vapors at the mercury resonance line of 253.6 nm. A simple mercury vapor meter or detector equipped with a variable span recorder is also satisfactory.

Aeration Apparatus The apparatus, shown in Fig. 15, consists of a flowmeter (*a*), capable of measuring at a flow rate of 1 ft^3 per h, connected via a three-way stopcock (*b*), with Teflon plug, to 125-ml gas washing bottles (*c* and *d*), followed by a drying tube packed with glass wool (*e*), and finally a suitable quartz liquid absorption cell (*f*), terminating with a vent (*g*). (NOTE: The absorption cell will vary in optical pathlength depending upon the type of mercury detection instrument used.) Bottle *c* is fitted with an extra-coarse fritted bubbler (Corning 31770 125 EC or equivalent), and the bottle is marked with a 60-ml calibration line. The drying tube *e* is lightly packed with glass wool or magnesium perchlorate. Bottle *c* is used for the test solution, and bottle *d*, which remains empty throughout the procedure, is used to collect water droplets. Alternatively, an apparatus embodying the principle of the assembly described and illustrated may be used. The aerating medium may be either compressed air or compressed nitrogen.

Standard Preparation Transfer 1.71 g of mercuric nitrate, $Hg(NO_3).H_2O$, to a 1000-ml volumetric flask, dissolve in a mixture of 100 ml of water and 2 ml of nitric acid, dilute to volume with water, and mix. Discard after 1 month. Transfer 10.0 ml of this solution to a second 1000-ml volumetric flask, acidify with 5 ml of dilute sulfuric acid solution (1 in 5), dilute to volume with water, and mix. Discard after 1 week. On the day of use, transfer 10.0 ml of the second solution to a 100-ml volumetric flask, acidify with 5 ml of dilute sulfuric acid (1 in 5), dilute to volume with water, and mix. Each ml of this solution contains 1 μg of Hg. Transfer 2.0 ml of this solution (2 μg of Hg) to a 50-ml beaker, and add 20 ml of water, 1 ml of dilute sulfuric acid solution (1 in 5), and 1 ml of potassium permanganate solution (1 in 25). Cover the beaker with a watch glass, boil for a few seconds, and cool.

Sample Preparation Prepare as directed in the individual monograph.

Procedure Assemble the aerating apparatus as shown in Fig. 15, with bottles *c* and *d* empty and stopcock *b* in the bypass position. Connect the apparatus to the absorption cell (*f*) in the instrument, and adjust the air or nitrogen flow rate so that, in the following procedure, maximum absorption and reproducibility are obtained without excessive foaming in the test solution. Obtain a baseline reading at 253.6 nm, following the manufacturer's instructions for operating the instrument. Treat the *Standard Preparation* as follows: Destroy the excess permanganate by adding a 1 in 10 solution of hydroxylamine hydrochloride, dropwise, until the solution is colorless. Immediately wash the solution into bottle *c* with water, and dilute to the 60-ml mark with water. Add 2 ml of 10% stannous chloride solution (prepared fresh each week by dissolving 20 g of $SnCl_2.2H_2O$ in 40 ml of warm hydrochloric acid and diluting with 160 ml of water), and immediately reconnect bottle *c* to the aerating apparatus. Turn stopcock *b* from the bypass to the

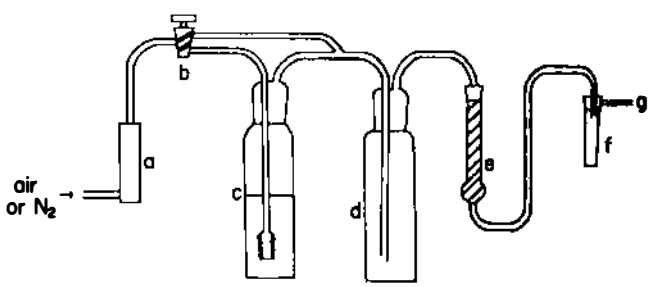


FIGURE 15 Aeration Apparatus for Mercury Limit Test

aerating position, and obtain the reading on the recorder. Disconnect bottle *c* from the aerating apparatus, discard the *Standard Preparation* mixture, wash bottle *c* with water, and repeat the foregoing procedure using the *Sample Preparation*; any absorbance produced by the *Sample Preparation* does not exceed that produced by the *Standard Preparation*.

Methoxyl Determination

Apparatus The apparatus for methoxyl determination, as shown in Fig. 16, consists of a boiling flask, *A*, fitted with a capillary side arm to provide an inlet for carbon dioxide and connected to a column, *B*, which separates aqueous hydriodic acid from the more volatile methyl iodide. After the methyl iodide passes through a suspension of aqueous red phosphorus in the scrubber trap, *C*, it is absorbed in the bromine–acetic acid absorption tube, *D*. The carbon dioxide is introduced from a device arranged to minimize pressure fluctuations and connected to the apparatus by a small capillary containing a small plug of cotton.

Reagents

Acetic Potassium Acetate Dissolve 100 g of potassium acetate in 1000 ml of a mixture consisting of 900 ml of glacial acetic acid and 100 ml of acetic anhydride.

Bromine–Acetic Acid Solution On the day of use, dissolve 5 ml of bromine in 145 ml of the *Acetic Potassium Acetate* solution.

Hydriodic Acid Use special-grade hydriodic acid suitable for alkoxyl determinations, or purify reagent grade as follows: Distil over red phosphorus in an all-glass apparatus, passing a slow stream of carbon dioxide through the apparatus until the distillation is terminated and the receiving flask has completely cooled.

Caution: Use a safety shield and conduct the distillation in a hood.

Collect the colorless, or almost colorless, constant-boiling acid distilling between 126° and 127°. Store the acid in a cool, dark place in small, brown, glass-stoppered bottles previously flushed with carbon dioxide and finally sealed with paraffin.

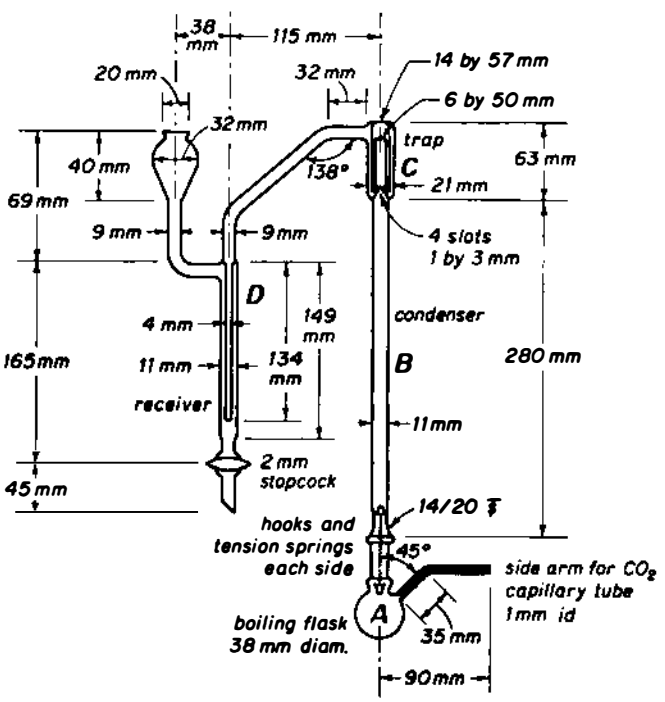


FIGURE 16 Distillation Apparatus for Methoxyl Determination

Procedure Fill trap *C* half full with a suspension of about 60 mg of red phosphorus in 100 ml of water, introduced through the funnel on tube *D* and the side arm that connects with the trap at *C*. Rinse tube *D* and the side arm with water, collecting the rinsings in trap *C*, then charge absorption tube *D* with 7 ml of *Bromine–Acetic Acid Solution*. Place the sample, accurately weighed in a tared gelatin capsule, in the boiling flask *A*, along with a few glass beads or boiling stones, then add 6 ml of *Hydriodic Acid*. Connect the flask to the condenser, using a few drops of the acid to seal the junction, and begin passing the carbon dioxide through the apparatus at the rate of about two bubbles per second. Heat the flask in an oil bath at 150°, continue the reaction for 40 min, and drain the contents of absorption tube *D* into a 500-ml Erlenmeyer flask containing 10 ml of sodium acetate solution (1 in 4). Rinse tube *D* with water, collecting the rinsings in the flask, and dilute to about 125 ml with water. Discharge the reddish brown color of bromine by adding formic acid dropwise, with swirling, then add 3 drops in excess. Usually a total of 12 to 15 drops of formic acid is required. Allow the flask to stand for 3 min, add 15 ml of diluted sulfuric acid TS and 3 g of potassium iodide, and titrate immediately with 0.1 *N* sodium thiosulfate, adding starch TS near the endpoint. Perform a blank determination with the same quantities of the same reagents, including the gelatin capsule, and in the same manner, and make any necessary correction. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 0.517 mg (517 μg) of methoxyl groups (—OCH_3).

Nitrogen Determination (Kjeldahl Method)

Caution: Provide adequate ventilation in the laboratory, and do not permit accumulation of exposed mercury.

NOTE: All reagents should be nitrogen-free, where available, or otherwise very low in nitrogen content.

Method I

This method should be used unless otherwise directed in the individual monograph. It is not applicable to certain nitrogen-containing compounds that do not yield their entire nitrogen upon digestion with sulfuric acid.

A. Nitrites and Nitrates Absent Unless otherwise directed, transfer from 700 mg to 2.2 g of the sample into a 500- to 800-ml Kjeldahl digestion flask of hard, moderately thick, well-annealed glass, wrapping the sample, if solid or semisolid, in nitrogen-free filter paper to facilitate the transfer if desired. Add 700 mg of mercuric oxide or 650 ml of metallic mercury, 15 g of powdered potassium sulfate or anhydrous sodium sulfate, and 25 ml of 93% to 98% sulfuric acid. (If a sample weight greater than 2.2 g is used, increase the sulfuric acid by 10 ml for each additional g of sample.) Place the flask in an inclined position, and heat gently until frothing ceases, adding a small amount of paraffin, if necessary, to reduce frothing.

Caution: The digestion should be conducted in a fume hood, or the digestion apparatus should be equipped with a fume exhaust system.

Boil briskly until the solution clears, and then continue boiling for 30 min longer (or for 2 h for samples containing organic material). Cool, add about 200 ml of water, mix, and then cool to below 25°. Add 25 ml of sulfide or thiosulfate solution (40 g of K_2S, 40 g of Na_2S, or 80 g of $Na_2S_2O_3.5H_2O$ in 1000 ml of water), and mix to precipitate the mercury. Add a few granules of zinc to prevent bumping, tilt the flask, and cautiously pour sodium hydroxide pellets, or a 2 in 5 sodium hydroxide solution, down the inside of the flask so that it forms a layer under the acid solution, using a sufficient amount (usually about 25 g of solid NaOH) to make the mixture strongly alkaline. Immediately connect the flask to a distillation apparatus consisting of a Kjeldahl connecting bulb and a condenser, the delivery tube of which extends well beneath the surface of a measured excess of 0.5 *N* hydrochloric or sulfuric acid contained in a 500-ml flask. Add from 5 to 7 drops of methyl red indicator (1 g of methyl red in 200 ml of alcohol) to the receiver flask. Rotate the Kjeldahl flask to mix its contents thoroughly, and then heat until all of the ammonia has distilled, collecting at least 150 ml of distillate. Wash the tip of the delivery tube, collecting the washings in the receiving flask, and titrate the excess acid with 0.5 *N* sodium hydroxide. Perform a blank determination, substituting 2 g of sucrose for the sample, and make any necessary correction (see page 2). Each ml of 0.5 *N* acid consumed is equivalent to 7.003 mg of nitrogen. (NOTE: If it is known that the substance to be determined has a low nitrogen content, 0.1 *N* acid and alkali may be used, in which case each ml of 0.1 *N* acid consumed is equivalent to 1.401 mg of nitrogen.)

B. Nitrites and Nitrates Present (NOTE: This procedure is not applicable to liquids or to materials having a high chlorine to nitrate ratio.) Unless otherwise directed, transfer from 700 mg to 2.2 g of the sample into the Kjeldahl flask, and add 40 ml of 93% to 98% sulfuric acid containing 2 g of salicylic acid. Mix thoroughly by shaking, and then allow to stand for 30 min or more, with occasional shaking. Add 5 g of $Na_2S_2O_3.5H_2O$, or 2 g of zinc dust (as an impalpable powder, not granules or filings), shake, and allow to stand for 5 min. Heat over a low flame until frothing ceases, then remove the heat, add 700 mg of mercuric oxide (or 650 mg of metallic mercury) and 15 g of powdered potassium sulfate (or anhydrous sodium sulfate), and boil briskly until the solution clears. Continue boiling for 30 min longer (or for 2 h for samples containing organic material), and then continue as directed under *A* beginning with "Cool, add about 200 ml of water. . . . "

Method II (Semimicro)

Transfer an accurately weighed or measured quantity of the sample, equivalent to about 2 or 3 mg of nitrogen, to the digestion flask of a semimicro Kjeldahl apparatus. Add 1 g of a powdered mixture of potassium sulfate and cupric sulfate (10 to 1), using a fine jet of water to wash down any material adhering to the neck of the flask, and then pour 7 ml of sulfuric acid down the inside wall of the flask to rinse it. Add cautiously down the inside of the flask 1 ml of 30% hydrogen peroxide, swirling the flask during the addition.

Caution: Do not add any peroxide during the digestion.

Heat over a free flame or an electric heater until the solution has attained a clear blue color and the walls of the flask are free from carbonized material. Cautiously add 20 ml of water, cool, then add through a funnel 30 ml of sodium hydroxide solution (2 in 5), and rinse the funnel with 10 ml of water. Connect the flask to a steam distillation apparatus, and immediately begin the distillation with steam. Collect the distillate in 15 ml of boric acid solution (1 in 25) to which has been added 3 drops of methyl red–methylene blue TS and enough water to cover the end of the condensing tube. Continue passing the steam until 80 to 100 ml of distillate has been collected, then remove the absorption flask, rinse the end of the condenser tube with a small quantity of water, and titrate with 0.01 *N* sulfuric acid. Each ml of 0.01 *N* acid is equivalent to 140 μg of nitrogen.

When more than 2 or 3 mg of nitrogen is present in the measured quantity of the substance is to be determined, 0.02 or 0.1 *N* sulfuric acid may be used in the titration if at least 15 ml of titrant is required. If the total dry weight of the material taken is greater than 100 mg, increase proportionately the quantities of sulfuric acid and sodium hydroxide added before distillation.

Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) may be defined as the phenomenon observed when atomic nuclei in molecules are placed in a static magnetic field, H_0, and are excited by radio frequency radiation that is polarized in a plane normal to H_0. NMR is a useful technique for the qualitative and quantitative analysis of a large number of organic compounds. It has been applied, for example, to the examination of certain FCC substances lacking useful ultraviolet chromophores (Turczan, Goldwitz, and Medwick, "Nuclear Magnetic Resonance Analysis of Bulk Food Additive Chemicals. 1. Food Chemicals Codex Chemicals, Group 1," *J. Agric. Food Chem.*, *25*, 594–602, 1977). The brief discussion presented herein may encourage

other investigators to determine NMR's usefulness in the analysis of FCC substances.

Theory

The origin of NMR depends on the nuclei of certain isotopes that possess spin and consequently possess a magnetic dipole moment and angular momentum. This angular momentum is proportional to a nuclear spin quantum number, I. The actual value of I of any given nucleus depends on the mass number and the atomic number, as follows. When the mass number of an isotope is odd and the atomic number is odd or even, the spin quantum number is half-integral, i.e., 1/2, 3/2, 5/2, etc. When both the mass number and the atomic number of an isotope are even, the spin quantum number is zero. Finally, when the mass number of an isotope is even and the atomic number is odd, the spin quantum number is integral, i.e., 1, 2, 3. . . . The nucleus may assume $2I + 1$ orientations in the applied magnetic field. Thus, nuclei with $I = 1/2$, e.g., ^{1}H, ^{13}C, ^{19}F, and ^{31}P, will have two orientations with respect to the magnetic field. When the orientation is parallel to the magnetic field, the nucleus is in the ground state; when the orientation is antiparallel to the magnetic field, the nucleus is in the excited state.

If the nuclei are in a magnetic field, absorption of radio frequency radiation will cause some of the nuclei to achieve the excited state by absorption of energy of the correct frequency. For example, for ^{1}H in the usual organic compounds in a magnetic field of 14,000 gauss, the frequency of such energy is about 60 megacycles per second or 60 megahertz (60 MHz).

The energy difference between the ground state and excited state in NMR is very small at room temperature. Consequently, there is only a very slight excess of nuclei in the ground state (the Boltzmann distribution excess is $10^{-3}\%$). It is this excess that is responsible for the observed absorption of radiation. Since the excess of nuclei is very slight, it is important that too large an amount of energy not be introduced into the system. If this occurs, all of the excess nuclei are in the excited state and the intensity of the absorption signal may decrease or even vanish. This is the phenomenon of saturation, a situation to be avoided if the quantitative nature of energy absorption by nuclei is to be preserved.

The value of NMR spectra in qualitative determinations arises from the nature of the proton resonances. Depending on the nature of the immediate molecular environment, protons will resonate at characteristic frequencies, allowing protons in different environments to be differentiated.

The interaction of protons gives rise to the phenomenon of splitting, behavior that makes an NMR spectrum complex. If a proton on a carbon interacts with n other equivalent protons on an adjacent carbon, the resonance pattern of the proton will consist of $n + 1$ lines whose individual intensities are given by the coefficients of the expansion $(1 + x)^n$. Thus when $n = 2$, line intensity ratios of 1:2:1 result; when $n = 3$, line intensity ratios of 1:3:3:1 result; and so on. If one proton, A, interacts with n other protons with unique interaction or coupling with each, then the signal for A will have 2^n lines, all of equal intensity. The separation distance or splitting of the lines in the multiplets is measured in hertz and is called the spin-spin coupling constant. Thus, for example, if the separation of the three individual peaks of a triplet is the same as the separation of the four individual peaks of a quartet, then the interaction of a methylene and a methyl is suggested. In ethanol, the methyl resonance is split to form a triplet by the methylene whereas the methylene resonance is split to form a quartet by the methyl group. The complementary relationship between these two groups of protons is established by the coupling constant. For most ethyl groups, the coupling constant J = 6–8 Hz. These rules are, however, only strictly valid for certain spectra, since one important criterion for application of these rules is that all of the couplings must be much less than the chemical shift separations. If this criterion is not obeyed, the spectra will show more lines than these rules will predict and the line intensities will be distorted.

There are other considerations to splitting. If a spectrum of ethanol is examined, the resonance ascribable to the hydroxyl proton is observed as a singlet. This is different from the anticipated hydroxyl triplet, a result of splitting by the adjacent methylene protons. A highly purified sample of ethanol, on the other hand, exhibits the hydroxyl triplet and a quartet of doublets for the methylene. The explanation for the different observations is chemical exchange. The exchange refers to the fact that the same proton may be bonded to a number of different ethanol molecules. In very pure ethanol this exchange is slow and the spectrum is able to present a picture with the hydroxyl proton behaving as expected; however, any acidic or basic impurities cause the exchange rate to increase, and the methylene signal becomes a sharp quartet indicating no interaction with the hydroxyl protons. A D_2O solution of ethanol would produce a single proton resonance for the hydroxyl at approximately the same chemical shift as any HDO impurity.

The experimental technique may introduce extraneous peaks into an NMR spectrum. Normally, the NMR sample tube is spun during the recording of the spectrum in order to eliminate magnetic field inhomogeneities and increase resolution. If the frequency of spinning is too low, the magnetic field is still somewhat inhomogeneous and a proton resonance absorption is accompanied by unexpected signals of low intensity appropriately called spinning side bands. These side bands are located symmetrically on both sides of the main proton resonance at a distance from the main resonance equal to the spinning frequency or some integral multiple of that frequency. Since the side band location depends on the spinning frequency, these bands are easily identified. Uneven spinning may also cause side bands.

The magnitude of the resonance intensity of a proton singlet or multiplet is directly proportional to the number of protons. Thus if the area of the singlets or multiplets in a spectrum is integrated, it is possible to assign relative values to the areas and determine the number of protons that a particular multiplet represents. This information simplifies interpretation of spectra and the identification of compounds.

The use of NMR for qualitative analysis involves several properties of the spectrum. First, the chemical shift establishes the general nature of the proton. Then the multiplicity of the proton resonance indicates the nature of the proton environment and the interaction of protons. For the simplest cases, the first-order spectra follow idealized rules and may be interpreted

directly. More complex spectra may prove to be too complex for non–computer assisted interpretation, and in these cases, qualitative analysis may be established by use of reference standard materials. Finally, the ability to be able to integrate resonance areas and to be able to compare areas with each other and to determine the relative numbers of protons is of great importance in some interpretations.

The ability to integrate areas under the resonance peaks has quantitative applications. In the relative method of quantitative analysis, integral areas from two different proton types within the same molecule are compared. The ratio of the two areas must be a particular value to indicate a satisfactory composition. In the absolute method of quantitative analysis, a known quantity of an organic compound is in the same solution with a known quantity of internal standard. An internal standard is a pure organic compound with a structure such that the integral for the area of the resonance peak(s) of a single kind of proton may be used as an absolute measure of the protons. The integral area for one kind of analyte protons may be compared with the integral area for the internal standard protons and quantitative analysis accomplished. Since the proton response in terms of integral area is a constant, it is convenient to define a proton milliequivalent, designated here by the symbol ^{1}H NMR meq. The ^{1}H NMR meq is the number obtained by dividing the formula weight of a compound in mg by the number of protons that give rise to the area to be integrated.

Several factors must be considered when selecting a particular resonance peak or cluster of resonance peaks for quantitative measurement. First, the analytically significant moiety giving rise to the resonance peak or peaks should be stable under analytical conditions and should not be disturbed by proton exchange processes during the time of analysis. Second, in general, the strongest resonance peak or multiplet is chosen, provided that it is an independent signal standing at least 0.5 ppm, if possible, from any other signal. In many cases, a molecule may provide more than one analytically useful resonance signal. Use of the strongest signal clearly results in the most sensitive measurement, since the ^{1}H NMR meq is smallest. In those instances where the strongest resonance is interfered with, an alternative choice is made. Third, the internal standard should be a compound that possesses a strong resonance signal, preferably a singlet, in the proximity of the chosen resonance signal of the article undergoing analysis.

Instruments

The NMR spectrometers available commercially are of either the continuous wave (CW) or Fourier transform (FT) type and vary as to magnetic field strength and, accordingly, the resonance frequency imposed by the ratio frequency oscillator. The FT instrument systems that permit signal enhancement are particularly useful when weak resonance signals are encountered, as in the case of ^{13}C, an isotope present in nature only to the extent of 1.11%, or in those cases when very dilute samples of strongly absorbing nuclei are studied.

The available spectrometers have different radio frequency oscillators, e.g., 60 MHz, 90 MHz, or 200 MHz. This variation makes it convenient to express field strength in a manner independent of oscillator frequency. Thus, the field position or chemical shift of a resonance is generally specified in ppm, using the delta (δ) scale defined by $\delta = \Delta\nu \times 10^6/\nu_0$, where $\Delta\nu$ is the absorption frequency difference between the analyte and the reference, in Hz; ν_0 is the oscillator frequency, in Hz; and 10^6 is a constant of convenience. Since the available spectrometers are not able to produce a specific frequency accurately enough, the chemical shift of a particular proton is related to an NMR reference such as tetramethylsilane (TMS) for nonpolar solvents and sodium 2,2-dimethyl-2-sila-pentane-5-sulfonate (DSS) for D_2O.

Experimental Procedures

The procedures outlined below are suitable for use with any CW NMR spectrometer in studies on FCC articles. The analyst should refer to the instrument manufacturer's instructions regarding operation of the instrument, selection of solvent, and other details beyond the scope of this introductory discussion.

Qualitative Analysis Unless otherwise directed, weigh 60 to 90 mg of the analyte into a 15- × 40-mm amber vial, and add about 600 µl of an appropriate solvent. For nonpolar organic solvents (i.e., $CDCl_3$, CCl_4, DMSO-d_6, DMF-d_7), add about 5 µl of tetramethylsilane (TMS), cap the vial, mix, and transfer 400 to 500 µl of the solution to a 5-mm × 17.5-cm NMR sample tube. Using field sweep and external lock, scan the analyte from 0 to about 8 ppm at a scan rate of about 3 to 5 min for full-scale recording, adjusting the amplification so that all peaks remain on scale. Adjust the spinning rate so that no spinning side bands interfere with the peaks of interest.

In adjusting resolution take care to ensure that the TMS peak shows definite ringing. Ringing is the repeated excursion or "wiggling" of the recorder trace after the magnetic field has passed through a resonance value and the peak value has been recorded. The ringing decays exponentially, finally reaching the baseline. Ringing is a good indication of a homogeneous field.

After the initial scan, check for peaks above 8 ppm by offsetting the instrument by 5 ppm. Record any peaks from 13 ppm to 5 ppm so that the peak positions occurring in both scans may be matched, since the TMS position may shift slightly in the higher range.

After recording the spectrum, set the recorder sweep time to about 1 min, check and reset the phase, and record the total integral, choosing an integrator amplitude setting such that the total integrator response is observed on the chart.

Compare the area of each set of peaks to the area of an assigned peak, and determine the number of nuclei contributing to each set.

Note peak positions, in ppm, from the TMS peak. For accurate peak matching where accuracy of 0.5 to 1.0 Hz is needed, reset the instrument calibration on internal lock and again scan.

When $CDCl_3$, CCl_4, C_6D_6, or $(CD_3)_2CO$ have been used as the solvent, add 2 drops of D_2O to the tube, shake for about 30 s, and again scan after resetting the zero position. Check the second scan for the absence of any peaks whose disappearance could be ascribable to exchange with D_2O, noting the additional peak for HDO. (NOTE: When NMR shift reagents are used, as

may be required in special situations, an additional peak results from the reagent and some degree of broadening occurs.)

Quantitative Analysis by NMR Absolute Method Select a solvent, *Internal Standard*, and *NMR Reference* as appropriate for the substance being determined.

Test Preparation Weigh accurately a quantity of the FCC article, corresponding to about 5.5 ^{1}H NMR meq of the analyte and to about 5.5 ^{1}H meq of the *Internal Standard*, and transfer to a glass-stoppered graduated centrifuge tube. Add 2 to 3 ml of the solvent, insert the stopper, and shake to dissolve. Add about 1% of *NMR Reference* (if it will not interfere with subsequent measurements), and shake.

Procedure Transfer about 0.4 ml of the *Test Preparation* to a standard 5-mm NMR sample tube, and record the spectrum, adjusting the spin rate so that no spinning side bands interfere with the peaks of interest. Measure the area under each of the peaks specified in the individual monographs by integrating no fewer than five times. Record the average area of the *Internal Standard* peak as A_S and that of the *Test Preparation* peak as A_U.

Calculate the quantity, in mg, of the analyte in the *Test Preparation* by the formula

$$W_S \times (A_U/A_S) \times (E_U/E_S),$$

in which W_S is the weight, in mg, of *Internal Standard* taken, and E_U and E_S are the ^{1}H NMR meq weights of the analyte and the *Internal Standard*, respectively.

Quantitative Analysis by NMR Relative Method Select a solvent and *NMR Reference* as appropriate for the substance being determined, and prepare the *Test Preparation* as directed under *Quantitative Analysis by NMR Absolute Method*.

Procedure Transfer about 0.4 ml of the *Test Preparation* to a standard 5-mm NMR sample tube, and record the spectrum, adjusting the spin rate so that no spinning side bands interfere with the peaks of interest. Measure the area under each of the peaks specified in the individual monograph by integrating no fewer than five times. Record the average areas resulting from the resonances of the groups designated in the individual monograph as A_1 and A_2.

Calculate the quantity, in mole percent, of analyte in the *Test Preparation* by the formula

$$\frac{100(A_1/n_1)}{(A_1/n_1) + (A_2/n_2)},$$

in which n_1 and n_2 are, respectively, the numbers of protons in the designated groups.

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Oil Content of Synthetic Paraffin

Apparatus

Filter Stick Use either a 10-mm diameter sintered-glass filter stick of 10- to 15-μm maximum pore diameter, or a filter stick made of stainless steel and having a 0.5-in. disk of 10- to 15-μm maximum pore diameter. Determine conformance with the pore diameter specified as follows: Clean sintered-glass filter sticks by soaking in hydrochloric acid, or stainless steel sticks by soaking in nitric acid, wash with water, rinse with acetone, and dry in air followed by drying in an oven at 105° for 30 min.

Thoroughly wet the clean filter stick by soaking in water, and then connect it with an apparatus (see Fig. 17) consisting of a mercury-filled manometer, readable to 0.5 mm; a clean and filtered air supply; a drying bulb filled with silica gel; and a needle-valve type air pressure regulator. Apply pressure slowly from the air source, and immerse the filter just below the surface of water contained in a beaker.

NOTE: If a head of liquid is noted above the surface of the filter after it is inserted into the water, the back pressure thus produced should be subtracted from the observed

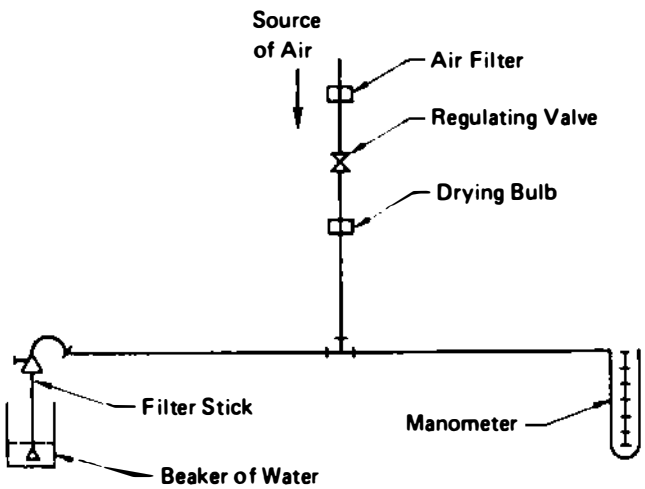


FIGURE 17 Assembly for Checking Pore Diameter of Filter Sticks

pressure when the pore diameter is calculated as directed below.

Increase the air pressure to 10 mm below the acceptable pressure limit, and then increase the pressure at a slow, uniform rate of about 3 mm of Hg per min until the first bubble passes through the filter. This can be conveniently observed by placing the beaker over a mirror. Read the manometer when the first bubble passes off the underside of the filter. Calculate the pore diameter, in μm, by the formula $2180/p$, in which p is the observed pressure, in mm, corrected for any back pressure as mentioned above.

Filtration Assembly Connect the *Filter Stick* with an air pressure inlet tube and delivery nozzle and ground-glass joint to fit a 25- × 170-mm test tube as shown in Fig. 18. If a stainless steel *Filter Stick* is used, make the connection to the test tube by means of a cork.

Cooling Bath Use a suitable insulated box having 1-in. holes in the center to accommodate any desired number of test tubes. The bath may be filled with a suitable medium such as kerosene and may be cooled by circulating a refrigerant through coils, or by using solid carbon dioxide, to produce a temperature of −30° ± 2°F.

Air Pressure Regulator Use a suitable pressure-reduction valve, or other suitable regulator, that will supply air to the *Filtration Assembly* at the volume and pressure required to give an even flow of filtrate (see *Procedure*). Connect the regulator with rubber tubing to the end of the *Filter Stick* in the *Filtration Assembly*.

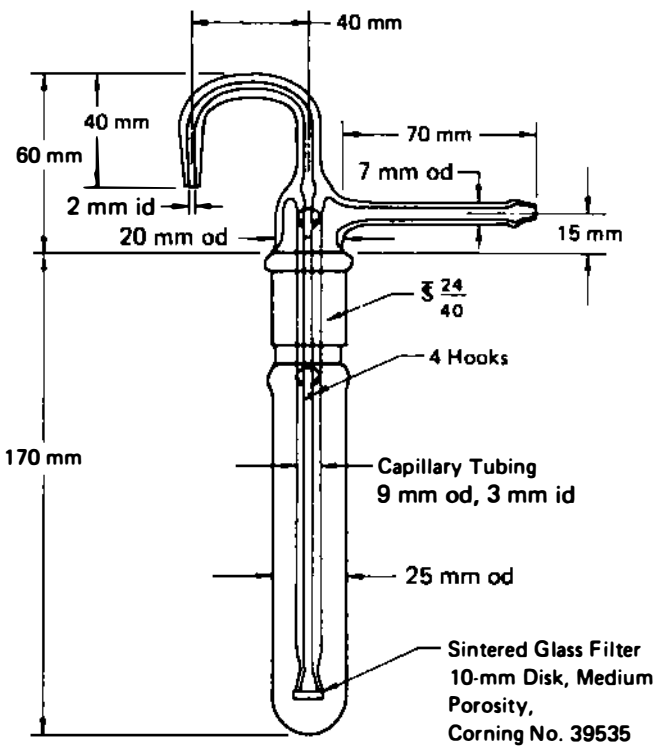


FIGURE 18 Filtration Assembly for Determination of Oil Content

Thermometer Use an ASTM Oil in Wax Thermometer having the range of −35° to +70°F and conforming to the requirements for an ASTM 71F thermometer (see page 547).

Weighing Bottles Use glass-stoppered conical bottles having a capacity of 15 ml. The bottles are used as evaporating flasks in the *Procedure*.

Evaporation Assembly The assembly consists of an evaporating cabinet capable of maintaining a temperature of 95° ± 2°F around the evaporation flasks, and air jets (4 ± 0.2 mm id) for delivering a stream of clean, dry air vertically downward into the flasks. In the *Procedure* below, support each jet so that the tip is 15 ± 5 mm above the surface of the liquid at the start of the evaporation. Supply the air (purified by passage through a tube of 1-cm bore packed loosely to a height of 20 cm with absorbent cotton) at the rate of 2 to 3 L per min per jet. The cleanliness of the air should be checked periodically to ensure that not more than 0.1 mg of residue is obtained when 4 ml of methyl ethyl ketone is evaporated as directed in the *Procedure*.

Wire Stirrer Use a 250-mm length of stiff iron or nichrome wire of about No. 20 B & S gauge. Form a 10-mm diameter loop at each end, and bend the loop at the bottom end so that the plane of the loop is perpendicular to the length of the wire.

Sample Selection If the sample is about 1 kg or less, obtain a representative portion by melting the entire sample and stirring thoroughly. For samples greater than about 1 kg, exercise special care to ensure that a truly representative portion is obtained, noting that the oil may not be distributed uniformly throughout the sample and that mechanical operations may have expressed some of the oil.

Procedure Melt a representative portion of the sample in a beaker, using a water bath or oven maintained at 160° to 210°F. As soon as the sample is completely melted, thoroughly mix it by stirring. Preheat a dropper pipet, provided with a rubber bulb and calibrated to deliver 1 ± 0.05 g of molten sample, and withdraw a 1-g portion of the sample as soon as possible after it has melted. Hold the pipet in a vertical position, and carefully transfer its contents into a clean, dry test tube previously weighed to the nearest mg. Evenly coat the bottom of the tube by swirling, allow the tube to cool, and weigh to the nearest mg. Calculate the sample weight, in g, and record it as *B* (see *Calculation*). Pipet 15 ml of methyl ethyl ketone (ASTM Specification D 740 or equivalent) into the tube, and immerse the tube up to the top of the liquid in a hot water or steam bath. Stir with an up-and-down motion with the *Wire Stirrer*, and continue heating and stirring until a homogeneous solution is obtained, exercising care to avoid loss of solvent by prolonged boiling. (NOTE: If it appears that a clear solution will not be obtained, stir until any undissolved material is well dispersed so as to produce a slightly cloudy solution.)

After the sample solution is prepared, plunge the test tube into an 800-ml beaker of ice water, and continue to stir until the contents are cold. Remove the stirrer, then remove the test tube from the bath, dry the outside of the tube with a cloth, and weigh to the nearest 100 mg. Calculate the weight, in g, of solvent in the test tube, and record it as *C* (see *Calculation*). Place the tube in the *Cooling Bath*, maintained at −30° ± 2°F, and stir continuously with the *Thermometer* until the temperature reaches −25° ± 0.5°F, maintaining the slurry at a uniform

consistency and taking precautions to prevent the sample from setting up on the walls of the tube or forming crystals.

Place the *Filter Stick* in a test tube and cool at −30° ± 2°F in the *Cooling Bath* for a minimum of 10 min. Immerse the cooled *Filter Stick* in the sample, then connect the *Filtration Assembly*, seating the ground-glass joint of the filter so as to make an airtight seal. Placed an unstoppered *Weighing Bottle*, previously weighed together with the glass stopper to the nearest 0.1 mg, under the delivery nozzle of the *Filtration Assembly*. (NOTE: Suitable precautions and proper analytical technique should be applied to ensure the accuracy of the weight of the bottle. Prior to determining its weight, the bottle and its stopper should have been cleaned and dried, then rinsed with methyl ethyl ketone, wiped dry on the outside, dried in the *Evaporation Assembly* for about 5 min, and cooled. Then allow it to stand for about 10 min near the balance before weighing.)

Apply air pressure to the *Filtration Assembly*, immediately collect about 4 ml of filtrate in the *Weighing Bottle*, and release the air pressure to permit the liquid to drain back slowly from the delivery nozzle. Stopper the bottle, and weigh it to the nearest 10 mg without waiting for it to come to room temperature. Remove the stopper, transfer the bottle to the *Evaporation Assembly* maintained at 95° ± 2°F, and place it under an air jet centered inside the neck, with the tip 15 ± 5 mm above the surface of the liquid. After the solvent has evaporated (usually less than 30 min time), stopper the bottle, and allow it to stand near the balance for about 10 min before it is weighed to the nearest 0.1 mg. Repeat the evaporation procedure for 5-min periods until the loss between successive weighings is not more than 0.2 mg. Determine the weight of solvent evaporated, in g, by subtracting the weight of the bottle plus oil residue from the weight of the bottle plus filtrate, and record the result as *D* (see *Calculation*).

Calculation Calculate the percentage, by weight, of oil in the sample by the formula

$$(100AC/BD) - 0.15,$$

in which *A*, *B*, *C*, and *D* are as indicated in the *Procedure* and 0.15 is a factor to correct for solubility of the sample in the solvent at −25°F.

Oleoresins

COLOR VALUE

Sample Preparation Transfer 70 to 100 mg of the sample, previously mixed well by shaking and accurately weighed, into a 100-ml volumetric flask, dissolve in acetone, dilute to volume with acetone, and mix. Allow the solution to stand for 2 min, then pipet 10 ml into a second 100-ml volumetric flask, dilute to volume with acetone, and mix.

Procedure Determine the absorbance of the *Sample Preparation* with a suitable spectrophotometer in a 1-cm cell at 460 nm, using acetone as the blank. Record the value obtained as A_S. In the same manner, determine the absorbance of a National Bureau of Standards Standard Glass Filter 2030, and record the value obtained as A_F. [NOTE: The recommended range for absorbance values is between 0.30 and 0.70. Solutions having absorbances greater than 0.70 should be diluted with acetone to one-half the original concentration, and those having absorbances less than 0.30 should be discarded and the *Sample Preparation* prepared with a larger sample. Appropriate adjustments should be made in the sample weight (*W*) used in the *Calculation* below.]

Calculation Determine the instrument correction factor, *F*, by the formula A_N/A_F, in which A_N is the absorbance of the filter as stated by the National Bureau of Standards. Calculate the color value of the sample by the formula

$$(A_S \times 164 \times F)/W,$$

in which *W* is the weight of sample taken, in g.

CURCUMIN CONTENT

Standard Preparation Transfer about 250 mg of pure curcumin, accurately weighed, into a 100-ml volumetric flask, and record the weight as *W*, in mg. Dissolve in acetone, dilute to volume with acetone, and mix. Pipet a 1-ml portion of this solution into a second 100-ml volumetric flask, dilute to volume with acetone, and mix. Finally, pipet a 5-ml portion of the last solution into a 50-ml volumetric flask, dilute to volume with acetone, and mix.

Sample Preparation Transfer an accurately weighed amount of the sample, equivalent to about 250 mg of curcumin, into a 100-ml volumetric flask, and record the weight as *w*, in mg. Dissolve in acetone, dilute to volume with acetone, and mix. Pipet a 1-ml portion of this solution into a second 100-ml volumetric flask, dilute to volume with acetone, and mix. Finally, pipet a 5-ml portion of the last solution into a 50-ml volumetric flask, dilute to volume with acetone, and mix.

Procedure Determine the absorbance of each solution in 1-cm cells at the wavelength of maximum absorption at about 421 nm with a suitable spectrophotometer, using acetone as the blank. Calculate the percentage of curcumin in the sample by the formula

$$100 \times (W/w) \times (A_U/A_S),$$

in which A_U is the absorbance of the *Sample Preparation*, and A_S is the absorbance of the *Standard Preparation*. (NOTE: The absorbance readings should be made as soon as possible after the solutions are prepared to avoid color loss.)

PIPERINE CONTENT

Stock Standard Solution Purify piperine by repeated crystallization from isopropanol until a product having a melting range of 129° to 130° is obtained. Transfer 100.0 mg of the crystals, accurately weighed, into a 100-ml volumetric flask, dissolve in ethylene dichloride, dilute to volume with ethylene dichloride, and mix. Pipet 10.0 ml of this solution into a second 100-ml volumetric flask, dilute to volume with ethylene dichloride, and mix.

Standard Dilutions Pipet 1.0, 3.0, 5.0, and 10.0 ml of the *Stock Standard Solution* (corresponding to 0.1, 0.3, 0.5, and 1.0 mg of piperine, respectively) into separate 100-ml volumetric flasks, dilute each flask to volume with ethylene dichloride, and mix. Determine the absorbance of each dilution at once, as directed in the *Procedure*.

Sample Preparation Heat a portion of the sample to 100° on a steam bath or in an oven (but not on a hot plate), mix with a glass stirring rod, and transfer 100 mg, accurately weighed, into a 100-ml volumetric flask. Dissolve in ethylene dichloride, dilute to volume with ethylene dichloride, and mix. Pipet 1.0 ml of this solution into a second 100-ml volumetric flask, dilute to volume with ethylene dichloride, and mix. Determine the absorbance of the solution at once, as directed in the *Procedure*.

Procedure Determine the absorbance of the *Sample Preparation* and of each of the *Standard Dilutions* in 1-cm cells at the wavelength of maximum absorption at about 342 nm with a suitable spectrophotometer, using ethylene dichloride as the blank. Prepare a standard curve of concentration, in mg per 100 ml, versus absorbance for the four *Standard Dilutions*, including the absorbance at zero concentration obtained with the blank. From the standard curve, determine the concentration of piperine in the *Sample Preparation*, and record the value as C, in mg per 100 ml. Calculate the percentage of piperine in the sample by the formula $100 \times (100C/W)$, in which W is the weight of sample taken, in mg.

RESIDUAL SOLVENT

This procedure is for the determination of acetone, ethylene dichloride, hexane, isopropanol, methanol, methylene chloride, and trichloroethylene residues.

Distilling Head Use a Clevenger trap designed for use with oils heavier than water. A suitable design is shown in Fig. 19a, page 529.

Toluene The toluene used for this analysis should not contain any of the solvents determined by this method. The purity may be determined by gas chromatographic analysis, using one of the following columns or their equivalent: (1) 17% by weight of Ucon 75-H-90,000 on 35/80-mesh Chromosorb W; (2) 20% Ucon LB-135 on 35/80-mesh Chromosorb W; (3) 15% Ucon LB-1715 on 60/80-mesh Chromosorb W; or (4) Porapak Q 50/60 mesh. Follow the conditions described under *Procedure*, and inject the same amount of toluene as will be injected in the analysis of the solvents. If impurities interfering with the test are present, they will appear as peaks occurring before the toluene peak and should be removed by fractional distillation.

Benzene The benzene used for this analysis should be free from interfering impurities. The purity may be determined as described under *Toluene*.

Detergent and **Antifoam** Any such products that are free from volatile compounds may be used. If volatile compounds are present, they may be removed by prolonged boiling of the aqueous solutions of the products.

Reference Solution A Prepare a solution in *Toluene* containing 2500 ppm of benzene. If the toluene available contains benzene as the only impurity, the benzene level can be determined by gas chromatography and sufficient benzene added to bring the level to 2500 ppm.

Reference Solution B Prepare a solution containing 0.63% v/w of acetone in water.

Sample Preparation A (all solvents except methanol) Place 50.0 g of the sample, 1.00 ml of *Reference Solution A*, 10 g of anhydrous sodium sulfate, 50 ml of water, and a small amount each of *Detergent* and *Antifoam* in a 250-ml round-bottom flask with a 24/40 ground-glass neck. Attach the *Distilling Head*, a 400-mm water-cooled condenser, and a receiver, and collect approximately 15 ml of distillate. Add 15 g of anhydrous potassium carbonate to the distillate, cool while shaking, and allow the phases to separate. All of the solvents except methanol will be present in the toluene layer, which is used in the *Procedure*. Draw off the aqueous layer for use in *Sample Preparation B*.

Sample Preparation B (methanol only) Place the aqueous layer obtained from *Sample Preparation A* in a 50-ml round-bottom distilling flask with a 24/40 ground-glass neck, add a few boiling chips and 1.00 ml of *Reference Solution B*, and collect approximately 1 ml of distillate, which will contain any methanol from the sample, together with acetone as the internal standard. The distillate is used in the *Procedure*.

Procedure Use a gas chromatograph equipped with a hot-wire detector and a suitable sample-injection system or on-column injection. Under typical conditions, the instrument contains a 1/4-in. (od) × 6- to 8-ft column maintained isothermally at 70° to 80°. The flow rate of dry carrier gas is 50 to 80 ml per min, and the sample size is 15 to 20 µl (for the hot-wire detector). The column selected for use in the chromatograph depends on the components to be analyzed and, to a certain extent, on the preference of the analyst. The columns 1, 2, 3, and 4, as described under *Toluene*, may be used as follows: (1) This column separates acetone and methanol from their aqueous solution. It may be used for the separation and analysis of hexane, acetone, and trichloroethylene in the toluene layer from *Sample Preparation A*. The elution order is acetone, methanol, and water, or hexane, acetone, isopropanol plus methylene chloride, benzene, trichloroethylene, and ethylene dichloride plus toluene. (2) This column separates methylene chloride and isopropanol, and ethylene dichloride. The elution order is hexane plus acetone, methylene chloride, isopropanol, benzene, ethylene dichloride, trichloroethylene, and toluene. (3) This is the best general purpose column, except for the determination of methanol. The elution order is hexane, acetone, benzene, ethylene dichloride, and toluene. (4) This column is used for the determination of methanol, which elutes just after the large water peak.

Calibration Determine the response of the detector for known ratios of solvents by injecting known mixtures of solvents and benzene in toluene. The levels of the solvents and benzene in toluene should be of the same magnitude as they will be present in the sample under analysis.

Calculate the areas of the solvents with respect to benzene, and then calculate the calibration factor, F, as follows:

$$F\ (\text{solvent}) = \frac{\text{wt \% solvent}}{\text{wt \% benzene}} \times \frac{\text{area of benzene}}{\text{area of solvent}}.$$

The recovery of the various solvents from the oleoresin sample, with respect to the recovery of benzene, is as follows: hexane, 52%; acetone, 85%; isopropanol, 100%; methylene chloride, 87.5%; trichloroethylene, 113%; ethylene dichloride, 102%; and methanol, 87%.

Calculation Calculate the ppm of residual solvent (except methanol) by the formula

$$\text{Res. solv.} = \frac{43.4 \times F(\text{solvent}) \times 100}{\text{\% recovery of solvent}} \times \frac{\text{area of solvent}}{\text{area of benzene}},$$

in which 43.4 is the ppm of benzene internal standard, related to the 50-g oleoresin sample taken for analysis. Calculate the ppm of residual methanol by the formula

$$\text{Methanol} = \frac{100 \times F\ (\text{methanol})}{0.87} \times \frac{\text{area of methanol}}{\text{area of benzene}},$$

in which 100 is the ppm of acetone internal standard, related to the 50-g oleoresin sample taken for analysis.

SCOVILLE HEAT UNITS

Sample Preparation Transfer 200 mg of the sample into a 50-ml volumetric flask, dilute to volume with alcohol, and mix thoroughly by shaking. Allow the insolubles to settle before use.

Sucrose Solution Prepare a suitable volume of a 10% w/v solution of sucrose in water.

Standard Solution Add 0.15 ml of the *Sample Preparation* to 140 ml of the *Sucrose Solution*, and mix. This solution contains the equivalent of 240,000 Scoville Heat Units.

Test Solutions If the oleoresin sample is claimed to contain more than 240,000 Scoville Heat Units, prepare one or more dilutions according to the following table.

Scoville Heat Units	Standard Solution (ml)	Sucrose Solution (ml)
360,000	20	10
480,000	20	20
600,000	20	30
720,000	20	40
840,000	20	50
960,000	20	60
1,080,000	20	70
1,200,000	20	80
1,320,000	20	90
1,440,000	20	100
1,560,000	20	110
1,680,000	20	120
1,800,000	20	130
1,920,000	20	140
2,040,000	20	150

If the oleoresin sample is claimed to contain less than 240,000 Scoville Heat Units, prepare one or more dilutions according to the following table.

Scoville Heat Units	Sample Preparation (ml)	Sucrose Solution (ml)
100,000	0.15	60
117,500	0.15	70
170,000	0.15	100
205,000	0.15	120

Procedure Select five panel members who are thoroughly experienced with this method. Instruct the panelists to swallow 5 ml of the solution corresponding to the claimed content of Scoville Heat Units. The sample passes the test if three of the five panel members perceive a pungent or stinging sensation in the throat.

VOLATILE OIL CONTENT

Weigh accurately an amount of sample sufficient to yield 2 to 5 ml of volatile oil, and transfer with the aid of water into a 1000- or 2000-ml round-bottom shortneck flask with a 24/40 ground-glass neck. Add a magnetic stirring bar and about 500 ml of water, and connect a Clevenger trap of the proper type (see Figs. 19a and 19b) and a 400-mm water-cooled condenser. Heat

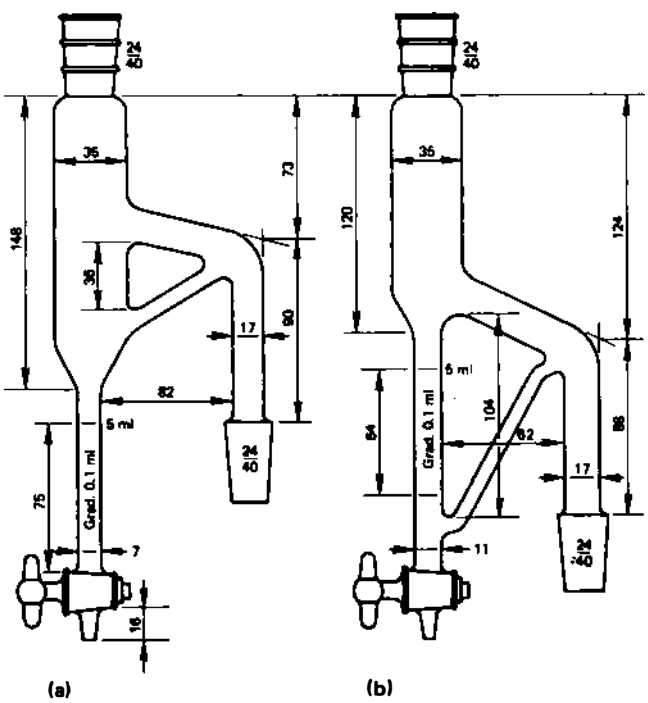


FIGURE 19 Clevenger Traps (All measurements are in mm.) (a) Oils Heavier Than Water; (b) Oils Lighter Than Water

the flask with stirring, and distil at a rate of 1 to 1.5 drops per second until two consecutive readings taken at 1-h intervals show no change of oil volume in the trap. Cool to room temperature, allow to stand until the oil layer is clear, and read the volume of oil collected, estimating to the nearest 0.02 ml. Calculate the percentage (v/w) of volatile oil in the sample by the formula $100 \times (V/W)$, in which V is the volume of oil collected, in ml, and W is the weight of sample taken, in g.

Optical Rotation

Many chemicals in a pure state or in solution are optically active in the sense that they cause incident polarized light to emerge in a plane forming a measurable angle with the plane of the incident light. When this effect is large enough for precise measurement, it may serve as the basis for an assay or an identity test. In this connection, the optical rotation is expressed in degrees, as either *angular rotation* (observed) or *specific rotation* (calculated with reference to the specific concentration of 1 g of solute in 1 ml of solution, measured under stated conditions).

Specific rotation usually is expressed by the term $[\alpha]_x^t$, in which t represents, in degrees centigrade, the temperature at which the rotation is determined, and x represents the characteristic spectral line or wavelength of the light used. Spectral lines most frequently employed are the D line of sodium (doublet at 589.0 and 589.6 nm) and the yellow green line of mercury at 546.1 nm. The specific gravity and the rotatory power vary appreciably with the temperature.

The accuracy and precision of optical rotatory measurements will be increased if they are carried out with due regard for the following general considerations.

The source of illumination should be supplemented by a filtering system capable of transmitting light of a sufficiently monochromatic nature. Precision polarimeters generally are designed to accommodate interchangeable disks to isolate the D line from sodium light or the 546.1-nm line from the mercury spectrum. With polarimeters not thus designed, cells containing suitably colored liquids may be employed as filters (see A. Weissberger, *Technique of Organic Chemistry*, Vol. I, Part II, Third Edition, Interscience Publishers, Inc., New York, 1960).

Special attention should be paid to temperature control of the solution and of the polarimeter. Observations should be accurate and reproducible to the extent that differences between replicates, or between observed and true values of rotation (the latter value having been established by calibration of the polarimeter scale with suitable standards), calculated in terms of either specific rotation or angular rotation, whichever is appropriate, shall not exceed one fourth of the range given in the individual monograph for the rotation of the article being tested. Generally, a polarimeter accurate to 0.05° of angular rotation, and capable of being read with the same precision, suffices for *Food Chemicals Codex* purposes; in some cases, a polarimeter accurate to 0.01°, or less, of angular rotation, and read with comparable precision, may be required.

Polarimeter tubes should be filled in such a way as to avoid creating or leaving air bubbles, which interfere with the passage of the beam of light. Interference from bubbles is minimized with tubes in which the bore is expanded at one end. However, with tubes of uniform bore, such as semimicro- or micro-tubes, care is required for proper filling. At the time of filling, the tubes and the liquid or solution should be at a temperature not higher than that specified for the determination, to guard against the formation of a bubble upon cooling and contraction of the contents.

In closing tubes having removable end plates fitted with gaskets and caps, the latter should be tightened only enough to ensure a leak-proof seal between the end plate and the body of the tube. Excessive pressure on the end plate may set up strains that result in interference with the measurements. In determining the specific rotation of a substance of low rotatory power, it is desirable to loosen the caps and tighten them again between successive readings in the measurement of both the rotation and the zero point. Differences arising from end plate strain thus generally will be revealed and appropriate adjustments to eliminate the cause may be made.

Procedure In the case of a solid, dissolve the substance in a suitable solvent, reserving a separate portion of the latter for a blank determination. Make at least five readings of the rotation of the solution, or of the substance itself if liquid, at 25° or the temperature specified in the individual monograph. Replace the solution with the reserved portion of the solvent (or, in the case of a liquid, use the empty tube), make the same number of readings, and use the average as the zero point value. Subtract the zero point value from the average observed rotation if the two figures are of the same sign, or add if opposite in sign, to obtain the corrected observed rotation.

Calculation Calculate the specific rotation of a liquid substance, or of a solid in solution, by application of one of the following formulas: (I) for liquid substances,

$$[\alpha]_x^t = a/ld;$$

(II) for solutions of solids,

$$[\alpha]_x^t = 100a/lpd = 100a/lc;$$

in which a is the corrected observed rotation, in degrees, at temperature t; l is the length of the polarimeter tube, in dm; d is the specific gravity of the liquid or solution at the temperature of observation; p is the concentration of the solution expressed as the number of g of substance in 100 g of solution; and c is the concentration of the solution expressed as the number of g of substance in 100 ml of solution. The concentrations p and c should be calculated on the dried or anhydrous basis, unless otherwise specified.

Oxygen Flask Combustion

Apparatus The apparatus consists of a heavy-walled, deeply lipped or cupped, conical flask of a volume suitable for the complete combustion of the sample in which the particular element is being determined (e.g., see *Selenium Limit Test*, page 537). The flask is fitted with a ground-glass stopper to which is fused a sample carrier consisting of heavy-gauge platinum wire and a piece of welded platinum gauze measuring about 1.5 × 2 cm. A suitable apparatus may be obtained as Catalog Nos. 6513-C20 (500-ml capacity) and 6513-C30 (1000-ml capacity) from Arthur H. Thomas Co., P.O. Box 779, Philadelphia, Pa. 19105. Equivalent apparatus available from other sources, or other suitable apparatus embodying the principles described herein, may also be used.

Procedure

Caution: The analyst should wear safety glasses and should use a suitable safety shield between himself and the apparatus. Further safety measures should be observed as necessary to ensure maximum protection of the analyst. Furthermore, the flask must be scrupulously clean and free from even traces of organic solvents. Samples containing water of hydration or more than 1% of moisture should be dried at 140° for 2 h before combustion, unless otherwise directed.

Accurately weigh the amount of sample specified in the monograph or general test. Solids should be weighed on a 4-cm square piece of halide-free paper, which should be folded around the sample. Liquid samples not exceeding 0.2 ml in volume should be weighed in tared cellulose acetate capsules [available as Catalog Nos. 6513-C80 (100 capsules) and 6513-C82 (1000 capsules) from the Arthur H. Thomas Co.]; gelatin capsules are satisfactory for liquid samples exceeding 0.2 ml in volume. (NOTE: Gelatin capsules may contain significant amounts of combined halide or sulfur, in which case a blank determination should be made as necessary.) Place the sample, together with a filter paper fuse-strip, in the platinum gauze sample holder. Place the absorbing liquid, as specified in the individual monograph or general test, in the flask, moisten the joint of the stopper with water, and flush the air from the flask with a stream of rapidly flowing oxygen, swirling the liquid to facilitate its taking up oxygen. (NOTE: Saturation of the liquid with oxygen is essential for successful performance of this procedure.) Ignite the fuse-strip by suitable means. If the strip is ignited outside the flask, immediately plunge the sample holder into the flask, invert the flask so that the absorption solution makes a seal around the stopper, and hold the stopper firmly in place. If the ignition is carried out in a closed system, the inversion of the flask may be omitted. After combustion is complete, shake the flask vigorously, and allow to stand for not less than 10 min with intermittent shaking. Then continue as directed in the individual monograph or general test chapter.

pH Determination

For FCC purposes, the pH of an aqueous solution may be determined accurately by potentiometry using a pH meter, or less accurately but more conveniently using pH indicator papers. Although some discussion and detail are presented here, for additional information the reader is referred to the text by Bates* for general background and to the paper by Lund† for consideration of pH meter standardization.

Potentiometric Method

The practical definition of pH in water may be given by the equation

$$pH = pH^0 + [(E - E^0)/0.0591],$$

where pH is the value for the solution being measured, pH^0 is the value for a standard buffer, E is the potential value for the solution being measured, E^0 is the potential value for the standard buffer, and 0.0591 is the value at 25°C of the Nernstian constant. The equation does not apply to solvents other than water, or to mixed solvents that include water. However, the pH meter gives reproducible readings in other solvent systems, on the basis of calibration with aqueous buffers, and while the pH readings lack thermodynamic significance they are useful in setting specifications.

The measurement of pH using a pH meter is a matter of comparing the meter reading of an unknown solution with the meter readings of standard buffers whose pH values are accurately known. Routine measurement uses only one buffer and an approximation of the electrode slope, usually made by a temperature compensator. For FCC purposes, pH measurement accurate to ±0.05 pH unit or better requires the use of two buffers that bracket, if possible, the expected pH range. All samples and buffers should be at the same temperature.

The choice and care of glass and reference electrodes must be carefully considered. The ordinary glass electrode begins to be sensitive to alkali metal cations at pH values above about 9, leading to the so-called alkaline error. Electrodes with a greatly reduced alkaline error should be used for readings in the alkaline range. Store the electrodes in distilled water when not in use, in order to avoid dehydration. "Flow-type" electrodes may be used if evidence of validity of pH measurement with the electrode is demonstrated.

The measurement of the pH of "lightly buffered solutions" (distilled water or solutions of nonionic organic compounds in distilled water) is a particularly difficult measurement. The addition of 0.3 ml of a saturated solution of potassium chloride per 100 ml of distilled water helps by providing a small amount of electrolyte. However, it will usually be necessary to protect

*R.G. Bates, *Determination of pH*, Second Edition, Wiley, New York, 1973.

†W. Lund, "The Standardization of pH Meters," *J. Chem. Ed.*, *56*, 129, 1979.

the solution being measured from the carbon dioxide in air by use of a blanket of nitrogen during the measurement. Measure the pH of successive portions of the distilled water or test solutions, with vigorous agitation, until the observed results for two successive portions agree within 0.1 pH unit.

Procedure Use a suitable pH meter and follow the manufacturer's instructions. Each time the electrodes are used, rinse them with distilled or deionized water and carefully blot them dry with clean absorbent tissue. Form a fresh reference electrode liquid junction. Rinse the sample vessel three times with each new solution to be introduced.

Choose two standard buffers to bracket, if possible, the anticipated pH of the unknown. Warm or cool these standards as necessary to match within 2°C the temperature of the unknown, and initially set the temperature compensator to that temperature. Immerse the electrodes in a portion of the first standard buffer, and following the manufacturer's instructions adjust the appropriate standardization control (knob, switch, or button) until the pH reading is that of the buffer. Repeat this procedure with fresh portions of the first standard buffer until two successive readings are within ±0.02 pH unit without an adjustment of the standardization control.

Rinse the electrodes, blot dry, and immerse them in a portion of the second standard buffer of lower pH. Do not change the setting of the standardization control. Following the manufacturer's instructions, adjust the slope control (thumbwheel switch, knob, or temperature compensator) until the exact buffer pH is displayed.

Repeat the sequence of standardization with both buffers until the pH readings are within ±0.02 pH unit for both buffers without any adjustment of either control. The pH of the unknown solution may then be measured.

Always restandardize the instrument after even a short period during which the amplifier is turned off.

pH Indicator Test Papers

There are presently available test papers that have impregnated acid-base indicators and are very convenient for the determination of the approximate pH of an aqueous solution. Indicator test papers that cover a wide pH range or that cover a narrow pH range may be obtained. The maximum accuracy attainable with these systems is ±0.25 pH unit. For FCC purposes, pH determination using pH indicator test papers may be specified where the potentiometric method cannot be used.

The accepted test procedure using a pH indicator test paper is to use a clean glass rod to remove a drop of the solution whose pH is being determined and place it on a test paper strip. When the indicator color is achieved, compare the color with the color comparison chart and determine by color similarity the pH of the solution. If the pH is alkaline, take care to minimize absorption of carbon dioxide by the solution on the pH paper, as this will result in pH change. Immersion of the test paper strip in the solution whose pH is to be determined is not recommended since some of the indicator may dissolve in the solution.

Readily Carbonizable Substances

Reagents

Sulfuric Acid TS Add a quantity of sulfuric acid of known concentration to sufficient water to adjust the final concentration to between 94.5% and 95.5% of H_2SO_4. Since the acid concentration may change upon standing or upon intermittent use, the concentration should be checked frequently and solutions assaying more than 95.5% or less than 94.5% discarded or adjusted by adding either diluted or fuming sulfuric acid, as required.

Cobaltous Chloride CS Dissolve about 65 g of cobaltous chloride ($CoCl_2.6H_2O$) in enough of a mixture of 25 ml of hydrochloric acid and 975 ml of water to make 1000 ml. Pipet 5 ml of this solution into a 250-ml iodine flask, add 5 ml hydrogen peroxide TS and 15 ml of sodium hydroxide solution (1 in 5), boil for 10 min, cool, and add 2 g of potassium iodide and 20 ml of dilute sulfuric acid (1 in 4). When the precipitate has dissolved, titrate the liberated iodine with 0.1 *N* sodium thiosulfate. The titration is sensitive to air oxidation and should be blanketed with carbon dioxide. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 23.79 mg of $CoCl_2.6H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water to make each ml contain 59.5 mg of $CoCl_2.6H_2O$.

Cupric Sulfate CS Dissolve about 65 g of cupric sulfate ($CuSO_4.5H_2O$) in enough of a mixture of 25 ml of hydrochloric acid and 975 ml of water to make 1000 ml. Pipet 10 ml of this solution into a 250-ml iodine flask, add 40 ml of water, 4 ml of acetic acid, and 3 g of potassium iodide, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 24.97 mg of $CuSO_4.5H_2O$. Adjust the final volume of the solution by the addition of enough of the mixture of hydrochloric acid and water to make each ml contain 62.4 mg of $CuSO_4.5H_2O$.

Ferric Chloride CS Dissolve about 55 g of ferric chloride ($FeCl_3.6H_2O$) in enough of a mixture of 25 ml of hydrochloric acid and 975 ml of water to make 1000 ml. Pipet 10 ml of this solution into a 250-ml iodine flask, add 15 ml of water, 5 ml of hydrochloric acid, and 3 g of potassium iodide, and allow the mixture to stand for 15 min. Dilute with 100 ml of water, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the indicator. Perform a blank determination with the same quantities of the same reagents and in the same manner, and make any necessary correction. Each ml of 0.1 *N* sodium thiosulfate is equivalent to 27.03 mg of $FeCl_3.6H_2O$. Adjust the final volume of the solution by addition of the mixture of hydrochloric acid and water to make each ml contain 45.0 mg of $FeCl_3.6H_2O$.

Platinum-Cobalt CS Transfer 1.246 g of potassium chloroplatinate, K_2PtCl_6, and 1.00 g of crystallized cobaltous chloride, $CoCl_2.6H_2O$, into a 1000-ml volumetric flask, dissolve in about 200 ml of water and 100 ml of hydrochloric acid, dilute to volume with water, and mix. This solution has a color of 500 APHA units. (NOTE: Use this solution only when specified in an individual monograph.)

Procedure Unless otherwise directed, add the specified quantity of the substance, finely powdered if in solid form, in small portions to the comparison container, which is made of colorless glass resistant to the action of sulfuric acid and contains the specified volume of *Sulfuric Acid TS*.

Stir the mixture with a glass rod until solution is complete, allow the solution to stand for 15 min, unless otherwise directed, and compare the color of the solution with that of the specified matching fluid in a comparison container that also is of colorless glass and has the same internal and cross-section dimensions, viewing the fluids transversely against a background of white porcelain or white glass.

When heat is directed in order to effect solution of the substance in the *Sulfuric Acid TS*, mix the sample and the acid in a test tube, heat as directed, cool, and transfer the solution to the comparison container for matching.

Matching Fluids[a]

Matching Fluid	Parts of Cobaltous Chloride CS	Parts of Ferric Chloride CS	Parts of Cupric Sulfate CS	Parts of Water
A	0.1	0.4	0.1	4.4
B	0.3	0.9	0.3	8.5
C	0.1	0.6	0.1	4.2
D	0.3	0.6	0.4	3.7
E	0.4	1.2	0.3	3.1
F	0.3	1.2	0.0	3.5
G	0.5	1.2	0.2	3.1
H	0.2	1.5	0.0	3.3
I	0.4	2.2	0.1	2.3
J	0.4	3.5	0.1	1.0
K	0.5	4.5	0.0	0.0
L	0.8	3.8	0.1	0.3
M	0.1	2.0	0.1	2.8
N	0.0	4.9	0.1	0.0
O	0.1	4.8	0.1	0.0
P	0.2	0.4	0.1	4.3
Q	0.2	0.3	0.1	4.4
R	0.3	0.4	0.2	4.1
S	0.2	0.1	0.0	4.7
T	0.5	0.5	0.4	3.6

[a] Solutions A–D, very light brownish yellow.
Solutions E–L, yellow through reddish yellow.
Solutions M–O, greenish yellow.
Solutions P–T, light pink.

Matching Fluids For purposes of comparison, a series of 20 matching fluids, each designated by a letter of the alphabet, is provided, the composition of each being as indicated in the accompanying table. To prepare the matching fluid specified, pipet the prescribed volumes of the colorimetric test solutions (CS) and water into one of the matching containers, and mix the solutions in the container.

Refractive Index

The refractive index of a transparent substance is the ratio of the velocity of light in air to its velocity in that material under like conditions. It is equal to the ratio of the sine of the angle of incidence made by a ray in air to the sine of the angle of refraction made by the ray in the material being tested. The refractive index values specified in this Codex are for the D line of sodium (589 nm), unless otherwise specified. The determination should be made at the temperature specified in the individual monograph, or at 25° if no temperature is specified. This physical constant is used as a means for identification of, and detection of impurities in, volatile oils and other liquid substances. The Abbé refractometer, or other refractometers of equal or greater accuracy, may be employed at the discretion of the operator.

Residue on Ignition (Sulfated Ash)

Method I (for Solids)

Transfer the quantity of the sample directed in the individual monograph to a tared 50- to 100-ml platinum dish or other suitable container, and add sufficient diluted sulfuric acid TS to moisten the entire sample. Heat gently, using a hot plate, an Argand burner, or an infrared heat lamp, until the sample is dry and thoroughly charred, then continue heating until all of the sample has been volatilized or nearly all of the carbon has been oxidized, and cool. Moisten the residue with 0.1 ml of sulfuric acid, and heat in the same manner until the remainder of the sample and any excess sulfuric acid have been volatilized. Finally, ignite in a muffle furnace at 800° ± 25° for 15 min, or longer if necessary to complete ignition, cool in a desiccator, and weigh.

Method II (for Liquids)

Unless otherwise directed, transfer the required weight of the sample to a suitable tared container, add 10 ml of diluted sulfuric acid TS, and mix thoroughly. Evaporate the sample completely by heating gently without boiling, and cool. Finally, ignite in a muffle furnace at 800° ± 25° for 15 min, cool in a desiccator, and weigh.

Rosins and Related Substances

ACID VALUE

The acid value is defined as the number of mg of potassium hydroxide required to neutralize the free acids in 1 g of the test substance.

Procedure Unless otherwise directed in the individual monograph, transfer about 4 g of the sample, previously crushed into small lumps and accurately weighed, into a 250-ml Erlenmeyer flask, and add 50 to 75 ml of a 2 to 1 mixture of benzene-methanol, previously neutralized to phenolphthalein TS with sodium hydroxide. Dissolve the sample by shaking or heating gently, if necessary, then add about 0.5 ml of phenolphthalein TS, and titrate with 0.5 *N* alcoholic potassium hydroxide to the first pink color that persists for 30 s. Calculate the acid value by the formula $56.1V \times N/W$, in which V is the exact volume, in ml, and N is the exact normality, respectively, of the potassium hydroxide solution, and W is the weight of the sample, in g.

SOFTENING POINT

Drop Method

The *drop softening point* is defined as that temperature at which a given weight of rosin or rosin derivative begins to drop from the bulb of a special thermometer mounted in a test tube that is immersed in a constant-temperature bath.

Apparatus The apparatus illustrated in Fig. 20 consists of the components described in the following paragraphs.

Thermometer A special total-immersion softening point thermometer,* covering the range from 0° to 250° and graduated in 1° divisions, should be employed. The bulb should be 5/8 ± 1/32 in. in length (16 mm) and 1/4 ± 1/64 in. in diameter (6.4 mm).

Heating Bath Use an 800- or 1000-ml beaker containing a suitable heating medium. For rosins having a softening point below 80°, use water; for those having softening points above 80°, use glycerin, mineral oil, or other suitable vegetable oil, depending upon the temperature range required. The temperature of the heating medium must be maintained within ± 1° of the temperature specified in the individual monograph. The medium must be stirred constantly during the test with a suitable mechanical stirrer to ensure uniform heating of the medium.

Test Tube Use a standard 22- × 175-mm test tube with rim, fitted with a cork stopper as shown in Fig. 20.

Sample Preparation Place about 20 g of the sample in a 50-ml beaker, and heat in an oven, on a sand bath or hot plate, or in an oil bath until its softening point is exceeded, but not more than 25° to 30° above its softening point or for any longer than necessary. Tare the softening point thermometer, and cautiously warm the bulb over a flame or hot plate until it registers 15° to 20° above the expected softening point of the sample. Immediately dip the thermometer bulb into the melted sample, withdraw, and rotate it so that a uniform film of the molten sample is deposited over the surface of the bulb, care being taken not to extend the film higher than the top of the bulb. Quickly place the thermometer on a balance, and weigh. The weight of the sample on the thermometer bulb should be between 500 and 550 mg. If the weight is low, again dip the bulb in the molten sample; if the weight is high, pull off some of the sample with the fingers. When the correct sample weight has been obtained, mold the sample uniformly around the bulb by rolling on the palm of the hand or between the fingers. The sample must be of uniform thickness over the bulb, and it must not extend up onto the thermometer stem (see Fig. 20). (If the film of the sample is not uniform when cooled, it should be completely removed from the bulb and a new one applied. Do not reheat the film and try to remold.) Allow the film and thermometer to cool to room temperature, allowing about 15 min for cooling. (NOTE: If samples having high softening points crack or "check" on the thermometer bulb upon cooling to room temperature, prepare another sample film and cool only to about 50° below the expected softening point.)

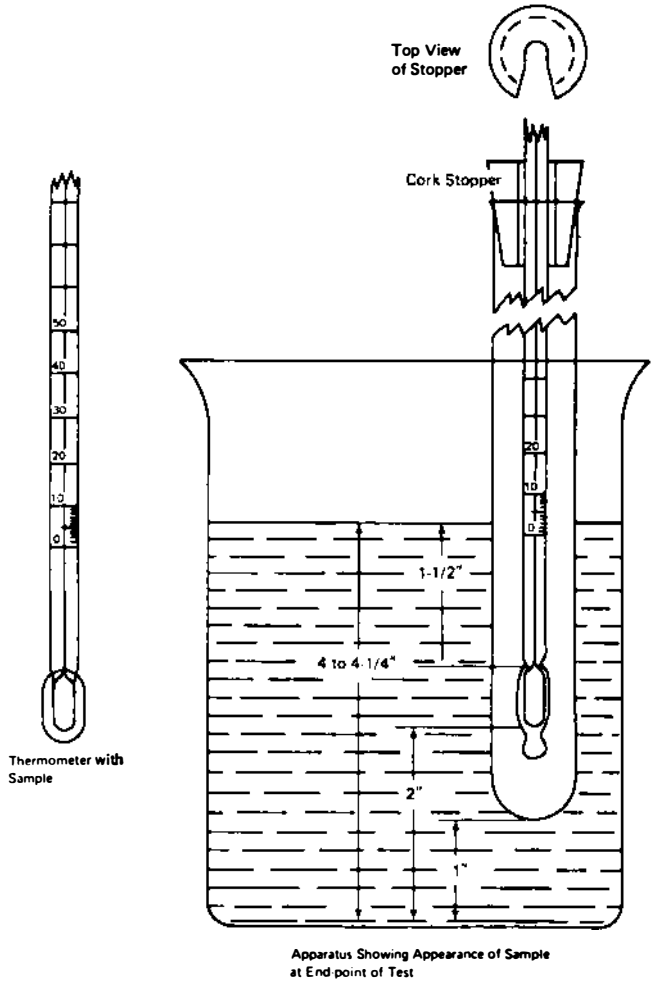


FIGURE 20 Apparatus for Drop Softening Point Determination

Procedure Fill the glass beaker to a depth of not less than 4 in. (100 mm) or more than 4.25 in. (108 mm) with a suitable heating medium, support the beaker over a Bunsen burner, hot plate, or other suitable source of heat, and insert the bath stirrer and a bath temperature thermometer. Place the stirrer to one side so that the impeller clears the side of the beaker and is about 0.5 in. (13 mm) above the bottom of the beaker. Start the

*Available from the Taylor Instrument Co., 95 Ames Street, Rochester, N.Y. 14601.

stirrer, heat the bath to the temperature specified in the monograph, and maintain this temperature within ±1° throughout the test.

Insert the prepared sample thermometer in the test tube, supporting it with a notched cork stopper so that the lower end of the bulb is 1 in. (25 mm) from the bottom of the test tube. Place the test tube in the bath so that the bottom of the thermometer bulb is 2 in. (51 mm) from the bottom of the beaker; the top of the bulb should be about 1.5 in. (38 mm) below the liquid level of the bath. Stir the bath in order to keep its temperature uniform throughout. Observe the sample thermometer, and record as the softening point the reading at which the elongated drop of sample on the end of the bulb first becomes constricted (see Fig. 20). Report the softening point to the nearest 0.5°.

Precautions: If the rosin crystallizes, thus making it difficult to obtain the correct softening point, prepare a new sample by heating the rosin rapidly, yet cautiously, over a flame to a temperature of 160° to 170° in order to destroy all crystal nuclei; then dip the thermometer bulb into the molten resin, remove momentarily, and rotate the thermometer to provide a uniform resin film on the bulb as it partially cools in air; dip the bulb in the melted sample repeatedly until the proper amount of resin has been deposited on the bulb. Results should not be reported if a crystal-free sample cannot be obtained.

Ring-and-Ball Method

The *ring-and-ball softening point* is defined as the temperature at which a disk of the sample held within a horizontal ring is forced downward a distance of 1 in. (25.4 mm) under the weight of a steel ball as the sample is heated at a prescribed rate in a water or glycerin bath.

Apparatus The apparatus illustrated in Figs. 21 and 22 consists of the components described in the following paragraphs.

Ring A brass-shouldered ring conforming to the dimensions shown in Fig. 21*a* should be used. If desired, the ring may be attached by brazing or other convenient manner to a brass wire of about 13 B & S gauge (0.06 to 0.08 in., or 1.52 to 2.03 mm, in diameter) as shown in Fig. 22*a*.

Ball A steel ball, 3/8 in. (9.53 mm) in diameter, weighing between 3.45 and 3.55 g, should be used.

Ball-Centering Guide A guide for centering the ball, constructed of brass and having the general shape and dimensions illustrated in Fig. 21*c*, may be used if desired.

Container Use a heat-resistant glass vessel, such as an 800-ml low-form Griffin beaker, not less than 3.34 in. (8.5 cm) in diameter and not less than 5 in. (12.7 cm) in depth from the bottom of the flare.

Support for Ring and Thermometer Any convenient device for supporting the ring and thermometer may be used, provided that it meets the following requirements: (1) the ring shall be supported in a substantially horizontal position; (2) when using the apparatus shown in Fig. 21*d*, the bottom of the ring shall be 1.0 in. (25.4 mm) above the horizontal plate below it, the

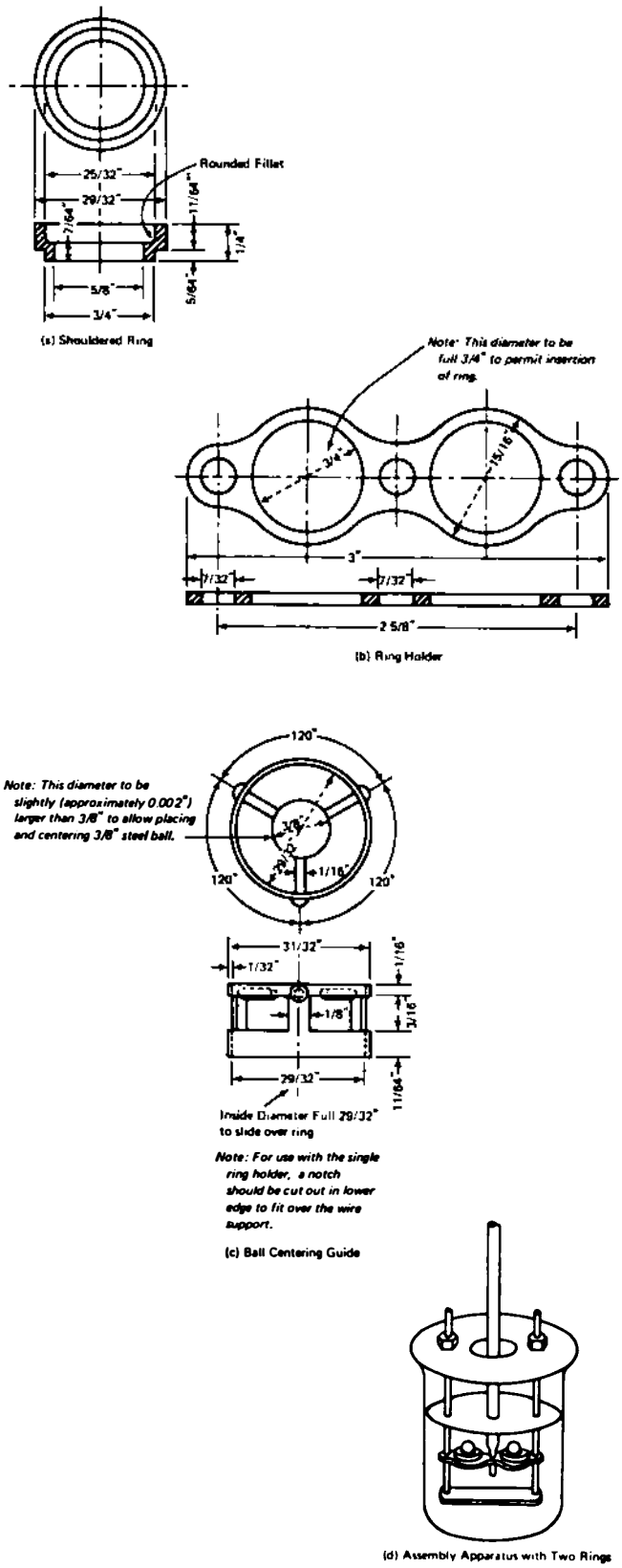


FIGURE 21 Shouldered Ring, Ring Holder, Ball-Centering Guide, and Assembly of Apparatus Showing Two Rings

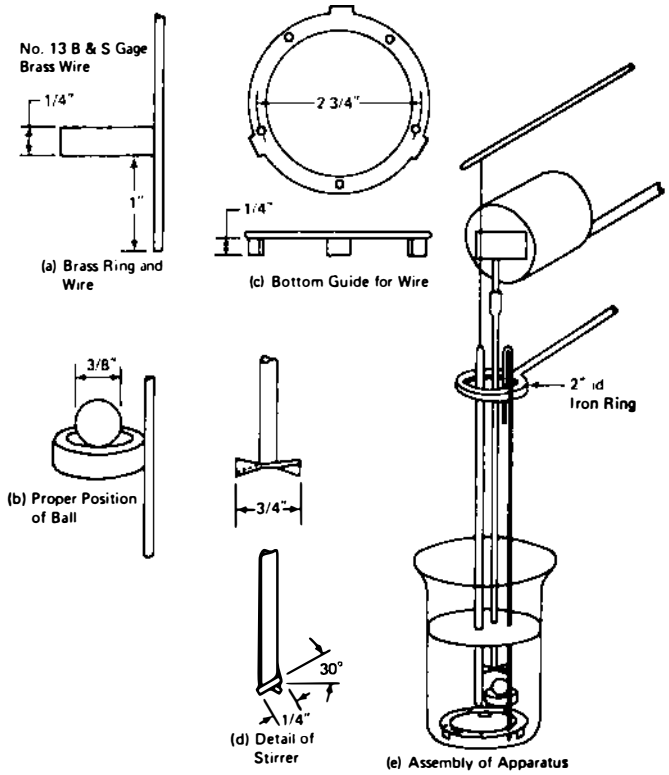


FIGURE 22 Assembly of Apparatus Showing Stirrer and Single-Shouldered Ring

bottom surface of the horizontal plate shall be at least 0.5 in. (13 mm) and not more than 0.75 in. (18 mm) above the bottom of the container, and the depth of the liquid in the container shall be not less than 4.0 in. (10.2 cm); (3) when using the apparatus shown in Fig. 22*e*, the bottom of the ring shall be 1.0 in. (25.4 mm) above the bottom of the container, with the bottom end of the rod resting on the bottom of the container, and the depth of the liquid in the container shall be not less than 4.0 in. (10.2 cm), as shown in Figs. 22*a*, *b*, and *c*; and (4) in both assemblies, the thermometer shall be suspended so that the bottom of the bulb is level with the bottom of the ring and within 0.5 in. (13 mm) but not touching the ring.

Thermometers Depending upon the expected softening point of the sample, use either an ASTM 15C or 15F low-softening-point thermometer (−2° to 80°C) or an ASTM 16C or 16F high-softening-point thermometer (30° to 200°C), as described under *Thermometers*, page 547.

Stirrer Use a suitable mechanical stirrer rotating between 500 and 700 rpm. To ensure uniform heat distribution in the heating medium, the direction of the shaft rotation should move the liquid upward. (See Fig. 22*d* for recommended dimensions.)

Sample Preparation Select a representative sample of the material under test consisting of freshly broken lumps free of oxidized surfaces. Scrape off the surface layer of samples received as lumps immediately before use, avoiding inclusion of finely divided material or dust. The amount of sample taken should be at least twice that necessary to fill the desired number of rings, but in no case less than 40 g. Immediately melt the sample in a clean container, using an oven, hot plate, or sand or oil bath to prevent local overheating. Avoid incorporating air bubbles in the melting sample, which must not be heated above the temperature necessary to pour the material readily without inclusion of air bubbles. The time from the beginning of heating to the pouring of the sample shall not exceed 15 min. Immediately before filling the rings, preheat them to approximately the same temperature at which the sample is to be poured. While being filled, the rings should rest on an amalgamated brass plate. Pour the sample into the rings so as to leave an excess on cooling. Cool for at least 30 min, and then cut the excess material off cleanly with a slightly heated knife or spatula. Use a clean container and a fresh sample if the test is repeated.

Procedure

Materials Having Softening Points above 80°C Fill the glass vessel with glycerin to a depth of not less than 4.0 in. (10.2 cm) and not more than 4.25 in. (10.8 cm). The starting temperature of the bath shall be 32°C (90°F). For resins (including rosin), the glycerin should be cooled to not less than 45°C (81°F) below the anticipated softening point, but in no case lower than 35°C (95°F). Position the axis of the stirrer shaft near the back wall of the container, with the blades clearing the wall and with the bottom of the blades 0.75 in. (18 mm) above the top of the ring. Unless the ball-centering guide is used, make a slight indentation in the center of the sample by pressing the ball or a rounded rod, slightly heated for hard materials, into the sample at this point. Suspend the ring containing the sample in the glycerin bath so that the lower surface of the filled ring is 1.0 in. (25.4 mm) above the surface of the lower horizontal plate (see Fig. 21*d*), which is at least 0.5 in. (13 mm) and not more than 0.75 in. (18 mm) above the bottom of the glass vessel, or 1.0 in. (25.4 mm) above the bottom of the container (see Fig. 22*e*). Place the ball in the glycerin but not on the test specimen. Suspend an ASTM high-softening-point thermometer (16C or 16F) in the glycerin so that the bottom of its bulb is level with the bottom of the ring and within 0.5 in. (13 mm) but not touching the ring. Maintain the initial temperature of the glycerin for 15 min, and then, using suitable forceps, place the ball in the center of the upper surface of the sample in the ring. Begin stirring, and continue stirring at 500 to 700 rpm until completion of the determination. Apply heat at such a rate that the temperature of the glycerin is raised 5°C (or 10°F) per min, avoiding the effects of drafts by using shields if necessary. [NOTE: The rate of rise of the temperature shall be uniform and shall not be averaged over the test period. Reject all tests in which the rate of rise exceeds ±0.5°C (or ± 1°F) for any min period after the first three.] Record as the softening point the temperature of the thermometer at the instant the sample touches the lower horizontal plate (see Fig. 21*d*) or the bottom of the container (see Fig. 22*e*). Make no correction for the emergent stem of the thermometer.

Materials Having Softening Points of 80°C or Below Follow the above procedure, except use an ASTM low-softening-point thermometer (15C or 15F) and use freshly boiled water cooled to 5°C (41°F) as the heating medium. For resins (including rosins), use water cooled to not less than 45°C (81°F) below the anticipated softening point, but in no case lower than 5°C (41°F).

VISCOSITY

Unless otherwise directed in the individual monograph, transfer the sample into an 8-oz wide-mouth glass jar, 10.8 cm (4.25 in.) high and 5.7 cm (2.75 in.) in inside diameter, equipped with a screw lid. Condition the sample in a water bath at 25° ± 0.2° for 2 h (±5 min), taking care to prevent water from coming into contact with the sample. Insert a No. 4 spindle in a Brookfield Model RVF viscometer,* or equivalent, and move the jar into place under the spindle, adjusting the elevation of the jar so that the upper surface of the sample is in the center of the shaft indentation and the spindle is in the center of the jar. (NOTE: The viscometer must be kept level at all times during the test procedure.) Set the viscometer to rotate at 20 rpm, and allow the spindle to rotate until a constant dial reading is obtained. The viscosity, in poises, is the dial reading on the 100 scale, or the dial reading on the 500 scale divided by 5.

Selenium Limit Test

Reagents and Solutions

2,3-Diaminonaphthalene Solution On the day of use, dissolve 100 mg of 2,3-diaminonaphthalene ($C_{10}H_{10}N_2$) and 500 mg of hydroxylamine hydrochloride ($NH_2OH.HCl$) in sufficient 0.1 *N* hydrochloric acid to make 100 ml.

Selenium Stock Solution Transfer 40.0 mg of powdered metallic selenium into a 1000-ml volumetric flask, and dissolve in 100 ml of dilute nitric acid (1 in 2), warming gently on a steam bath to effect solution. Cool, dilute with water to volume, and mix.

Selenium Standard Solution Pipet 5.0 ml of *Selenium Stock Solution* into a 200-ml volumetric flask, dilute to volume with water, and mix. Each ml of this solution contains the equivalent of 1 μg of selenium (Se).

Method I

Standard Preparation Pipet 6.0 ml of *Selenium Standard Solution* into a 150-ml beaker, add 50 ml of 0.25 *N* nitric acid, and mix.

Sample Preparation Using a 1000-ml combustion flask and 25 ml of 0.5 *N* nitric acid as the absorbing liquid, proceed as directed under *Oxygen Flask Combustion*, page 531, using the amount of sample specified in the individual monograph (and the magnesium oxide or other reagent, where specified). (NOTE: If the sample contains water of hydration or more than 1% of moisture, dry it at 140° for 2 h before combustion, unless otherwise directed.) Upon completion of combustion, place a few ml of water in the cup or lip of the combustion flask, loosen the stopper of the flask, and rinse the stopper, sample holder, and sides of the flask with about 10 ml of water. Transfer the solution, with the aid of about 20 ml of water, into a 150-ml beaker, heat gently to boiling, boil for 10 min, and cool.

Procedure Treat the *Sample Preparation*, the *Standard Preparation*, and 50 ml of 0.25 *N* nitric acid, to serve as the blank, similarly and in parallel as follows: Add dilute ammonium hydroxide (1 in 2) to adjust the pH of the solution to 2.0 ± 0.2. Dilute with water to 60.0 ml, and transfer to a low-actinic separator with the aid of 10.0 ml of water, adding the 10.0 ml of rinsings to the separator. Add 200 mg of hydroxylamine hydrochloride, swirl to dissolve, immediately add 5.0 ml of *2,3-Diaminonaphthalene Solution*, insert the stopper, and swirl to mix. Allow the solution to stand at room temperature for 100 min. Add 5.0 ml of cyclohexane, shake vigorously for 2 min, and allow the layers to separate. Discard the aqueous phases, and centrifuge the cyclohexane extracts to remove any traces of water. Determine the absorbance of each extract in a 1-cm cell at the maximum at about 380 nm with a suitable spectrophotometer, using the blank to set the instrument. The absorbance of the extract from the *Sample Preparation* is not greater than that from the *Standard Preparation* when a 200-mg sample is tested, or not greater than one-half the absorbance of the extract from the *Standard Preparation* when a 100-mg sample is tested.

Method II

Standard Preparation Pipet 6.0 ml of *Selenium Standard Solution* into a 150-ml beaker, add 50 ml of 2 *N* hydrochloric acid, and mix.

Sample Preparation Transfer the amount of sample specified in the individual monograph into a 150-ml beaker, dissolve in 25 ml of 4 *N* hydrochloric acid, swirling if necessary to effect solution, heat gently to boiling, and digest on a steam bath for 15 min. Remove from heat, add 25 ml of water, and allow to cool to room temperature.

Procedure Place the beakers containing the *Standard Preparation* and the *Sample Preparation* in a fume hood, and to a third beaker add 50 ml of 2 *N* hydrochloric acid to serve as the blank. Cautiously add 5 ml of ammonium hydroxide to each beaker, mix, and allow the solution to cool. Treat each solution, similarly and in parallel, as directed under *Procedure* in *Method I*, beginning with "Add dilute ammonium hydroxide (1 in 2). . . . "

Sieve Analysis of Granular Metal Powders

(Based on ASTM Designation: B 214)

Apparatus

Sieves Use a set of standard sieves, ranging from +80 mesh to −325 mesh, conforming to the specifications in ASTM Designation: E 11 (Sieves for Testing Purposes).

Sieve Shaker Use a mechanically operated sieve shaker that imparts to the set of sieves a horizontal rotary motion of

*Available from Brookfield Engineering Laboratories, Inc., Stoughton, Mass.

between 270 and 300 rotations per min and a tapping action of between 140 and 160 taps per min. The sieve shaker is fitted with a plug to receive the impact of the tapping device. The entire apparatus is mounted rigidly by bolting to a solid foundation, preferably of concrete. Preferably a time switch is provided to ensure accuracy of duration of the test.

Procedure Assemble the sieves in consecutive order as to size of openings, with the coarsest sieve (+80 mesh) at the top, and place a solid-collecting pan below the bottom sieve (−325 mesh). Place 100.0 g of the test sample on the top sieve, and close the sieve with a solid cover. Securely fasten the assembly to the sieve shaker, and operate the shaker for 15 min. Remove the most coarse sieve from the nest, gently tap its contents to one side, and pour the contents onto a glazed paper. Using a soft brush, transfer onto the next finer sieve any material adhering to the bottom of the sieve and frame. Place the sieve just removed upside down on the paper containing the retained portion, and tap the sieve. Weigh the paper and its contents to the nearest 100 mg, and record the net weight of the fraction obtained. Repeat this process for each sieve in the nest and for the portion of the sample that has been collected in the bottom pan. Record the total of the fractions retained on the sieves as T and that portion collected in the pan as t. The combined total, S, of $T + t$ is not less than 99.0 g. Add the weight $(100.0 - S)$ to the fraction t collected in the pan. The sum,

$$t + (100.0 - S),$$

represents the portion of the sample that has passed through the −325-mesh sieve.

Solidification Point

Scope This method is designed to determine the solidification point of food-grade chemicals having appreciable heats of fusion. It is applicable to chemicals having solidification points between −20° and +150°. Necessary modifications will be noted in individual monographs.

Definition *Solidification Point* is an empirical constant defined as the temperature at which the liquid phase of a substance is in approximate equilibrium with a relatively small portion of the solid phase. It is measured by noting the maximum temperature reached during a controlled cooling cycle after the appearance of a solid phase.

Soldification point is distinguished from freezing point in that the latter term applies to the temperature of equilibrium between the solid and liquid state of pure compounds.

Some chemical compounds have two temperatures at which there may be a temperature equilibrium between the solid and liquid state depending upon the crystal form of the solid that is present.

Apparatus The apparatus illustrated in Figs. 23 and 24 consists of the components described in the following paragraphs.

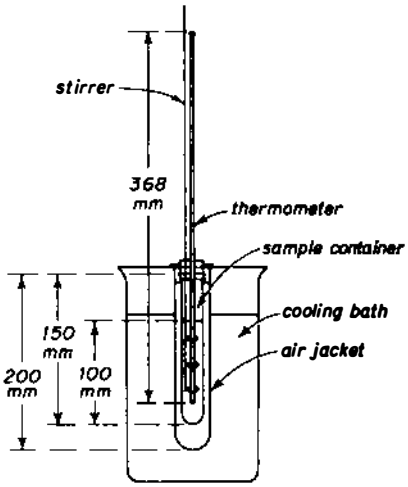


FIGURE 23 Apparatus for Determination of Solidification Point

Thermometer A thermometer having a range not exceeding 30°, graduated in 0.1° divisions, and calibrated for 76 mm immersion should be employed. A satisfactory series of thermometers, covering a range from −20° to +150°, is available as ASTM-E1 89C through 96C (see *Thermometers*, page 547). A thermometer should be so chosen that the solidification point is not obscured by the cork stopper of the sample container.

Sample Container Use a standard glass 25- × 150-mm test tube with lip, fitted with a two-hole cork stopper to hold the thermometer in place and to allow stirring with stirrer.

Air Jacket For the air jacket use a standard glass 38- × 200-mm test tube with lip, fitted with a cork or rubber stopper bored with a hole into which the sample container can easily be inserted up to the lip.

Cooling Bath Use a 2000-ml beaker or similar suitable container as a cooling bath. Fill it with an appropriate cooling medium such as glycerin, mineral oil, water, water and ice, or alcohol–dry ice.

Stirrer The stirrer (Fig. 24) consists of a 1-mm diameter (B & S gauge 18), corrosion-resistant wire bent into a series of

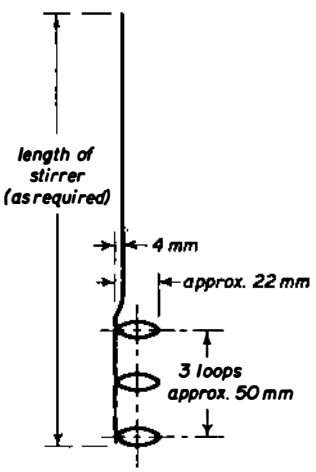


FIGURE 24 Stirrer for Solidification Point Determination

three loops about 25 mm apart. It should be made so that it will move freely in the space between the thermometer and the inner wall of the sample container. The shaft of the stirrer should be of a convenient length designed to pass loosely through a hole in the cork holding the thermometer. Stirring may be hand operated or mechanically activated at 20 to 30 strokes per min.

Assembly Assemble the apparatus in such a way that the cooling bath can be heated or cooled to control the desired temperature ranges. Clamp the air jacket so that it is held rigidly just below the lip, and immerse it in the cooling bath to a depth of 160 mm.

Preparation of Sample The solidification point is usually determined on chemicals as they are received. Some may be hygroscopic, however, and require special drying. Where this is necessary it will be noted in the monograph.

Products that are normally solid at room temperature must be carefully melted at a temperature about 10° above the expected solidification point. Care should be observed to avoid heating in such a way as to decompose or distil any portion of a sample.

Procedure Adjust the temperature of the cooling bath to about 5° below the expected solidification point. Fit the thermometer and stirrer with a cork stopper so that the thermometer is centered and the bulb is about 20 mm from the bottom of the sample container. Transfer a sufficient amount of the sample, previously melted if necessary, into the sample container to fill it to a depth of about 90 mm when in the molten state. Place the thermometer and stirrer in the sample container, and adjust the thermometer so that the immersion line will be at the surface of the liquid and the end of the bulb 20 ± 4 mm from the bottom of the sample container. When the temperature of the sample is about 5° above the expected solidification point, place the assembled sample tube in the air jacket.

Allow the sample to cool while stirring, at the rate of 20 to 30 strokes per min, in such a manner that the stirrer does not touch the thermometer. Stir the sample continuously during the remainder of the test.

The temperature at first will gradually fall, then will become constant as crystallization starts and continues under equilibrium conditions, and finally will start to drop again. Some chemicals may supercool slightly below (0.5°) the solidification point; as crystallization begins the temperature will rise and remain constant as equilibrium conditions are established. Other products may cool more than 0.5° and cause deviation from the normal pattern of temperature change. If the temperature rise exceeds 0.5° after the initial crystallization begins, repeat the test and seed the melted compound with small crystals of the sample at 0.5° intervals as the temperature approaches the expected solidification point. Crystals for seeding may be obtained by freezing a small sample in a test tube directly in the cooling bath. It is preferable that seed of the stable phase be used from a previous determination.

Observe and record the temperature readings at regular intervals until the temperature rises from a minimum, due to supercooling, to a maximum and then finally drops. The maximum temperature reading is the solidification point. Readings 10 s apart should be taken in order to establish that the temperature is at the maximum level and continues until the drop in temperature is established.

Spectrophotometry and Light Scattering

Definitions

Absorption spectrophotometry is the measurement of the selective absorption by atoms, molecules, or ions of electromagnetic radiations having a definite and narrow wavelength range, approximating monochromatic light.

Absorption spectrophotometry encompasses the following wavelength regions: ultraviolet (185 nm to 380 nm), visible (380 nm to 780 nm), near-infrared (780 nm to 3000 nm), and infrared (2500 nm to 40 μm). *Colorimetry* has been commonly accepted as the measurement of "filtered" light in the visible region; however, it is more prudent to restrict its use to those applications where human perception of color is involved, i.e., the visible region. *Atomic absorption spectroscopy* is the measurement of the radiation absorbed by the unexcited atoms of a chemical substance that has been aspirated into a flame or, in the absence of a flame, directly into the path of radiation. *Flame emission spectroscopy* (*flame photometry*) is the measurement of the intensity of radiation emitted from electronically excited atoms or molecular species. The excitation is brought about by aspirating a solution of the sample into a hot flame. *Fluorescence spectrophotometry* or "fluorometry" is the measurement of light emitted from a chemical substance while it is being exposed to electromagnetic radiation. The maximum intensity of the emitted fluorescence is usually at a wavelength longer (i.e., of lower energy) than the exciting radiation. *Turbidimetry* and *nephelometry* are two light-scattering techniques that involve the measurement of light scattered due to its passage through a transparent medium containing a suspended particulate phase. As a result of this scattering, an attenuation or decrease in intensity is suffered by the beam along its axis of travel. Turbidimetry involves the measurement of the degree of attenuation of the light beam by particles suspended in a medium, the measurement being made in the axis of the transmitted beam. Nephelometry involves the measurement of the light scattered by the suspended particles, the measurement being made at right angles to the incident beam.

Terminology

Radiant power, P, is the energy of radiation per second that reaches certain areas of a detector. Incident radiant power is usually given the symbol P_0. Alternate terminology is radiation intensity with symbols I and I_0.

Absorbance, $A = \text{Log}_{10} (P_0/P)$, is the logarithm to the base 10 of the quotient of the incident radiant power upon a specimen divided by the radiant power transmitted by the specimen. Former terms were optical density "D," absorbancy, and extinction.

Transmittance, $T = (P/P_0)$, is the quotient of the radiant power transmitted by a specimen divided by the incident power upon the specimen. Transmittance is often expressed as a percentage and is related to the absorbance by the equation $\text{Log}_{10}T = A$, or $A = 2 - \text{Log}_{10}\%T$. Other terms are transmission and transmittancy.

Absorptivity, $a = A/bc$, is the quotient of the absorbance, A, divided by the product of the absorption pathlength, b, in cm, and the concentration, c, of the specimen, expressed in g per 1000 ml. In general, the absorptivity of a substance is a constant and is independent of the intensity of the incident radiation, pathlength, and concentration.

Molar absorptivity, ϵ, is the quotient of the absorbance, A, divided by the product of the absorption pathlength, in cm, and the specimen concentration, expressed in moles per liter. Former terms were molar absorbancy index, molar extinction coefficient, and molar absorption coefficient.

Absorption spectrum is a graphic representation of the absorbance of a specimen or any of its functions, e.g., transmittance, as the ordinate and the wavelength of the incident radiation as the abscissa.

Fluorescence intensity, I, is a descriptive term for the fluorescence activity of a substance and is commonly expressed in units related to the detector response. An alternate term is fluorescence power, with the symbol F.

Fluorescence excitation spectrum is a graphic representation of the incident (activating) radiation intensity as the ordinate and its wavelength as the abscissa.

Fluorescence emission spectrum is a graphic representation of the radiation intensity emitted by an activated species for a specific excitation wavelength as the ordinate and its wavelength as the abscissa.

Turbidance is the light-scattering effect of the suspended particles in a turbid medium.

Turbidity, τ, is a measure of the attenuation in the incident beam power per unit length of a turbid medium. The former term is turbidity coefficient.

Theory and Formulas for Calculations

When electromagnetic radiation travels through a medium containing atoms, molecules, or ions of a chemical substance, radiation at certain frequencies may be partially or totally removed in a process called "absorption." As a result of this absorption, these species are activated from their lowest energy state (ground state) to higher energy states (excited states). For absorption to occur, the energy of the exciting radiation must match the quantized energy difference between the ground state and one of the excited states of the specimen. In atomic absorption, excitation occurs only through electronic transition. Visible and ultraviolet radiation can excite only the outermost or bonding electrons to a higher energy level. Inner-shell electrons are excited only by X-ray radiation (less than 1 nm).

In the case of polyatomic molecules, vibrational and rotational transitions can occur in addition to electronic excitation, and as a result the molecular spectrum consists of closely spaced absorption bands instead of the sharp lines generally observed in the atomic absorption spectrum. Pure vibrational transitions can be achieved by infrared radiation in the range of 1 to 15 μm, while changes in rotational levels are detectable in the region from 10 to 100 μm.

The decrease in the radiant power of a monochromatic beam of light has been found to be proportional to both the distance the radiation traveled through the absorbing medium and the concentration of the absorbing species encountered in that medium. This decrease in energy can be described quantitatively by the Beer-Lambert law:

$$\text{Log}_{10}(P_0/P) = \text{Log}_{10}\,(1/T) = A = abc.$$

Therefore if the absorptivity and the cell thickness are kept constant during a specific determination, a plot of the absorbance as the ordinate versus concentration as the abscissa should yield a linear relationship. The practical application of the Beer-Lambert law, however, necessitates the use of a reference standard solution of known concentration in order to compare its absorbance with that of the sample solution of unknown concentration. If absorption measurements are conducted in two matching cells having the same pathlengths, the absorptivity, a, and the cell thickness, b, will be the same. Therefore the following general formula can be used for the calculation of the unknown concentration of the sample solution,

$$C_U = C_S \times (A_U/A_S),$$

where C_U is the concentration of the sample solution, C_S is the known concentration of the standard solution, A_U is the absorbance of the sample solution, and A_S is the absorbance of the standard solution.

The Beer-Lambert law is usually satisfactory, provided a thorough understanding of its limitations is taken into consideration. Some of these are of such a fundamental nature that they constitute a real limitation of the law; they are due to the fact that the law does not take into consideration the effects of temperature, wavelength, or solute-solvent and solute-solute interactions, e.g., association, dissociation, chemical reaction, etc. Due to these limitations, the law usually applies only to dilute solutions, where these interactions are insignificant. Another limitation to the Beer-Lambert law is due to the inability of most instruments to provide monochromatic radiation.

Fluorescence can be observed in a number of gaseous, liquid, or solid substances. However, it is only applied analytically to a relatively small number of organic compounds. Fluorescence occurs when a molecule absorbs sufficient radiation at a certain wavelength to promote it to an excited singlet state with higher levels of energy. The gained energy is released as radiation or "fluorescence" of wavelengths longer than the incident radiation. In most cases, in order for fluorescence to occur the electronic transition involved is a $\pi \rightarrow \pi^*$ system. To a lesser extent, $\eta \rightarrow \pi^*$ and $\eta \rightarrow \sigma^*$ transitions occur. There is a delay between the absorption and emission of radiation of about 10^{-9} s. This short delay period distinguishes fluorescence from phosphorescence, which has a delay period of about 10^{-3} s and is due to release of weaker radiations from an excited triplet state and not a singlet state as is true of fluorescence. The effect of concentration on the fluorescence intensity can be described by a slightly modified version of the Beer-Lambert law. A linear

relationship exists between the fluorescence intensity, I, of the solution and the concentration of the emitting species:

$$I = 2.3K \times \epsilon bcP_0,$$

where K is a constant dependent upon the quantum efficiency of the fluorescence process and instrumental parameters. At constant P_0, a simple relationship as in the Beer-Lambert law can be obtained: $I = Kc$. Thus a plot of the fluorescence intensity of a solution as the ordinate versus concentration of the emitting species as the abscissa should be linear at low concentrations.

When light passes through a transparent medium containing a suspended particulate phase, scattering occurs in all directions, and as a result the beam loses power along its axis of travel. For dilute suspensions and under fixed conditions (particles, shape, size, refractive index, wavelength of radiation), the loss in radiation intensity can be related to the number of particles (or concentration, c) by an equation similar to the Beer-Lambert law,

$$\text{Log}_{10}(P_0/P) = kbc,$$

where $\tau = kc/2.303$. Therefore, in *turbidimetric analysis* a plot is constructed with standard solutions with $\text{Log}_{10}(P_0/P)$ as the ordinate and c as the abscissa (P_0 is determined by using the solvent as reference). In *nephelometric analysis*, the radiation intensity scattered at right angles to the incident beam is plotted as the ordinate versus concentration as the abscissa.

Apparatus

The fundamental principles of optics and electronics that are used in manufacturing spectrophotometers are common to all regions of the spectrum from the vacuum ultraviolet to the far-infrared. However, due to important differences in detail, spectrophotometers are commercially available for use in the visible; in the visible and ultraviolet; in the visible, ultraviolet, and near-infrared; and in the infrared regions of the spectrum. In selecting the type of spectrophotometer to be employed, several factors have to be considered, including the nature of the specimen to be analyzed, the degree of accuracy required, sensitivity, and selectivity. The essential parts of all spectrophotometers include a stable source of radiant energy; a device that permits the selection of a defined wavelength region such as a prism or grating monochromator: a slit for limiting the suitable bandwidth; a transparent container for sample and solvent; a radiation detector; and an indicator that may be a meter, a recorder, a digital counter, a printer, or a computer. Radiation sources commonly employed are hydrogen or deuterium lamps for the ultraviolet region, tungsten lamps for the visible, and a Nernst glower, a globar, or an incandescent wire for the infrared. Quartz or fused-silica cells or cuvettes can be used in the ultraviolet, visible, or near-infrared regions. For infrared spectrophotometry, cells or plates made of sodium chloride are usually used. The radiation detector of ultraviolet and visible radiation is usually a photomultiplier tube with associated amplifiers.

Two types of spectrophotometers are available: a single-beam spectrophotometer, which adapts well to quantitative analysis that involves single-wavelength measurements, and a double-beam spectrophotometer, which is particularly useful for qualitative analysis and where continuous monitoring of absorbance is required. Some spectrophotometers are manually operated, while others are equipped for automatic and continuous recordings. Spectrophotometers employing the latest technology can be interfaced to a digital computer through an analog-digital converter for the direct determination of difference spectra of analytes as well as for the storage of reference spectra. Fourier transform infrared spectrophotometry is different from the regular dispersion type in that it employs an interferometric technique, whereby polychromatic radiations pass through the specimen to a detector on an intensity and frequency basis. In order to process such complicated spectral data, interfacing with a digital computer is a requirement.

Instruments for atomic absorption measurements have the same basic components as other spectrophotometers except for the radiation source and the sample container. The most common radiation source is the hollow-cathode lamp, the cathode of which is usually made of the element to be analyzed. The sample is aspirated as a fine mist into a flame that is produced by an optimized mixture of air and acetylene or other suitable gases. The flame thus serves a function similar to that of the sample cell in ordinary absorption spectrophotometry. Photomultiplier tubes are used as detectors, with the electronics designed to accept the modulated radiation source output, thereby negating the continuous signal from the flame. Therefore only changes in the signal from the hollow-cathode lamp are monitored by the detector. These changes are proportional to the number of atoms in the analyte. Both single- and double-beam atomic absorption spectrophotometers are available.

The apparatus for fluorescence intensity measurement is either a fluorometer, which employs filters to restrict the bandwidth of both the excitation and emission beams, or a spectrofluorometer, where prism or grating monochromators are used to limit either the excitation beam, the emission beam, or both. Since the spectrofluorometer requires a more intense radiation source than the spectrophotometers, either a mercury lamp with its strong discrete lines or a xenon lamp with its energy continuum from the ultraviolet to the infrared is used. Cells for fluorometric measurement are constructed of glass or silica, and the cell compartment is designed to allow a minimum of scattered light to reach the photomultiplier. To minimize scattering interferences, the detector is placed at right angles to the incident excitation beam.

For turbidimetric measurements, a conventional photometer with a tungsten source is usually employed. However, it is preferable to make the measurements in the blue region of a mercury arc. For nephelometric measurements, standard fluorometers are commonly used.

Procedures

Instruction manuals supplied by manufacturers should always be consulted for such matters as care, calibration, handling techniques, and operating procedures. Calibration of both the wavelength and the photometric scales should be conducted at fixed intervals. For wavelength calibration in the ultraviolet and

visible regions, a quartz-mercury arc and a holmium oxide glass filter are the most common standards employed. For the near-infrared and infrared regions, a polystyrene film may be used. The photometric scale can be checked by a number of standard inorganic glass filters or by standard solutions of known transmittance.

In absorption spectrophotometry, comparisons of the sample and reference standard are best made at or within ± 1 nm of the wavelength at which maximum absorption occurs. If matched cells are unavailable, both cells are filled with the selected solvent and any difference in absorption should be corrected instrumentally or mathematically. The solvent should be transparent in the spectral range of interest. Water, lower alcohols, chloroform, aliphatic hydrocarbons, and many other organic solvents can be used as solvents for ultraviolet and visible measurements. For best results, the concentration of the sample solution should produce an absorbance in the range of about 0.2 to 0.7. For the infrared region, however, few solvents are suitable for sample preparation (carbon disulfide, chloroform, and carbon tetrachloride are the most frequently used). In some cases, the sample can be dispersed in mineral oil to form a mull, which is transferred to the salt plates. In most cases, however, the sample is dispersed in dried potassium bromide and the mixture is compressed into a tablet or pellet. Although the infrared region extends from 2 to 40 μm, for purposes of ascertaining compliance with a reference spectrum, the range from 2.5 to 15 μm (3800 to 650 cm^{-1}) is usually satisfactory.

For atomic absorption measurements, the solvent should not seriously interfere with the absorption or emission processes or with the production of neutral atoms. Also, both the analyte solution and the standard solution should be as much alike as possible, especially with respect to concentration, viscosity, and surface tension.

In fluorescence spectrophotometry, test solutions are usually very dilute (10^{-3} to 10^{-7} *M*) in order to minimize the "inner filter" effect caused by significant absorption of incident radiation by the sample near the cell surface. Other undesirable effects of highly concentrated solutions in fluorometry are the "self-quenching" and "self-absorption" phenomena that cause significant deviation from linearity. Test solutions used in fluorometry should also be free from any dust and solid particles, as they cause interference in the measurement. In some cases, before any measurement it is advisable to remove dissolved oxygen from the test solutions, due to its quenching effect. Temperature control is usually needed for extremely sensitive determinations, and baseline correction may be critical.

In turbidimetric and nephelometric measurements, it is important to minimize the settling of the suspended particles. This is generally achieved through the addition of protective colloids.

When visual color and turbidity comparisons are made, matched color-comparison tubes that are of the same internal diameter must be used. The solutions to be compared should be at the same temperature (preferably room temperature). For color comparisons, the tubes are usually held vertically and illuminated from below. Viewing is done from above along the axis of the tube, against a white background. If the colors to be compared are too dark to be viewed downward through the depth of the solutions, they may be viewed horizontally across the diameter of the tubes, with the aid of a light source directed from the back of the tubes. If two layers are present, the designated layer must be viewed horizontally across the diameter of the tube.

For visual turbidity comparisons, the tubes should be viewed horizontally across the diameter of the tubes, with the aid of a light source directed at a right angle against the sides of the tubes.

When conducting limit tests involving the comparison of colors or turbidities, suitable detection instruments may be used in place of the unaided eye.

Applications

Ultraviolet and visible spectra provide only limited information about the chemical structure of a substance. However, because of the sensitivity of these techniques and the high degree of precision and accuracy in their measurements, they are employed extensively in assays and other quantitative determinations. Near-infrared and infrared spectra, on the other hand, are unique for a given chemical compound, except for optical isomers. Specificity makes the infrared spectrum one of the most valuable tools for structure elucidation and positive identification of complex organic molecules. Correlation charts and reference spectra of thousands of chemicals are readily available. The sensitivity of infrared analysis, however, is poor (about 1/100 to 1/1000 of ultraviolet), and therefore it has only a very limited application in quantitative analysis.

Atomic absorption is the technique of choice for the quantitative determination of most of the common elements, even those in complex matrices. Although interferences may occur in the determination of some elements due to chemical interaction between different atoms in the flame (e.g., cation-anion interference), they can usually be circumvented by preliminary treatment (e.g., addition of a complexing agent) or by the optimization of the instrumentation parameters (e.g., increasing the temperature of the flame to decrease anion-cation attraction).

Fluorescence spectrophotometry has the most inherent sensitivity of all the absorption and light-scattering techniques. Concentrations as low as 10^{-7} *M* can be quantitatively determined with high precision and accuracy. Fluorescence, however, is not as widespread as the other techniques because of the limited number of organic compounds in which fluorescence can be induced.

Light-scattering techniques, including turbidimetry and nephelometry, are very useful in the determination of weight-average molecular weights in dispersed colloidal systems. Several common ions can be determined using these techniques after their precipitation with suitable reagents. Generally, turbidimetry is adequate for the analysis of heavy suspensions where excessive scattering occurs. Nephelometry, on the other hand, is more suitable for the analysis of cloudy liquids where the attenuation of the radiant power is minimal.

Starches and Related Substances

ACETYL GROUPS

Transfer about 5 g of the sample, accurately weighed, into a 250-ml Erlenmeyer flask, suspend in 50 ml of water, add a few drops of phenolphthalein TS, and titrate with 0.1 *N* sodium hydroxide to a permanent pink endpoint. Add 25.0 ml of 0.45 *N* sodium hydroxide, stopper the flask, and shake vigorously for 30 min, preferably with a mechanical shaker. Remove the stopper, wash the stopper and sides of the flask with a few ml of water, and titrate the excess alkali with 0.2 *N* hydrochloric acid to the disappearance of the pink color, recording the volume, in ml, of 0.2 *N* hydrochloric acid required as *S*. Perform a blank titration of 25.0 ml of 0.45 *N* sodium hydroxide, and record the volume, in ml, of 0.2 *N* hydrochloric acid required as *B*. Calculate the percentage of acetyl groups by the formula

$$(B - S) \times N \times 0.043 \times 100/W,$$

in which *N* is the exact normality of the hydrochloric acid solution, and *W* is the weight of the sample, in g.

CRUDE FAT

Apparatus The apparatus consists of a Butt-type extractor,* as shown in Fig. 25, having a standard-taper 34/45 female joint at the upper end, to which is attached a Friedrichs- or Hopkins-type condenser, and a 24/40 male joint at the lower end, to which is attached a 125-ml Erlenmeyer flask.

*Available from H.S. Martin & Co., Evanston, Ill.

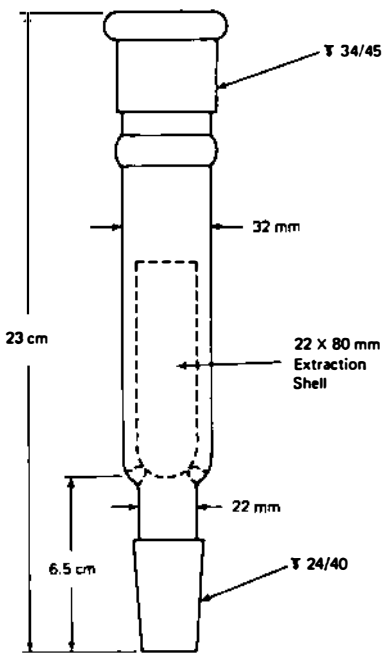


FIGURE 25 Butt-Type Extractor for Crude Fat Determination

Procedure Transfer about 10 g of the sample, previously ground to 20-mesh or finer and accurately weighed, to a 15-cm filter paper, roll the paper tightly around the sample, and place it in a suitable extraction shell. Plug the top of the shell with cotton previously extracted with hexane, and place the shell in the extractor. Attach the extractor to a dry 125-ml Erlenmeyer flask containing about 50 ml of hexane and to a water-cooled condenser, apply heat to the flask to produce 150 to 200 drops of condensed solvent per min, and extract for 16 h. Disconnect the flask, and filter the extract to remove any insoluble residue. Rinse the flask and filter with a few ml of hexane, combine the washings and filtrate in a tared flask, and evaporate on a steam bath until no odor of solvent remains. Dry in a vacuum for 1 h at 100°, cool in a desiccator, and weigh.

MANGANESE

Manganese Detection Instrument Use any suitable atomic absorption spectrophotometer equipped with a fast-response recorder or other readout device and capable of measuring the radiation absorbed by manganese atoms at the manganese resonance line of 279.5 nm.

Standard Preparations Transfer 1.000 g of manganese metal powder into a 1000-ml volumetric flask, dissolve by warming in a mixture of 10 ml of water and 10 ml of 0.5 *N* hydrochloric acid, cool, dilute to volume with water, and mix. Pipet 5.0 ml of this solution into a 50-ml volumetric flask, dilute to volume with water, and mix. Finally, pipet 5.0, 10.0, 15.0, and 25.0 ml of this solution into separate 1000-ml volumetric flasks, dilute each flask to volume with water, and mix. The final solutions contain 0.5, 1.0, 1.5, and 2.5 ppm of Mn, respectively.

Sample Preparation Transfer 10.000 g of the sample into a 200-ml Kohlrausch volumetric flask, previously rinsed with 0.5 *N* hydrochloric acid, add 140 ml of 0.5 *N* hydrochloric acid, and shake vigorously for 15 min, preferably with a mechanical shaker. Dilute to volume with 0.5 *N* hydrochloric acid, and shake. Centrifuge approximately 100 ml of the sample mixture in a heavy-walled centrifuge tube at 2000 rpm for 5 min, and use the clear supernatant liquid in the following *Procedure*.

Procedure Aspirate 0.5 *N* hydrochloric acid through the air-acetylene burner for 5 min, and obtain a baseline reading at 279.5 nm, following the manufacturer's instructions for operating the atomic absorption spectrophotometer being used for the analysis. Aspirate a portion of each *Standard Preparation* in the same manner, note the readings, then aspirate a portion of the *Sample Preparation*, and note the reading. Prepare a standard curve by plotting the ppm of Mn in each *Standard Preparation* against the respective readings. From the graph determine the ppm of Mn in the *Sample Preparation*, and multiply this value by 20 to obtain the ppm of Mn in the original sample taken for analysis.

PHOSPHORUS

Reagents

Ammonium Molybdate Solution (5%) Dissolve 50 g of ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in 900 ml of warm water, cool to room temperature, dilute to 1000 ml with water, and mix.

Ammonium Vanadate Solution (0.25%) Dissolve 2.5 g of ammonium metavanadate, NH_4VO_3, in 600 ml of boiling water, cool to 60° to 70°, and add 20 ml of nitric acid. Cool to room temperature, dilute to 1000 ml with water, and mix.

Zinc Acetate Solution (10%) Dissolve 120 g of zinc acetate dihydrate, $Zn(C_2H_3O_2)_2.2H_2O$, in 880 ml of water, and filter through Whatman No. 2V or equivalent filter paper before use.

Nitric Acid Solution (29%) Add 300 ml of nitric acid (sp. gr. 1.42) to 600 ml of water, and mix.

Standard Phosphorus Solution (100 µg P in 1 ml) Dissolve 438.7 mg of monobasic potassium phosphate, KH_2PO_4, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Standard Curve Pipet 5.0, 10.0, and 15.0 ml of the *Standard Phosphorus Solution* into separate 100-ml volumetric flasks. To each of these flasks, and to a fourth blank flask, add in the order stated 10 ml of *Nitric Acid Solution*, 10 ml of *Ammonium Vanadate Solution*, and 10 ml of *Ammonium Molybdate Solution*, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min. Determine the absorbance of each standard solution in a 1-cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. Prepare a standard curve by plotting the absorbance of each solution versus its concentration, in mg P per 100 ml.

Treated Sample Place 20 to 25 g of the starch sample in a 250-ml beaker, add 200 ml of a 7 to 3 methanol-water mixture, disperse the sample, and agitate mechanically for 15 min. Recover the starch by vacuum filtration in a 150-ml medium-porosity fritted-glass or Buchner funnel, and wash the wet cake with 200 ml of the methanol-water mixture. Reslurry the wet cake in the solvent, and wash it a second time in the same manner. Dry the filter cake in an air oven at a temperature below 50°, then grind the sample to 20-mesh or finer, and blend thoroughly. Determine the amount of dry substance by drying a 5-g portion in a vacuum oven, not exceeding 100 mm of Hg, at 120° for 5 h. (NOTE: The treatment outlined above is satisfactory for starch products that are insoluble in cold water. For pregelatinized starch and other water-soluble starches, prepare a 1% to 2% aqueous paste, place it in a cellophane tube, and dialyze against running distilled water for 30 to 40 h. Precipitate the starch by pouring the solution into 4 volumes of acetone per volume of paste, while stirring. Recover the starch by vacuum filtration in a medium-porosity fritted-glass or Buchner funnel, and wash the filter cake with absolute ethanol. Dry the filter cake, and determine the amount of dry substance as directed for water-insoluble starches.)

Sample Preparation Transfer about 10 g of the *Treated Sample*, calculated on the dry-substance basis and accurately weighed, into a Vycor dish, and add 10 ml of *Zinc Acetate Solution* in a fine stream, distributing the solution uniformly in the sample. Carefully evaporate to dryness on a hot plate, then increase the heat, and carbonize the sample on the hot plate or over a gas flame. Ignite in a muffle furnace at 550° until the ash is free from carbon (about 1 to 2 h), and cool. Wet the ash with 15 ml of water, and slowly wash down the sides of the dish with 5 ml of *Nitric Acid Solution*. Heat to boiling, cool, and quantitatively transfer the mixture into a 200-ml volumetric flask, rinsing the dish with three 20-ml portions of water and adding the rinsings to the flask. Dilute to volume with water, and mix. Transfer an accurately measured aliquot (V, in ml) of this solution, containing not more than 1.5 mg of phosphorus, into a 100-ml volumetric flask, and add 50 ml of water to a second flask to serve as a blank. To each flask add in the order stated 10 ml of *Nitric Acid Solution*, 10 ml of *Ammonium Vanadate Solution*, and 10 ml of *Ammonium Molybdate Solution*, mixing thoroughly after each addition. Dilute to volume with water, mix, and allow to stand for 10 min.

Procedure Determine the absorbance of the *Sample Preparation* in a 1-cm cell at 460 nm, with a suitable spectrophotometer, using the blank to set the instrument at zero. From the *Standard Curve*, determine the mg of phosphorus in the aliquot taken, recording this value as a. Calculate the amount, in ppm, of phosphorus (P) in the original sample by the formula

$$(a \times 200 \times 1000)/(V \times W),$$

in which W is the weight of the sample taken, in g.

PROPYLENE CHLOROHYDRIN

Special Apparatus

Gas Chromatograph Use a Hewlett-Packard Model 5750 or equivalent. A dual-column instrument equipped with a flame-ionization detector is recommended. An integrator should be part of the recording system.

Concentrator Use a Kuderna-Danish concentrator having a 500-ml flask, available from Kontes Glass Co., Vineland, N.J. (Catalog No. K-57000), or equivalent.

Pressure Bottles Use 200-ml pressure bottles, with a Neoprene washer, glass stopper, and attached wire clamp, available from Fisher Scientific Co. (Vitro 400, Catalog No. 3-100), or equivalent.

Gas Chromatography Column Use a stainless steel column, 3 m × 3.2 mm (od), packed with 10% Carbowax 20 M on 80/100-mesh Gas Chrom 2, or equivalent. After packing and prior to use, condition the column overnight at 200°, using a helium flow of 25 ml per min.

Reagents

Diethyl Ether Use anhydrous, analytical reagent-grade diethyl ether, available from Fisher Scientific Co. or J.T. Baker Co., or other suitable sources. (NOTE: Some lots of diethyl ether contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. If the ether quality is unknown or suspect, concentrate 50 ml to a volume of about 1 ml in the *Concentrator*, and then chromatograph a 2.0-µl portion using the conditions outlined under the *Procedure*. If

the chromatogram is excessively noisy and contains signal peaks that overlap or interfere in the measurement of the peaks produced by the propylene chlorohydrin isomers, the ether should be redistilled.)

Florisil PR Use 60/100-mesh material, available from Floridin Co., 3 Penn Center, Pittsburgh, Pa. 15235, or an equivalent product available from Supelco, Bellefonte, Pa. 16823.

Propylene Chlorohydrins Use Eastman No. P1325 1-Chloro-2-propanol Practical, containing 25% 2-chloro-1-propanol, available from Eastman Kodak Co., Rochester, N.Y. 14650.

Standard Preparation Draw 25 µl of *Propylene Chlorohydrins* into a 50-µl syringe, weigh accurately, and discharge the contents into a 500-ml volumetric flask partially filled with water. Reweigh the syringe, and record the weight of the chlorohydrins taken. Dilute to volume with water, and mix. This solution contains about 27.5 mg of mixed chlorohydrins, or about 55 µg per ml. Prepare this solution fresh daily.

Sample Preparation Transfer a blended representative 50.0-g sample into a *Pressure Bottle*, and add 125 ml of 2 *N* sulfuric acid. Clamp the top in place, and swirl the contents until the sample is completely dispersed. Place the bottle in a boiling water bath, heat for 10 min, then swirl the bottle to mix the contents, and heat in the bath for an additional 15 min. Cool in air to room temperature, then neutralize the hydrolyzed sample to pH 7 with 25% sodium hydroxide solution, and filter through Whatman No. 1 paper, or equivalent, in a Buchner funnel, using suction. Wash the bottle and filter paper with 25 ml of water, and combine the washings with the filtrate. Add 30 g of anhydrous sodium sulfate, and stir with a magnetic stirring bar for 5 to 10 min, or until the sodium sulfate is completely dissolved. Transfer the solution into a 500-ml separator equipped with a Teflon plug, rinse the flask with 25 ml of water, and combine the washings with the sample solution. Extract with five 50-ml portions of *Diethyl Ether*, allowing at least 5 min in each extraction for adequate phase separation. Transfer the combined ether extracts in a *Concentrator*, place the graduated receiver of the concentrator in a water bath maintained at 50° to 55°, and concentrate the extract to a volume of 4 ml. (NOTE: Ether extracts of samples may contain foreign residues that interfere with the analysis and/or the interpretation of the chromatograms. These residues are believed to be degradation products arising during the hydrolysis treatment. Analytical problems created by their presence can be avoided through application of a cleanup treatment performed as follows: Concentrate the ether extract to about 8 ml, instead of 4 ml specified above. Add 10 g of *Florisil*, previously heated to 130° for 16 h just before use, to a chromatographic tube of suitable size, then tap gently, and add 1 g of anhydrous sodium sulfate to the top of the column. Wet the column with 25 ml of *Diethyl Ether*, and quantitatively transfer the concentrated extract to the column with the aid of small portions of the ether. Elute with three 25-ml portions of the ether, collect all of the eluate, transfer it to a *Concentrator*, and concentrate to a volume of 4 ml.)

Cool the extract to room temperature, transfer it quantitatively to a 5.0-ml volumetric flask with the aid of small portions of *Diethyl Ether*, dilute to volume with the ether, and mix.

Control Preparations Transfer 50.0-g portions of unmodified (underivatized) waxy corn starch into five separate *Pressure Bottles*, and add 125 ml of 2 *N* sulfuric acid to each bottle. Add 0.0, 0.5, 1.0, 2.0, and 5.0 ml of the *Standard Preparation* to the bottles, respectively, giving propylene chlorohydrin concentrations, on the starch basis, of 0, 0.5, 1, 2, and 5 ppm, respectively. Calculate the exact concentration in each bottle from the weight of *Propylene Chlorohydrins* used in making the *Standard Preparation*. Clamp the tops in place, swirl until the contents of each bottle are completely dissolved, and proceed with the hydrolysis, neutralization, filtration, extraction, extract concentration, and final dilution as directed under *Sample Preparation*.

Procedure The analysis is performed by gas chromatography with the *Gas Chromatograph* and *Gas Chromatography Column* previously described. The operating conditions may be varied, depending upon the particular instrument used, but a suitable chromatogram is obtained with the Hewlett-Packard Model 5750 using a column oven temperature of 110°, isothermal; injection port temperature of 210°; detector temperature of 240°; and hydrogen (30 ml per min), helium (25 ml per min), or air (350 ml per min) as the carrier gas. A 1.0-mV full-scale recorder is recommended; range, attenuation, and chart speed should be selected to optimize signal characteristics.

Inject 2.0-µl aliquots of each of the concentrated extracts, prepared as directed under *Control Preparations*, allowing sufficient time between injections for signal peaks corresponding to the two chlorohydrin isomers to be recorded (and integrated) and for the column to be purged. Record and sum the signal areas (integrator outputs) from the two chlorohydrin isomers for each of the controls.

Using identical operating conditions, inject a 2.0-µl aliquot of the concentrated extract prepared as directed under *Sample Preparation*, and record and sum the signal areas (integrator outputs) from the sample.

Calculation Prepare a calibration plot on linear coordinate graph paper by plotting the summed signal areas for each of the controls against the calculated propylene chlorohydrin concentrations, in ppm, derived from the actual weight of chlorohydrin isomers used. Using the summed signal areas corresponding to the 1-chloro-2-propanol and 2-chloro-1-propanol from the sample, determine the concentration of mixed propylene chlorohydrins, in ppm, in the sample by reference to the calibration plot. [NOTE: After gaining experience with the procedure and demonstrating that the calibration plot derived from the control samples is linear and reproducible, the number of controls can be reduced to one containing about 5 ppm of mixed propylene chlorohydrin isomers. The propylene chlorohydrin level in the sample can then be calculated as follows:

$$\text{Propylene chlorohydrins, ppm} = (C \times a)/A,$$

in which C is the concentration, in ppm, of propylene chlorohydrins (sum of isomers) in the control; a is the sum of the signal areas produced by the propylene chlorohydrin isomers in the sample; and A is the sum of the signal areas produced by the propylene chlorohydrin isomers in the control.]

SULFUR DIOXIDE

Apparatus Use a modified Monier-Williams apparatus* for the determination of sulfurous acid, or construct the apparatus as shown in Fig. 26. The assembly consists of a 1000-ml three-neck round-bottom distillation flask having 24/40 standard-taper ground-glass joints. A 30-cm Allihn condenser is attached in the reflux position to an outer neck of the flask, and the other end of the condenser is connected with 1/4-in. Tygon or silicon tubing (preboiled with 1 in 20 hydrochloric acid solution and rinsed with water) to the absorption tube assembly (having 35/20 ball joints or the equivalent). Connect the center neck of the flask with a 125-ml cylindrical separator, and attach a piece of tubing to a short U-tube inserted through a rubber stopper in the neck of the separator. Attach a curved glass inlet tube, reaching nearly to the bottom of the flask, to the other outer neck of the flask, and connect the inlet tube to a 250-ml gas-washing bottle with a piece of the tubing. The gas-washing bottle, in turn, is connected by tubing to a nitrogen cylinder.

Grind 4.5 g of pyrogallol (pyrogallic acid) with 5 ml of water in a small mortar, and transfer the slurry to the gas-washing bottle. Grind the residue again, and transfer it quantitatively to the bottle with two 5-ml portions of water. Pass nitrogen from the cylinder to the bottle to flush out air, and then add to the bottle, through a long-stem funnel, a cooled solution of 65 g of potassium hydroxide in about 85 ml of water. Place the head of the bottle in position, and bubble nitrogen through it to remove air from the headspace. Clamp off the tubing on both sides of the bottle, and connect it to the glass inlet tube of the distillation flask. The gas-washing bottle must be prepared with fresh pyrogallol solution as described on the day of use.

To each U-tube of the absorption tube assembly add the following: two pieces of 8-mm glass rod about 25 mm in length, 10 ml of 3-mm glass beads at the exit side, 10.0 ml of 3% hydrogen peroxide solution, and 1 drop of methyl red TS.

Assemble all pieces of the apparatus, and check for leaks by blowing gently into the tubing attached to the neck of the separator. While blowing, close the stopcock of the separator. Let stand for a few min; if the liquid levels in the U-tubes equalize, reseal all connections and test again. If the system is airtight, proceed as directed below.

Procedure Disperse about 100 g of the sample, accurately weighed, in 300 ml of recently boiled and cooled water, and transfer the slurry to the flask with the aid of water, using a large-bore funnel. Dilute to about 400 ml with water, and reseal the separator. Add 90 ml of 4 *N* hydrochloric acid to the separator, and force the acid into the flask by blowing gently into the tube in the neck of the separator. Close the stopcock of the separator.

Unclamp the tubing on both sides of the gas-washing bottle, and start the nitrogen flow at a steady stream of bubbles. Heat the distilling flask with a heating mantle to cause refluxing in approximately 20 min. When steady refluxing is reached, apply the line voltage to the mantle and reflux for 1.75 h. Turn off the water in the condenser, and continue heating until the inlet joint of the first U-tube shows condensation and slight warming. Remove the separator, and turn off the heat.

When the joint at the top of the condenser cools, remove the connecting assembly and rinse it into the second U-tube, leaving the crossover tube attached to the exit joint of the first U-tube but disconnected from the entrance of the second U-tube. Rotate the crossover tube until the free end almost touches the entrance of the first U-tube. Add 1 drop of methyl red TS to the first U-tube, and titrate with 0.1 *N* sodium hydroxide just to a clear yellow color, mixing with a gentle rocking motion. After titrating the first U-tube, remove the crossover tube, attach it to the second U-tube exit, and titrate similarly. Record the sum of the two titers as *S*, in ml. Perform a blank determination, and record the volume of 0.1 *N* sodium hydroxide required as *B*. Calculate the percentage of sulfur dioxide in the sample by the formula

$$SO_2,\ \% = (S - B) \times 0.0032 \times 100W,$$

in which *W* is the weight of the sample taken, in g.

*Available from SGA Scientific, Inc., Bloomfield, N.J.

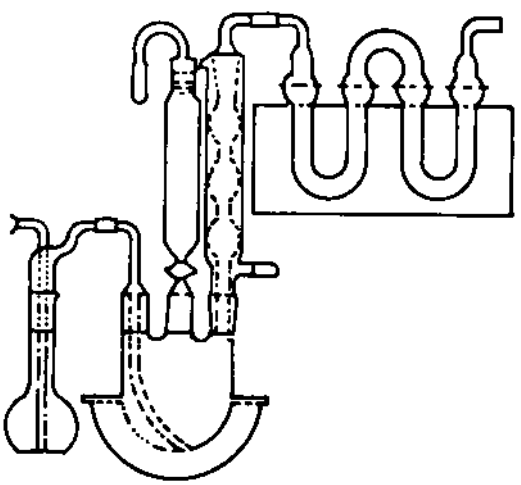


FIGURE 26 Modified Monier-Williams Apparatus

Sulfuric Acid Table

Be°	Sp. Gr.	Percent H_2SO_4	Be°	Sp. Gr.	Percent H_2SO_4
0	1.0000	0.00	13	1.0985	14.13
1	1.0069	1.02	14	1.1069	15.25
2	1.0140	2.08	15	1.1154	16.38
3	1.0211	3.13	16	1.1240	17.53
4	1.0284	4.21	17	1.1328	18.71
5	1.0357	5.28	18	1.1417	19.89
6	1.0432	6.37	19	1.1508	21.07
7	1.0507	7.45	20	1.1600	22.25
8	1.0584	8.55	21	1.1694	23.43
9	1.0662	9.66	22	1.1789	24.61
10	1.0741	10.77	23	1.1885	25.81
11	1.0821	11.89	24	1.1983	27.03
12	1.0902	13.01	25	1.2083	28.28

Sulfuric Acid Table (*continued*)

Be°	Sp. Gr.	Percent H_2SO_4	Be°	Sp. Gr.	Percent H_2SO_4
26	1.2185	29.53	49	1.5104	60.75
27	1.2288	30.79	50	1.5263	62.18
28	1.2393	32.05	51	1.5426	63.66
29	1.2500	33.33	52	1.5591	65.13
30	1.2609	34.63	53	1.5761	66.63
31	1.2719	35.93	54	1.5934	68.13
32	1.2832	37.26	55	1.6111	69.65
33	1.2946	38.58	56	1.6292	71.17
34	1.3063	39.92	57	1.6477	72.75
35	1.3182	41.27	58	1.6667	74.36
36	1.3303	42.63	59	1.6860	75.99
37	1.3426	43.99	60	1.7059	77.67
38	1.3551	45.35	61	1.7262	79.43
39	1.3679	46.72	62	1.7470	81.30
40	1.3810	48.10	63	1.7683	83.34
41	1.3942	49.47	64	1.7901	85.66
42	1.4078	50.87	64¼	1.7957	86.33
43	1.4216	52.26	64½	1.8012	87.04
44	1.4356	53.66	64¾	1.8068	87.81
45	1.4500	55.07	65	1.8125	88.65
46	1.4646	56.48	65¼	1.8182	89.55
47	1.4796	57.90	65½	1.8239	90.60
48	1.4948	59.32	66	1.8354	93.19

Source: Courtesy of the Manufacturing Chemists Association.

Specific gravity determinations were made at 60°F, compared with water at 60°F. The values given above for aqueous sulfuric acid solutions were adopted as standard in 1904 by the Manufacturing Chemists' Association of the United States.

From the specific gravities, the corresponding degrees Baumé were calculated by the following formula:

$$\text{Baumé} = 145 - (145/\text{sp. gr.})$$

Baumé hydrometers for use with this table must be graduated by the above formula, which formula should always be printed on the scale. Acids stronger than 66° Bé should have their percentage compositions determined by chemical analysis.

Thermometers

Thermometers suitable for *Food Chemicals Codex* use conform to the specifications of the American Society for Testing and Materials, ASTM Standards E 1, and are standardized in accordance with ASTM Method E 77.

The thermometers are of the mercury in glass type, and the column above the liquid is filled with nitrogen. They may be standardized for *total immersion* or for *partial immersion* and should be used as near as practicable under the same condition of immersion.

"Total immersion" means standardization with the thermometer immersed to the top of the mercury column, with the remainder of the stem and the upper expansion chamber exposed to the ambient temperature. "Partial immersion" means standardization with the thermometer immersed to the indicated immersion line etched on the front of the thermometer, with the remainder of the stem exposed to the ambient temperature. If used under any other condition of immersion, an emergent-stem correction is necessary to obtain correct temperature readings.

Thermometer Specifications

ASTM No. E-1-	Range °C	Range °F	Subdivisions °C	Subdivisions °F	Immersion (mm)
		For General Use			
1 C	−20 to +150	—	1	—	76
1 F	—	0 to 302	—	2	76
2 C	−5 to +300	—	1	—	76
2 F	—	20 to 580	—	2	76
3 C	−5 to +400	—	1	—	76
3 F	—	20 to 760	—	2	76
		For Determination of Softening Point			
15 C	−2 to +80	—	0.2	—	total
15 F	—	30 to 180	—	0.5	total
16 C	30 to 200	—	0.5	—	total
16 F	—	85 to 392	—	1	total
		For Determination of Kinematic Viscosity			
44 F	—	66.5 to 71.5	—	0.1	total
45 F	—	74.5 to 79.5	—	0.1	total
28 F	—	97.5 to 102.5	—	0.1	total
46 F	—	119.5 to 124.5	—	0.1	total
29 F	—	127.5 to 132.5	—	0.1	total
47 F	—	137.5 to 142.5	—	0.1	total
48 F	—	177.5 to 182.5	—	0.1	total
30 F	—	207.5 to 212.5	—	0.1	total
		For Determination of Distillation Range			
37 C	−2 to +52	—	0.2	—	100
38 C	24 to 78	—	0.2	—	100
39 C	48 to 102	—	0.2	—	100
40 C	72 to 126	—	0.2	—	100
41 C	98 to 152	—	0.2	—	100
102 C	123 to 177	—	0.2	—	100
103 C	148 to 202	—	0.2	—	100
104 C	173 to 227	—	0.2	—	100
105 C	198 to 252	—	0.2	—	100
106 C	223 to 277	—	0.2	—	100
107 C	248 to 302	—	0.2	—	100
		For Determination of Solidification Range			
89 C	−20 to +10	—	0.1	—	76
90 C	0 to 30	—	0.1	—	76
91 C	20 to 50	—	0.1	—	76
92 C	40 to 70	—	0.1	—	76
93 C	60 to 90	—	0.1	—	76
94 C	80 to 110	—	0.1	—	76
95 C	100 to 130	—	0.1	—	76
96 C	120 to 150	—	0.1	—	76
100 C	145 to 205	—	0.2	—	76
101 C	195 to 305	—	0.5	—	76
		For Special Use			
14 C[a]	38 to 82	—	0.1	—	79
38 C[b]	−2 to +68	—	0.2	—	45
18 C[c]	34 to 42	—	0.1	—	total
18 F[c]	—	94 to 108	—	0.2	total
22 C[c]	95 to 103	—	0.1	—	total
22 F[c]	—	204 to 218	—	0.2	total
23 C[d]	18 to 28	—	0.2	—	90

Thermometer Specifications (*continued*)

ASTM No. E-1-	Range °C	Range °F	Subdivisions °C	Subdivisions °F	Immersion (mm)
24 C[a]	30 to 54	—	0.2	—	90
54 F[b]	—	68 to 213	—	0.5	total
71 F[f]	—	−35 to +70	—	1	76

[a] For determination of melting range of Class III solids.
[b] For determination of the titer of fatty acids.
[c] For determination of Saybolt viscosity.
[d] For determination of Engler viscosity.
[e] For determination of congealing point.
[f] For determination of oil in wax.

In selecting a thermometer, careful consideration should be given to the conditions under which it is to be used. The preceding table lists several ASTM thermometers, together with their usual conditions of use, which may be required in *Food Chemicals Codex* tests. Complete specifications for these thermometers are given in "ASTM Standards on Thermometers."

Thiamin Assay

Special Solutions and Solvents

Potassium Chloride Solution Dissolve 250 g of potassium chloride, KCl, in sufficient water to make 1000 ml.

Acid Potassium Chloride Solution Add 8.5 ml of hydrochloric acid to 1000 ml of *Potassium Chloride Solution.*

Potassium Ferricyanide Solution, 1% Dissolve 1 g of potassium ferricyanide, $K_3Fe(CN)_6$, in sufficient water to make 100 ml. Prepare fresh on the day of use.

Oxidizing Reagent Mix 4.0 ml of *Potassium Ferricyanide Solution, 1%* with sufficient 3.5 *N* sodium hydroxide to make 100 ml. Use this solution within 4 h.

Quinine Sulfate Stock Solution Dissolve 10 mg of quinine sulfate, $(C_{20}H_{24}N_2O_2)_2.H_2SO_4.2H_2O$, in sufficient 0.1 *N* sulfuric acid to make 1000 ml. Preserve this solution, protected from light, in a refrigerator.

Quinine Sulfate Standard Solution Dilute 1 volume of *Quinine Sulfate Stock Solution* with 39 volumes of 0.1 *N* sulfuric acid. This solution fluoresces to approximately the same degree as the thiochrome obtained from 1 μg of thiamin hydrochloride and is used to correct the fluorophotometer at frequent intervals for variation in sensitivity from reading to reading within an assay. Prepare this solution fresh on the day of use.

Standard Thiamin Hydrochloride Stock Solution Transfer about 25 mg of USP Thiamine Hydrochloride Reference Standard, previously dried at 105° for 2 h and accurately weighed, to a 1000-ml volumetric flask, observing precautions to avoid absorption of moisture in weighing the dried standard. Dissolve the weighed standard in about 300 ml of dilute alcohol solution (1 in 5) adjusted to a pH of 4.0 with diluted hydrochloric acid TS, and add the acidified, dilute alcohol to volume. Store in a light-resistant container in a refrigerator. Prepare this stock solution fresh each month.

Standard Preparation Pipet a volume of *Standard Thiamin Hydrochloride Stock Solution*, equivalent to exactly 100 μg of USP Thiamine Hydrochloride Reference Standard, into a 100-ml volumetric flask, and dilute with *Acid Potassium Chloride Solution* to volume. Dilute exactly 10 ml of this solution with *Acid Potassium Chloride Solution* to 50.0 ml. Each ml of the resulting *Standard Preparation* contains 0.2 μg of USP Thiamine Hydrochloride Reference Standard.

Assay Preparation Prepare as directed in the individual monograph.

Procedure Into each of four or more tubes (or other suitable vessels), of about 40-ml capacity, pipet 5 ml of *Standard Preparation.* To each of three of these tubes add rapidly (within 1 to 2 s), with mixing, 3 ml of *Oxidizing Reagent*, and within 30 s add 20 ml of isobutyl alcohol, then mix vigorously for 90 s by shaking the capped tubes manually, or by bubbling a stream of air through the mixture. Prepare a blank in the remaining tube of the standard by substituting for the *Oxidizing Reagent* an equal volume of 3.5 *N* sodium hydroxide and proceeding in the same manner.

Into each of four or more similar tubes pipet 5 ml of the *Assay Preparation.* Treat these tubes in the same manner as directed for the tubes containing the *Standard Preparation.*

To each of the eight tubes add 2 ml of absolute alcohol, swirl for a few seconds, allow the phases to separate, and decant or draw off about 10 ml of the clear, supernatant isobutyl alcohol solution into standardized cuvettes, then measure the fluorescence in a suitable fluorophotometer. Use an input filter of narrow transmittance range with a maximum at about 365 nm, and an output filter of narrow transmittance range with a maximum at about 435 nm.

Calculation The number of μg of $C_{12}H_{17}ClN_4OS.HCl$ in each 5 ml of the *Assay Preparation* is given by the formula

$$(A - b)/(S - d),$$

in which A is the average of the fluorophotometer readings of the portions of the *Assay Preparation* treated with *Oxidizing Reagent*, b is the fluorophotometer reading of the *Assay Preparation* blank, S is the average of the fluorophotometer readings of the portions of the *Standard Preparation* treated with *Oxidizing Reagent*, and d is the fluorophotometer reading of the *Standard Preparation* blank.

Calculate the quantity, in mg, of $C_{12}H_{17}ClN_4OS.HCl$ (thiamin hydrochloride) in the assay material on the basis of the aliquots taken. Where indicated, the quantity, in mg, of $C_{12}H_{17}N_5O_4S$ (thiamin mononitrate) may be calculated by multiplying the quantity of $C_{12}H_{17}ClN_4OS.HCl$ found by 0.9706.

Viscosity of Dimethylpolysiloxane

Apparatus The Ubbelohde suspended level viscometer, shown in Fig. 27, is preferred for the determination of the viscosity of dimethylpolysiloxane. Alternatively, a Cannon-Ubbelohde viscometer may be used.

Select a viscometer having a minimum flow time of at least 200 s. For use in the range of 300 to 600 centistokes, a No. 3 size Ubbelohde, or a No. 400 size Cannon-Ubbelohde, viscometer is required. The viscometer should be fitted with holders that satisfy the dimensional positions of the separate tubes as shown in the diagram, and that hold the viscometer vertical. Filling lines in bulb *A* indicate the minimum and maximum volumes of liquid to be used for convenient operation. The volume of bulb *B* is approximately 5 ml.

Calibration of the Viscometer Determine the viscosity constant *C* for each viscometer by using an oil of known viscosity.* Charge the viscometer by tilting the instrument about 30 degrees from the vertical, with bulb *A* below the capillary, and then introduce enough of the sample into tube *1* to bring the level up to the lower filling line. The level should not be above the upper filling line when the viscometer is returned to the vertical position and the sample has drained from tube *1*. Charge the viscometer in such a manner that the U-tube at the bottom fills completely without trapping air.

*Oils of known viscosities, formerly supplied by the National Bureau of Standards, may be obtained from the Cannon Instrument Co., P.O. Box 812, State College, Pa. 16801. For determining the viscosity of dimethylpolysiloxane, choose an oil whose viscosity is as close as possible to that of the type of sample to be tested.

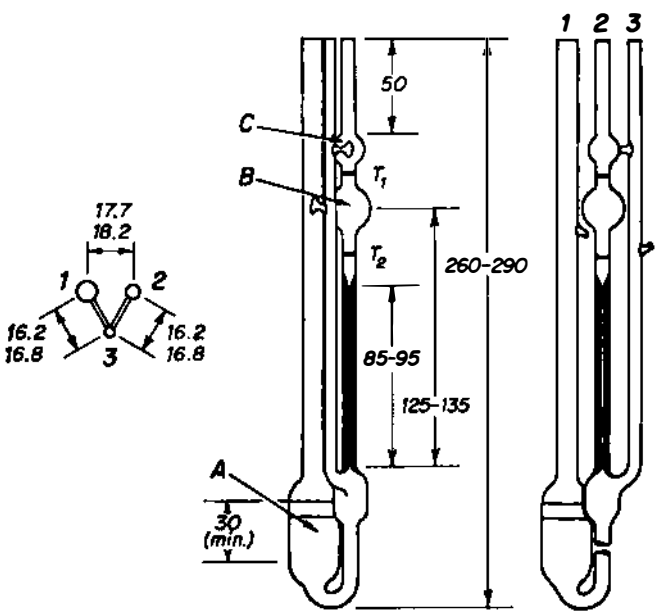


FIGURE 27 Ubbelohde Viscometer for Dimethylpolysiloxane (All dimensions are in mm.)

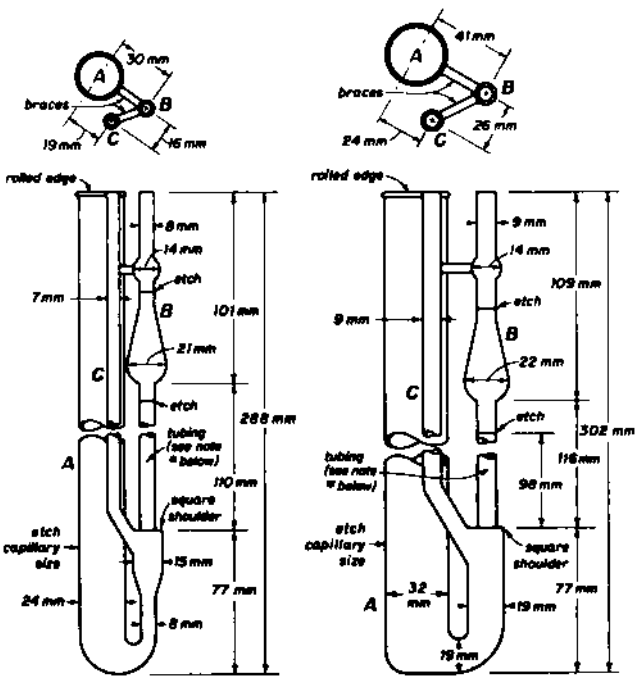
After the viscometer has been in a constant-temperature bath (25° ± 0.2°) long enough for the sample to reach temperature equilibrium, place a finger over tube *3* and apply suction to tube *2* until the liquid reaches the center of bulb *C*. Remove suction from tube *2*, then remove the finger from tube *3* and place it over tube *2* until the sample drops away from the lower end of the capillary. Remove the finger from tube *2*, and measure the time, to the nearest 0.1 s, required for the meniscus to pass from the first timing mark (T_1) to the second (T_2).

Calculate the viscometer constant *C* by the equation $C = cs/t_1$, in which *cs* is the viscosity, in centistokes, and t_1 is the efflux time, in seconds, for the standard liquid.

Determination of the Viscosity of Dimethylpolysiloxane Charge the viscometer with the sample in the same manner as described for the calibration procedure, determine the efflux time, t_2, and calculate the viscosity of the dimethylpolysiloxane by the formula $C \times t_2$.

Viscosity of Methylcellulose

Apparatus Viscometers used for the determination of the viscosity of methylcellulose and some related compounds are illustrated in Fig. 28 and consist of three parts: a large filling tube, *A*, an orifice tube, *B*, and an air vent to the reservoir, *C*.



Viscometer for Low Viscosity
*Precision bore capillary tubing 1.5 mm id for 15 cps, 1.8 mm id for 25 cps, 2.4 mm id for 100 cps, and 3.2 mm id for 400 cps viscosities.

Viscometer for High Viscosity
*Precision bore capillary tubing 5.0 mm id for 1500 cps and 6.0 mm id for 4000 cps viscosities.

FIGURE 28 Methylcellulose Viscometers

There are two basic types of viscometers, one for cellulose derivatives of a range between 1500 and 4000 centipoises, and the other for less viscous types. Each type of viscometer is modified slightly for the different viscosity types.

Calibration of the Viscometer Determine the viscometer constant *K* for each viscometer by the use of an oil of known viscosity.* Place an excess of the liquid that is to be tested (adjusted to 20° ± 0.1°) in the filling tube, *A*, and transfer it to the orifice tube, *B*, by gentle suction, taking care to keep the liquid free from air bubbles by closing the air vent tube, *C*. Adjust the column of liquid in tube *B* even with the top graduation line. Open both tubes *B* and *C* to permit the liquid to flow into the reservoir against atmospheric pressure. Failure to open air vent tube *C* before determining the viscosity will yield false values. Record the time in seconds for the liquid to flow from the upper mark to the lower mark in tube *B*.

Calculate the viscometer constant *K* from the equation $V = Kdt$, in which *V* is the viscosity of the liquid in centipoises, *K* is the viscometer constant, *d* is the specific gravity of the liquid tested at 20°/20°, and *t* is the time in seconds for the liquid to pass from the upper to the lower mark.

For the calibration, all values in the equation are known or can be determined except *K*, which must be solved. If a tube is repaired, it must be recalibrated to avoid obtaining significant changes in the value of *K*.

Determination of the Viscosity of Methylcellulose Prepare a 2% solution of methylcellulose or other cellulose derivative, by weight, as directed in the monograph. Place the solution in the proper viscometer and determine the time required to flow from the upper mark to the lower mark in orifice tube *B*. Separately determine the specific gravity at 20°/20°, and from the values of *d* and *t* thus determined calculate *V* from the equation under *Calibration of the Viscometer*.

Viscosity of Sodium Carboxymethylcellulose

Apparatus

Viscometer A Model LVG Brookfield or equivalent type viscometer should be used for the determination of viscosity of aqueous solutions of sodium carboxymethylcellulose within the range of 25 to 10,000 centipoises at 25°. Instruments of this type are provided with spindles for use in determining the viscosity of different viscosity types of sodium carboxymethylcellulose. The spindles and speeds for determining viscosity within different ranges are tabulated below.

*Oils of known viscosities, formerly supplied by the National Bureau of Standards, may be obtained from The Cannon Instrument Co., P.O. Box 812, State College, Pa. 16801. For determining the viscosity of methylcellulose, choose an oil whose viscosity is as close as possible to that of the type of sample to be tested.

Viscometer Spindles Required for Given Speeds

Viscosity Range (centipoises)	Spindle No.	Speed (rpm)	Scale	Factor
10–100	1	60	100	1
100–200	1	30	100	2
200–1000	2	30	100	10
1000–4000	3	30	100	40
4000–10,000	4	30	100	200

Mechanical Stirrer Use an agitator essentially as shown in Fig. 29 that can be attached to a variable-speed motor capable of turning up to 1500 rpm. (NOTE: Stirrers equipped with 1-1/2-in., 3-blade type, stainless steel propellers, such as A.H. Thomas Co. Catalog No. 9240-K, have also been found to be satisfactory).

Sample Container Use a glass jar about 5-1/4 in. deep (133 mm) having an outside diameter of approximately 2-3/8 in. (60 mm) and a capacity of about 8 oz (236 ml).

Sample Preparation Weigh accurately an amount of sample equivalent to 4.8 g of sodium carboxymethylcellulose on the dried basis, and record the actual quantity required as *S*, in g. Transfer to the sample container an accurately measured volume of water equivalent to 240 − *S* g. Position the stirrer in the sample container, allowing minimum clearance between the stirrer and the bottom of the container, and begin stirring. Slowly add the sample, and adjust the stirring speed to

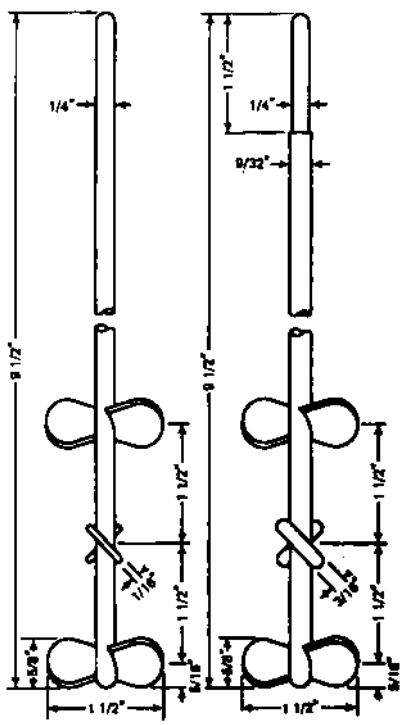


FIGURE 29 Agitator for Viscosity of Sodium Carboxymethylcellulose

approximately 800 ± 100 rpm. Mix for exactly 2 h. Do not allow the stirring speed to exceed 900 rpm. Remove the stirrer, and transfer the sample container to a constant-temperature water bath maintained at 25° ± 0.2°. Check the sample temperature at the end of 1 h to ensure that the test temperature has been reached.

Procedure Remove the sample container from the water bath, and measure the viscosity with the Brookfield viscometer, using the proper spindle and speed indicated in the accompanying table. Allow the spindle to rotate until a constant reading is obtained. Calculate the viscosity, in centipoises, by multiplying the reading observed by the appropriate factor from the table.

Vitamin A Assay

This procedure is provided for the determination of vitamin A intended for use as a food additive. It conforms to that which was adopted in 1956 for international use by the International Union of Pure and Applied Chemistry.

Complete the assay promptly and exercise care throughout the procedure to keep to a minimum exposure to atmospheric oxygen and other oxidizing agents and to actinic light, preferably by the use of an atmosphere of an inert gas and nonactinic glassware.

Special Reagents

Isopropyl Alcohol Use reagent-grade isopropyl alcohol. Redistil, if necessary, to meet the following requirements for spectral purity: When measured in a 1-cm quartz cell against water it shows an absorbance not greater than 0.05 at 300 nm and not greater than 0.01 between 320 and 350 nm.

Ether Use freshly redistilled reagent-grade ether, discarding the first and last 10% portions.

Procedure Transfer into a saponification flask an accurately weighed portion of the sample containing the equivalent of not less than 0.15 mg of retinol, but containing not more than 1 g of fat. If in solid form, heat the portion taken in 10 ml of water on a steam bath for about 10 min, crush the remaining solid with a blunt glass rod, and warm for about 5 min.

Add 30 ml of alcohol if the sample is liquid, or 23 ml of alcohol and 7 ml of glycerin if the sample is solid, followed by 3 ml of potassium hydroxide solution (9 in 10). Reflux under an all-glass condenser for 30 min. Cool the solution, add 30 ml of water, and transfer to a separator. Add 2 g of finely powdered sodium sulfate. Extract by shaking for 2 min with one 150-ml portion of ether and, if an emulsion forms, with three additional 25-ml portions of ether. Combine the ether extracts, if necessary, and wash by swirling gently with 50 ml of water. Repeat the washing more vigorously with three additional 50-ml portions of water. Transfer the washed ether extract to a 250-ml volumetric flask, and add ether to volume.

Evaporate a 25.0-ml portion of the ether extract to about 5 ml. *Without applying heat and with the aid of a stream of inert gas or vacuum*, continue the evaporation to about 3 ml. Dissolve the residue in sufficient isopropyl alcohol to give an expected concentration of the equivalent of 3 to 5 μg of retinol per ml or such that it will give an absorbance in the range of 0.5 to 0.8 at 325 nm. Determine the absorbances of the resulting solution at the wavelengths 310, 325, and 334 nm, with a suitable spectrophotometer fitted with matched quartz cells.

Calculation Calculate the retinol content as follows:

$$\text{Content (in mg)} = 0.549A_{325}/LC,$$

in which A_{325} is the observed absorbance at 325 nm, L is the length, in cm, of the absorption cell, and C is the amount of sample expressed as g in each 100 ml of the final isopropyl alcohol solution, provided that A_{325} has a value not less than $[A_{325}]/1.030$ and not more than $[A_{325}]/0.970$, where $[A_{325}]$ is the corrected absorbance at 325 nm and is given by the equation

$$[A_{325}] = 6.815A_{325} - 2.555A_{310} - 4.260A_{334},$$

in which A designates the absorbance at the wavelength indicated by the subscript.

Where $[A_{325}]$ has a value less than $A_{325}/1.030$, apply the following equation:

$$\text{Content (in mg)} = 0.549[A_{325}]/LC,$$

in which the values are as defined herein.

Confidence Interval The range of the limits of error, indicating the extent of discrepancy to be expected in the results of different laboratories at $P = 0.05$, is approximately ±8%.

Volumetric Apparatus

Most of the volumetric apparatus available in the United States is calibrated at 20°, although the temperatures generally prevailing in laboratories more nearly approach 25°, which is the temperature specified generally for tests and assays. This discrepancy is inconsequential provided the room temperature is reasonably constant and the apparatus has been calibrated accurately prior to and under the conditions of its intended use.

Before use, all volumetric ware must be cleaned in such a manner that, when rinsed with water, no droplet of water can be seen on the inside walls. Many kinds of "degreasing" solutions are available, and the user should consult the manufacturer's literature for the system of choice.

Use To attain the degree of precision required in many assays involving volumetric measurements and directing that a quantity be "accurately measured," the apparatus must be chosen and used with exceptional care. Where less than 10 ml of titrant is to be measured, a 10-ml buret or microburet generally is required.

The design of volumetric apparatus is an important factor in assuring accuracy. For example, the length of the graduated portions of graduated cylinders should be not less than five

times the inside diameter, and the tips of burets should permit an outflow of not more than 0.5 ml per second.

Pipets and burets must be allowed to drain properly in use. Usually, transfer pipets for dilute aqueous solutions should drain for the time specified by the manufacturer before the tip is touched to the wall of the vessel. Buret volumes should not be read immediately upon delivery of the titrant. A suitable length of time should elapse to allow the titrant retained on the walls to drain down. A time interval of 5 to 10 s is usually sufficient.

Standards of Accuracy The capacity tolerances for volumetric flasks, transfer pipets, and burets are those accepted by the National Bureau of Standards,* as indicated in the accompanying tables.

Volumetric Flasks

	Designated Volume (ml)						
	10	25	50	100	250	500	1000
Limit of error (ml)	0.02	0.03	0.05	0.08	0.12	0.15	0.30
Limit of error (%)	0.20	0.12	0.10	0.08	0.05	0.03	0.03

Transfer Pipets

	Designated Volume (ml)						
	1	2	5	10	25	50	100
Limit of error (ml)	0.006	0.006	0.01	0.02	0.03	0.05	0.08
Limit of error (%)	0.6	0.30	0.20	0.20	0.12	0.10	0.08

Burets

	Designated Volume (ml)		
	10 ("micro" type)	25	50
Subdivisions (ml)	0.02	0.10	0.10
Limit of error (ml)	0.02	0.03	0.05

The capacity tolerances for measuring (i.e., "graduated") pipets of up to and including 10-ml capacity are somewhat larger than those for the corresponding sizes of transfer pipets, namely, 0.01, 0.02, and 0.03 ml for the 2-, 5-, and 10-ml sizes, respectively.

Transfer and measuring pipets calibrated "to deliver" should be drained in a vertical position and then touched against the wall of the receiving vessel to drain the tips. Volume readings on burets should be estimated to the nearest 0.01 ml for 25- and 50-ml burets, and to the nearest 0.005 ml for 5- and 10-ml burets. Pipets calibrated "to contain" may be called for in special cases, generally for measuring viscous fluids. In such cases, the pipet should be washed clean, after draining, and the washings added to the measured portion.

*See "Testing of Glass Volumetric Apparatus," NBS Circ. 602, April 1, 1959. Apparatus meeting the specifications of NB SIR 74-461 ("The Calibration of Small Volumetric Laboratory Glassware"), as well as of ANSI/ASTM E 694-79 ("Specifications for Volumetric Ware"), is also acceptable.

Water Determination

Karl Fischer Titrimetric Method

Principle The Karl Fischer titrimetric method for the determination of water is based upon the quantitative reaction of water with an anhydrous solution of sulfur dioxide and iodine dissolved in pyridine and an alcohol. The sample may be titrated with the *Reagent* directly, or the analysis may be carried out by a residual titration procedure. In the residual titration, excess *Reagent* is added to the sample, sufficient time is allowed for the reaction to reach completion, and the unconsumed *Reagent* is titrated with a standard solution of water in methanol. The residual titration procedure is applicable generally and avoids the difficulties that may be encountered in the direct titration of substances from which the bound water is released slowly.

The stoichiometry of the reaction is not exact, and the reproducibility of a determination depends upon such factors as the relative concentrations of the *Reagent* ingredients, the nature of the inert solvent used to dissolve the sample, and the technique used in the particular determination. Therefore, an empirically standardized technique must be used in order to achieve the desired accuracy. Precision in the method is governed largely by the extent to which atmospheric moisture is excluded from the system. The titration of water is usually carried out using anhydrous methanol as the solvent for the sample; however, other suitable solvents may be used for special or unusual substances.

Apparatus and Endpoint Determination Any apparatus may be used that provides for adequate exclusion of atmospheric moisture and determination of the endpoint. In the case of a colorless solution that is titrated directly, the endpoint may be observed visually as a change in color from canary yellow to amber. The reverse is observed in the case of a sample that is titrated residually. More commonly, however, the endpoint is determined electrometrically with the use of dual platinum electrodes (about 5 mm square and about 2.5 cm apart) and a polarizing current of about 100 μA at an applied potential of about 200 mV. When completion of the reaction is reached, a change in the electrochemical properties of the solution is sensed by the electrodes, and the endpoint is indicated by the deflection of a microammeter or by means of some other current-sensing device or a potential-sensing device. With some automatic titrators, the abrupt change in current or potential at the endpoint serves to close a solenoid-operated valve that controls the buret delivering the titrant. Commercially available apparatus generally comprises a closed system consisting of one or two automatic burets and a tightly covered titration vessel fitted with the appropriate electrodes and a magnetic stirrer.

The air in the system is kept dry with a suitable desiccant such as phosphorus pentoxide, and the titration vessel may be purged by means of a stream of dry nitrogen or a current of dry air.

Preparation of the Fischer Reagent To a mixture of 670 ml of methanol and 170 ml of pyridine contained in a flask, add 125 g of iodine, immediately stopper the flask, and cool. Pass dry sulfur dioxide through 100 ml of pyridine contained in a 250-ml graduate and cooled in an ice bath until the volume of the solution attains 200 ml. Slowly add this solution, with shaking, to the cooled iodine mixture, stopper immediately, and shake well until the iodine is dissolved. Transfer the combined solution to the apparatus, preferably an automatic buret protected from moisture with desiccants such as phosphorus pentoxide, anhydrous calcium chloride, or silica gel, and allow to stand for 24 h or overnight before standardizing. Each ml of this reagent when freshly prepared is equivalent to approximately 5 mg of water. Since this solution deteriorates continuously, it should be standardized within 1 h before use, or daily if in continuous use. Protect from light while in use, and store bulk solutions in glass-stoppered containers and under refrigeration.

A stabilized Karl Fischer Reagent solution is commercially available that can be used satisfactorily instead of one prepared as directed herein.

Standardization of the Fischer Reagent

Primary Standardization Transfer 35 to 40 ml of methanol into the titration vessel of the *Apparatus*, and titrate with the *Reagent* to the endpoint color or to the electrometric endpoint.

(*a*) For determination of trace amounts of water (i.e., less than about 1% in the sample), sodium tartrate dihydrate may be used as a convenient water reference substance. If this method is used, quickly add 150 to 350 mg of sodium tartrate dihydrate, ($Na_2C_4H_4O_6.2H_2O$), accurately weighed, to the methanol, and again titrate to the endpoint with the *Reagent*. Calculate the water equivalence factor, *F*, in mg of water per ml of *Reagent*, by the formula

$$2 \times (18.02/230.08) \times (W/V),$$

in which *W* is the weight, in mg, of sodium tartrate dihydrate, *V* is the volume, in ml, of the *Reagent* consumed in the second titration, and 18.02 and 230.08 are, respectively, the molecular weights of water and sodium tartrate dihydrate.

(*b*) For the precise determination of more than trace amounts of water (i.e., more than about 1%), distilled water is used as the reference substance. If this method is used, quickly add 25 to 250 mg of distilled water, accurately weighed, to the methanol, and again titrate to the endpoint with the *Reagent*. Calculate the water equivalence factor, *F*, in mg of water per ml of *Reagent*, by the formula *W/V*, in which *W* is the weight, in mg, of distilled water, and *V* is the volume, in ml, of the *Reagent* consumed in the second titration.

Secondary Standardization Prepare a *Water-Methanol Solution* by diluting 2 ml of water to 1000 ml with methanol. Standardize this solution by titrating 25.0 ml with the *Reagent*, previously standardized as directed under *Primary Standardization* above. Calculate the water content, in mg per ml, of the *Water-Methanol Solution* by the formula *V'F*/25, in which *V'* is the volume, in ml, of the *Reagent* consumed, and *F* is the water equivalence factor of the *Reagent*, determined as directed under *Primary Standardization*. The water content of the *Water-Methanol Solution* should be determined weekly and the *Reagent* standardized against it periodically as needed.

Procedure

NOTE: Determine the water content by the *Direct Titration Procedure*, unless otherwise directed.

Direct Titration Unless otherwise directed, place about 35 to 40 ml of methanol in the titration vessel, and titrate with the *Reagent* to the endpoint, disregarding the volume consumed. Quickly transfer to the titration vessel an accurately weighed or measured quantity of the sample, preferably containing 10 to 50 mg of water, stir vigorously, and again titrate to the endpoint. The water content of the sample, in mg, is obtained by multiplying the volume of *Reagent* used in titrating the sample by the equivalence factor, *F*, of the *Reagent*.

Residual Titration Unless otherwise directed, place about 35 to 40 ml of methanol in the titration vessel, and titrate with the *Reagent* to the endpoint, disregarding the volume consumed. Quickly transfer to the titration vessel an accurately weighed or measured quantity of the sample, preferably containing 10 to 50 mg of water, stir vigorously, and add an accurately measured excess of the *Reagent*. Allow sufficient time for the reaction to reach completion, and titrate the unconsumed *Reagent* with standardized *Water-Methanol Solution* to the endpoint. The water content in the sample, in mg, is obtained by multiplying the net volume of the *Reagent* used in titrating the sample by the equivalence factor, *F*, of the *Reagent*.

Toluene Distillation Method

Principle This method determines water by distillation of a sample with an immiscible solvent, usually toluene.

Apparatus Glass distillation apparatus (see Fig. 30) provided with 24/40 ground-glass connections should be used. The components consist of a 500-ml short-neck, round-bottom flask connected by means of a trap to a 400-mm water-cooled condenser. The lower tip of the condenser should be about 7 mm above the surface of the liquid in the trap after distillation conditions have been established (see *Procedure*).

The trap should be constructed of well-annealed glass, the receiving end of which is graduated to contain 5 ml and subdivided into 0.1-ml divisions with each 1-ml line numbered from 5 ml beginning at the top. Calibrate the receiver by adding 1 ml of water, accurately measured, to 100 ml of toluene contained in the distillation flask. Conduct the distillation and calculate the volume of water obtained as directed in the *Procedure*. To the cooled apparatus add another ml of water and repeat the distillation. Continue in this manner until five 1-ml portions of water have been added. The error at any indicated capacity should not exceed 0.05 ml.

The source of heat is either an oil bath or an electric heater provided with a suitable means of temperature control. The distillation may be better controlled by insulating the tube

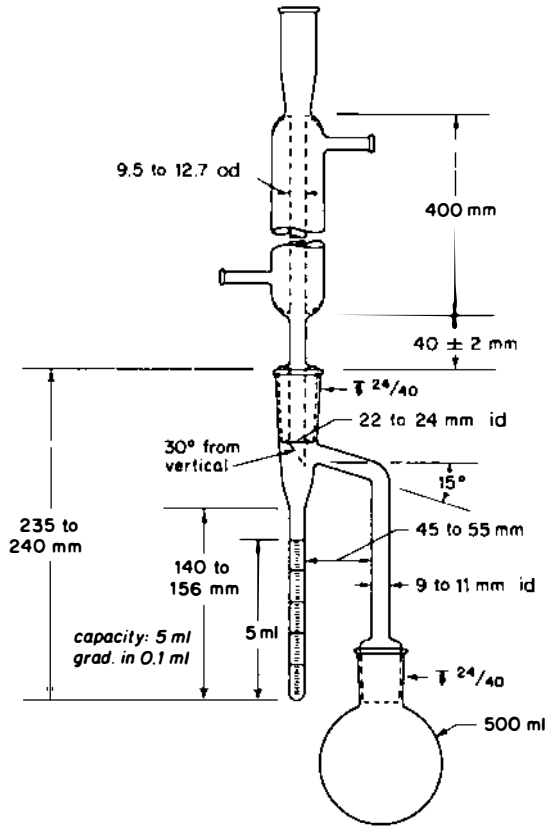


FIGURE 30 Moisture Distillation Apparatus

leading from the flask to the receiver. It is also advantageous to protect the flask from drafts.

Clean the entire apparatus with potassium dichromate–sulfuric acid cleaning solution, rinse thoroughly, and dry completely before using.

Procedure Place in the previously cleaned and dried flask a quantity of the substance, weighed accurately to the nearest 0.01 g, that is expected to yield from 1.5 to 4 ml of water. If the substance is of a pastelike consistency, weigh it in a boat of metal foil that will pass through the neck of the flask. If the substance is likely to cause bumping, take suitable precautions to prevent it. Transfer about 200 ml of ACS reagent-grade toluene into the flask, and swirl to mix it with the sample. Assemble the apparatus, fill the receiver with toluene by pouring it through the condenser until it begins to overflow into the flask, and insert a loose cotton plug in the top of the condenser. Heat the flask so that the distillation rate will be about 200 drops per min, and continue distilling until the volume of water in the trap remains constant for 5 min. Discontinue the heating, dislodge any drops of water that may be adhering to the inside of the condenser tube or receiver with a copper or nichrome wire spiral, and wash down with about 5 ml of toluene. Disconnect the receiver, immerse it in water at 25° for at least 15 min or until the toluene layer is clear, and then read the volume of water. Conduct a blank determination using the same volume of toluene as used when distilling the sample mixture, and make any necessary correction (see page 2).

Weights and Balances

Codex tests and assays are designed for use with three types of analytical balances, known as macro-, semimicro-, and micro-.

By custom, microbalances weigh objects with a sensitivity down to the μg range (or lower); semimicrobalances down to the 0.01-mg range; and analytical macrobalances down to the 0.1-mg range.

Tolerances The analytical weights meet the tolerances of the National Bureau of Standards for Class S if used without corrections, or meet the use tolerances for Class S-1 if used with corrections. Where quantities of 25 mg or less are to be "weighed accurately," any applicable corrections for weights should be used.

Use Where substances are to be "accurately weighed" in an assay or a test, the weighing is to be performed in such manner as to limit the error to 0.1% or less. For example, a quantity of 50 mg is to be weighed to the nearest 0.05 mg; a quantity of 0.1 g is to be weighed to the nearest 0.1 mg; and a quantity of 10 g is to be weighed to the nearest 10 mg.

Calibration All precision balances and weights should be calibrated periodically (preferably at least once a year) and a record kept of the calibration date and results. The user may have a set of weights calibrated by the nearest Department of Weights and Measurements (or its equivalent). This is usually done for little or no charge. Alternatively, an independent, outside company may be retained for the purpose of performing such calibrations.

Buoyancy Effect When a weighing is to be performed with an accuracy of 0.1% or better, the buoyancy effect should not be neglected. The equation to be used in correcting for this effect is

$$M_v = M_a[1 + 0.0012(1/D_o - D_w)],$$

in which M_v is the mass in vacuum; M_a is the mass in air; 0.0012 is the density of air; D_o is the density of the weighed object; and D_w is the density of the calibrated weights.

7 / *Solutions and Indicators*

Contents

Colorimetric Solutions (CS)

Colorimetric solutions are used in the preparation of colorimetric standards for certain chemicals, and for the carbonization tests with sulfuric acid that are specified in several monographs. Directions for the preparation of the primary colorimetric solutions and *Matching Fluids* are given under the test for *Readily Carbonizable Substances*, page 532. Store the solutions in suitably resistant, tight containers.

Comparison of colors as directed in the *Food Chemicals Codex* tests preferably is made in matched color-comparison tubes or in a suitable colorimeter under conditions that ensure that the colorimetric reference solution and that of the specimen under test are treated alike in all respects (see page 542).

Standard Buffer Solutions

Reagent Solutions Previously dry the crystalline reagents, except the boric acid, at 110° to 120°, and use water that has been previously boiled and cooled in preparing the solutions. Store the prepared reagent solutions in chemically resistant glass or polyethylene bottles, and use within 3 months. Discard if molding is evident.

Potassium Chloride, 0.2 M Dissolve 14.91 g of potassium chloride, KCl, in sufficient water to make 1000.0 ml.

Potassium Biphthalate, 0.2 M Dissolve 40.84 g of potassium biphthalate, $KHC_6H_4(COO)_2$, in sufficient water to make 1000.0 ml.

Potassium Phosphate, Monobasic, 0.2 M Dissolve 27.22 g of monobasic potassium phosphate, KH_2PO_4, in sufficient water to make 1000.0 ml.

Boric Acid–Potassium Chloride, 0.2 M Dissolve 12.37 g of boric acid, H_3BO_3, and 14.91 g of potassium chloride, KCl, in sufficient water to make 1000.0 ml.

Hydrochloric Acid, 0.2 M, and *Sodium Hydroxide, 0.2 M* Prepare and standardize as directed under *Volumetric Solutions*, page 564.

Procedure To prepare 200 ml of a standard buffer solution having a pH within the range 1.2 to 10.0, place 50.0 ml of the appropriate 0.2 *M* salt solution, prepared above, in a 200-ml volumetric flask, add the volume of 0.2 *M* hydrochloric acid or a sodium hydroxide specified for the desired pH in the accompanying table, dilute to volume with water, and mix.

Composition of Standard Buffer Solutions[a]

Hydrochloric Acid Buffer		Acid Phthalate Buffer		Neutralized Phthalate Buffer		Phosphate Buffer		Alkaline Borate Buffer	
To 50.0 ml of 0.2 *M* KCl add the ml of HCl specified		To 50.0 ml of 0.2 *M* $KHC_6H_4(COO)_2$ add the ml of HCl specified		To 50.0 ml of 0.2 *M* $KHC_6H_4(COO)_2$ add the ml of NaOH specified		To 50.0 ml of 0.2 *M* KH_2PO_4 add the ml of NaOH specified		To 50.0 ml of 0.2 *M* H_3BO_3-KCl add the ml of NaOH specified	
pH	0.2 *M* HCl (ml)	pH	0.2 *M* HCl (ml)	pH	0.2 *M* NaOH (ml)	pH	0.2 *M* NaOH (ml)	pH	0.2 *M* NaOH (ml)
1.2	85.0	2.2	49.5	4.2	3.0	5.8	3.6	8.0	3.9
1.3	67.2	2.4	42.2	4.4	6.6	6.0	5.6	8.2	6.0
1.4	53.2	2.6	35.4	4.6	11.1	6.2	8.1	8.4	8.6
1.5	41.4	2.8	28.9	4.8	16.5	6.4	11.6	8.6	11.8
1.6	32.4	3.0	22.3	5.0	22.6	6.6	16.4	8.8	15.8
1.7	26.0	3.2	15.7	5.2	28.8	6.8	22.4	9.0	20.8
1.8	20.4	3.4	10.4	5.4	34.1	7.0	29.1	9.2	26.4
1.9	16.2	3.6	6.3	5.6	38.8	7.2	34.7	9.4	32.1
2.0	13.0	3.8	2.9	5.8	42.3	7.4	39.1	9.6	36.9
2.1	10.2	4.0	0.1	—	—	7.6	42.4	9.8	40.6
2.2	7.8	—	—	—	—	7.8	44.5	10.0	43.7
						8.0	46.1	—	—

[a] Dilute all final solutions to 200.0 ml. (see *Procedure*). The standard pH values given in this table are considered to be reproducible to within ±0.02 of the pH unit specified at 25°.

Standard Solutions for the Preparation of Controls and Standards

The following solutions are used in tests for impurities that require the comparison of the color or turbidity produced in a solution of the test substance with that produced by a known amount of the impurity in a control. Directions for the preparation of other standard solutions are given in the monographs or under the general tests in which they are required (see also *Index*).

Ammonium Standard Solution (10 μg NH_4 in 1 ml) Dissolve 296.0 mg of ammonium chloride, NH_4Cl, in sufficient water to make 100.0 ml, and mix. Transfer 10.0 ml of this solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Barium Standard Solution (100 μg Ba in 1 ml) Dissolve 177.9 mg of barium chloride, $BaCl_2.2H_2O$, in water in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Iron Standard Solution (10 μg Fe in 1 ml) Dissolve 702.2 mg of ferrous ammonium sulfate, $Fe(NH_4)_2(SO_4)_2.6H_2O$, in 10 ml of diluted sulfuric acid TS in a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a 1000-ml volumetric flask, add 10 ml of diluted sulfuric acid TS, dilute to volume with water, and mix.

Magnesium Standard Solution (50 μg Mg in 1 ml) Dissolve 50.0 mg of magnesium metal, Mg, in 1 ml of hydrochloric acid in a 1000-ml volumetric flask, dilute to volume with water, and mix.

Phosphate Standard Solution (10 μg PO_4 in 1 ml) Dissolve 143.3 mg of monobasic potassium phosphate, KH_2PO_4, in water in a 100-ml volumetric flask, dilute to volume with water, and mix. Transfer 10.0 ml of this solution into a 1000-ml volumetric flask, dilute to volume with water, and mix.

Test Solutions (TS) and Other Reagents

Certain of the following test solutions are intended for use as acid-base indicators in volumetric analyses. Such solutions should be so adjusted that when 0.15 ml of the indicator solution is added to 25 ml of carbon dioxide–free water, 0.25 ml of 0.02 *N* acid or alkali, respectively, will produce the characteristic color change.

In general, the directive to prepare a solution "fresh" indicates that the solution is of limited stability and must be prepared on the day of use.

Acetic Acid TS, Diluted A solution containing about 6% (w/v) of CH_3COOH. Prepare by diluting 60.0 ml of glacial acetic acid, or 166.6 ml of 36% acetic acid (6 *N*), with sufficient water to make 1000 ml.

Alcohol (*Ethanol; Ethyl Alcohol; C_2H_5OH*) Use ACS reagent-grade *Ethyl Alcohol* (not less than 95.0%, by volume, of C_2H_5OH). (NOTE: For use in assays and tests involving ultraviolet spectrophotometry, use ACS reagent-grade *Ethyl Alcohol Suitable for Use in Ultraviolet Spectrophotometry.*)

Alcohol, Absolute (*Anhydrous Alcohol; Dehydrated Alcohol*) Use ACS reagent-grade *Ethyl Alcohol, Absolute* (not less than 99.5%, by volume, of C_2H_5OH).

Alcohol, Diluted A solution containing 41.0% to 42.0%, by weight, corresponding to 48.4% to 49.5%, by volume, at 15.56°, of C_2H_5OH.

Alcohol, 70% (at 15.56°) A 38.6:15 mixture (v/v) of 95% alcohol and water, having a specific gravity of 0.884 at 25°. To prepare 100 ml, dilute 73.7 ml of alcohol to 100 ml with water at 25°.

Alcohol, 80% (at 15.56°) A 45.5:9.5 mixture (v/v) of 95% alcohol and water, having a specific gravity of 0.857 at 25°. To prepare 100 ml, dilute 84.3 ml of alcohol to 100 ml with water at 25°.

Alcohol, 90% (at 15.56°) A 51:3 mixture (v/v) of 95% alcohol and water, having a specific gravity of 0.827 at 25°. To prepare 100 ml, dilute 94.8 ml of alcohol to 100 ml with water at 25°.

Alcohol, Aldehyde-Free Dissolve 2.5 g of lead acetate in 5 ml of water, add the solution to 1000 ml of alcohol contained in a glass-stoppered bottle, and mix. Dissolve 5 g of potassium hydroxide in 25 ml of warm alcohol, cool, and add slowly, without stirring, to the alcoholic solution of lead acetate. Allow to stand for 1 h, then shake the mixture vigorously, allow to stand overnight, decant the clear liquid, and recover the alcohol by distillation. Ethyl Alcohol FCC, Alcohol USP, or USSD #3A or #30 may be used. If the titration of a 250-ml sample of the alcohol by *Hydroxylamine Hydrochloride Solution* (see page 561) does not exceed 0.25 ml of 0.5 *N* alcoholic potassium hydroxide, the above treatment may be omitted.

Alcoholic Potassium Hydroxide TS See *Potassium Hydroxide TS, Alcoholic.*

Alkaline Cupric Tartrate TS (*Fehling's Solution*) See *Cupric Tartrate TS, Alkaline.*

Alkaline Mercuric-Potassium Iodide TS (*Nessler's Reagent*) See *Mercuric-Potassium Iodide TS, Alkaline.*

Ammonia–Ammonium Chloride Buffer TS (approximately pH 10) Dissolve 67.5 g of ammonium chloride, NH_4Cl, in water, add 570 ml of ammonium hydroxide (28%), and dilute with water to 1000 ml.

Ammonia TS A solution containing between 9.5% and 10.5%

of NH_3. Prepare by diluting 400 ml of ammonium hydroxide (28%) with sufficient water to make 1000 ml.

Ammonia TS, Stronger (*Ammonium Hydroxide, 28%, Stronger Ammonia Water*) A practically saturated solution of ammonia in water, containing between 28% and 30% of NH_3.

Ammoniacal Silver Nitrate TS Add ammonia TS, dropwise, to a 1 in 20 solution of silver nitrate until the precipitate that first forms is almost, but not entirely, dissolved. Filter the solution, and store in a dark bottle.

Caution: Ammoniacal silver nitrate TS forms explosive compounds on standing. Do not store this solution, but prepare a fresh quantity for each series of determinations. Neutralize the excess reagent and rinse all glassware with hydrochloric acid immediately after completing a test.

Ammonium Acetate TS Dissolve 10 g of ammonium acetate, $NH_4C_2H_3O_2$, in sufficient water to make 100 ml.

Ammonium Carbonate TS Dissolve 20 g of ammonium carbonate and 20 ml of ammonia TS in sufficient water to make 100 ml.

Ammonium Chloride TS Dissolve 10.5 g of ammonium chloride, NH_4Cl, in sufficient water to make 100 ml.

Ammonium Molybdate TS Dissolve 6.5 g of finely powdered molybdic acid (85%) in a mixture of 14 ml of water and 14.5 ml of stronger ammonia TS. Cool the solution, and add it slowly, with stirring, to a well-cooled mixture of 32 ml of nitric acid and 40 ml of water. Allow to stand for 48 h, and filter through a fine-porosity sintered-glass crucible lined at the bottom with a layer of glass wool. This solution deteriorates upon standing and is unsuitable for use if, upon the addition of 2 ml of sodium phosphate TS to 5 ml of the solution, an abundant yellow precipitate does not form at once or after slight warming. Store it in the dark. If a precipitate forms during storage, use only the clear, supernatant solution.

Ammonium Oxalate TS Dissolve 3.5 g of ammonium oxalate, $(NH_4)_2C_2O_4.H_2O$, in sufficient water to make 100 ml.

Ammonium Sulfanilate TS To 2.5 g of sulfanilic acid add 15 ml of water and 3 ml of ammonia TS, and mix. Add, with stirring, more ammonia TS, if necessary, until the acid dissolves, adjust the pH of the solution to about 4.5 with diluted hydrochloric acid TS, using bromocresol green TS as an outside indicator, and dilute to 25 ml.

Ammonium Sulfide TS Saturate ammonia TS with hydrogen sulfide, H_2S, and add two thirds of its volume of ammonia TS. Residue upon ignition: not more than 0.05%. The solution is not rendered turbid either by magnesium sulfate TS or by calcium chloride TS (*carbonate*). This solution is unsuitable for use if an abundant precipitate of sulfur is present. Store it in small, well-filled, dark amber–colored bottles in a cold, dark place.

Ammonium Thiocyanate TS Dissolve 8 g of ammonium thiocyanate, NH_4SCN, in sufficient water to make 100 ml.

Antimony Trichloride TS Dissolve 20 g of antimony trichloride, $SbCl_3$, in chloroform to make 100 ml. Filter if necessary.

Barium Chloride TS Dissolve 12 g of barium chloride, $BaCl_2.2H_2O$, in sufficient water to make 100 ml.

Barium Diphenylamine Sulfonate TS Dissolve 300 mg of *p*-diphenylamine sulfonic acid barium salt in 100 ml of water.

Benedict's Qualitative Reagent See *Cupric Citrate TS, Alkaline*.

Benzidine TS Dissolve 50 mg of benzidine in 10 ml of glacial acetic acid, dilute to 100 ml with water, and mix.

Bismuth Nitrate, TS Reflux 5 g of bismuth nitrate, $Bi(NO_3)_3.5H_2O$, with 7.5 ml of nitric acid and 10 ml of water until dissolved, cool, filter, and dilute to 250 ml with water.

Bromine TS (*Bromine Water*) A saturated solution of bromine, prepared by agitating 2 to 3 ml of bromine, Br_2, with 100 ml of cold water in a glass-stoppered bottle, the stopper of which should be lubricated with petrolatum. Store it in a cold place protected from light.

Bromocresol Blue TS Use *Bromocresol Green TS*.

Bromocresol Green TS Dissolve 50 mg of bromocresol green in 100 ml of alcohol, and filter if necessary.

Bromocresol Purple TS Dissolve 250 mg of bromocresol purple in 20 ml of 0.5 *N* sodium hydroxide, and dilute with water to 250 ml.

Bromophenol Blue TS Dissolve 100 mg of bromophenol blue in 100 ml of dilute alcohol (1 in 2), and filter if necessary.

Bromothymol Blue TS Dissolve 100 mg of bromothymol blue in 100 ml of dilute alcohol (1 in 2), and filter if necessary.

Calcium Chloride TS Dissolve 7.5 g of calcium chloride, $CaCl_2.2H_2O$, in sufficient water to make 100 ml.

Calcium Hydroxide TS A solution containing approximately 140 mg of $Ca(OH)_2$ in each 100 ml. To prepare, add 3 g of calcium hydroxide, $Ca(OH)_2$, to 1000 ml of water, and agitate the mixture vigorously and repeatedly during 1 h. Allow the excess calcium hydroxide to settle, and decant or draw off the clear, supernatant liquid.

Carr-Price Reagent See *Antimony Trichloride TS*.

Ceric Ammonium Nitrate TS Dissolve 6.25 g of ceric ammonium nitrate, $(NH_4)_2Ce(NO_3)_6$, in 100 ml of 0.25 *N* nitric acid. Prepare the solution fresh every third day.

Chlorine TS (*Chlorine Water*) A saturated solution of chlorine in water. Place the solution in small, completely filled, light-resistant containers. Chlorine TS, even when kept from light and air, is apt to deteriorate. Store it in a cold, dark place. For full strength, prepare this solution fresh.

Chromotropic Acid TS Dissolve 50 mg of chromotropic acid or its sodium salt in 100 ml of 75% sulfuric acid (made by adding cautiously 75 ml of 95% to 98% sulfuric acid to 33.3 ml of water).

Cobaltous Chloride TS Dissolve 2 g of cobaltous chloride, $CoCl_2.6H_2O$, in 1 ml of hydrochloric acid and sufficient water to make 100 ml.

Cobalt-Uranyl Acetate TS Dissolve, with warming, 40 g of uranyl acetate, $UO_2(C_2H_3O_2)_2.2H_2O$, in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 ml. Similarly, prepare a solution containing 200 g of cobaltous acetate, $Co(C_2H_3O_2)_2.4H_2O$, in a mixture of 30 g of glacial acetic acid and sufficient water to make 500 ml. Mix the two solutions while still warm, and cool to 20°. Maintain the temperature at 20° for about 2 h to separate the excess salts from solution, and then filter through a dry filter.

Congo Red TS Dissolve 500 mg of congo red in a mixture of 10 ml of alcohol and 90 ml of water.

Cresol Red TS Triturate 100 mg of cresol red in a mortar with 26.2 ml of 0.01 *N* sodium hydroxide until solution is complete, then dilute the solution with water to 250 ml.

Cresol Red–Thymol Blue TS Add 15 ml of thymol blue TS to 5 ml of cresol red TS, and mix.

Crystal Violet TS Dissolve 100 mg of crystal violet in 10 ml of glacial acetic acid.

Cupric Citrate TS, Alkaline (*Benedict's Qualitative Reagent*) With the aid of heat, dissolve 173 g of sodium citrate, $C_6H_5Na_3O_7.2H_2O$, and 117 g of sodium carbonate, $Na_2CO_3.H_2O$, in about 100 ml of water, and filter through paper, if necessary. In a separate container dissolve 17.3 g of cupric sulfate, $CuSO_4.5H_2O$, in about 700 ml of water, and slowly add this solution, with constant stirring, to the first solution. Cool the mixture, dilute to 1000 ml, and mix.

Cupric Nitrate TS Dissolve 2.4 g of cupric nitrate, $Cu(NO_3)_2.3H_2O$, in sufficient water to make 100 ml.

Cupric Sulfate TS Dissolve 12.5 g of cupric sulfate, $CuSO_4.5H_2O$, in sufficient water to make 100 ml, and mix.

Cupric Tartrate TS, Alkaline (*Fehling's Solution*) *The Copper Solution* (*A*): Dissolve 34.66 g of carefully selected, small crystals of cupric sulfate, $CuSO_4.5H_2O$, showing no trace of efflorescence or of adhering moisture, in sufficient water to make 500 ml. Store this solution in small, tight containers. *The Alkaline Tartrate Solution* (*B*): Dissolve 173 g of crystallized potassium sodium tartrate, $KNaC_4H_4O_6.4H_2O$, and 50 g of sodium hydroxide, NaOH, in sufficient water to make 500 ml. Store this solution in small, alkali-resistant containers. For use, mix exactly equal volumes of solutions *A* and *B* at the time required.

Cyanogen Bromide TS Dissolve 5 g of cyanogen bromide in water to make 50 ml.

Caution: Prepare this solution under a hood, as cyanogen bromide volatilizes at room temperature and the vapor is highly irritating and poisonous.

Denigès' Reagent See *Mercuric Sulfate TS*.

2,7-Dihydroxynaphthalene TS Dissolve 100 mg of 2,7-dihydroxynaphthalene in 1000 ml of sulfuric acid, and allow the solution to stand until the initial color disappears. If the solution is very dark, discard it and prepare a new solution from a different supply of sulfuric acid. This solution is stable for approximately 1 month if stored in a dark bottle.

Diphenylamine TS Dissolve 1 g of diphenylamine in 100 ml of sulfuric acid. The solution should be colorless.

Diphenylcarbazone TS Dissolve about 1 g of diphenylcarbazone ($C_{13}H_{12}N_4O$) in sufficient alcohol to make 100 ml. Store this solution in a brown bottle.

α,α′-Dipyridyl TS Dissolve 100 mg of α,α′-dipyridyl, $C_{10}H_8N_2$, in 50 ml of absolute alcohol.

Dithizone TS Dissolve 25.6 mg of dithizone in 100 ml of alcohol.

Eosin Y TS (adsorption indicator) Dissolve 50 mg of eosin Y in 10 ml of water.

Eriochrome Black TS Dissolve 200 mg of eriochrome black T and 2 g of hydroxylamine hydrochloride, $NH_2OH.HCl$, in sufficient methanol to make 50 ml, and filter. Store the solution in a light-resistant container and use within 2 weeks.

***p*-Ethoxychrysoidin TS** Dissolve 50 mg of *p*-ethoxychrysoidin monohydrochloride in a mixture of 25 ml of water and 25 ml of alcohol, add 3 drops of hydrochloric acid, stir vigorously, and filter if necessary to obtain a clear solution.

Fehling's Solution See *Cupric Tartrate TS, Alkaline*.

Ferric Ammonium Sulfate TS Dissolve 8 g of ferric ammonium sulfate, $FeNH_4(SO_4)_2.12H_2O$, in sufficient water to make 100 ml.

Ferric Chloride TS Dissolve 9 g of ferric chloride, $FeCl_3.6H_2O$, in sufficient water to make 100 ml.

Ferric Chloride TS, Alcoholic Dissolve 100 mg of ferric

chloride, $FeCl_3.6H_2O$, in 50 ml of absolute alcohol. Prepare this solution fresh.

Ferric Sulfate TS, Acid Add 7.5 ml of sulfuric acid to 100 ml of water, and dissolve 80 g of ferrous sulfate in the mixture with the aid of heat. Mix 7.5 ml of nitric acid and 20 ml of water, warm, and add to this the ferrous sulfate solution. Concentrate the mixture until, upon the sudden disengagement of ruddy vapors, the black color of the liquid changes to red. Test for the absence of ferrous iron, and, if necessary, add a few drops of nitric acid and boil again. When the solution is cold, add sufficient water to make 110 ml.

Ferrous Sulfate TS Dissolve 8 g of clear crystals of ferrous sulfate, $FeSO_4.7H_2O$, in about 100 ml of recently boiled and thoroughly cooled water. Prepare this solution fresh.

Formaldehyde TS A solution containing approximately 37.0% (w/v) of HCHO. It may contain methanol to prevent polymerization.

Hydrochloric Acid Use ACS reagent-grade *Hydrochloric Acid* (36.5% to 38.0% of HCl).

Hydrochloric Acid TS, Diluted A solution containing 10% (w/v) of HCl. Prepare by diluting 226 ml of hydrochloric acid (36%) with sufficient water to make 1000 ml.

Hydrogen Peroxide TS A solution containing between 2.5 and 3.5 g of H_2O_2 in each 100 ml. It may contain suitable preservatives, totaling not more than 0.05%.

Hydrogen Sulfide TS A saturated solution of hydrogen sulfide made by passing H_2S into cold water. Store it in small, dark amber–colored bottles, filled nearly to the top. It is unsuitable unless it possesses a strong odor of H_2S, and unless it produces at once a copious precipitate of sulfur when added to an equal volume of ferric chloride TS. Store in a cold, dark place.

Hydroxylamine Hydrochloride TS Dissolve 3.5 g of hydroxylamine hydrochloride, $NH_2OH.HCl$, in 95 ml of 60% alcohol, and add 0.5 ml of bromophenol blue solution (1 in 1000) and 0.5 *N* alcoholic potassium hydroxide until a greenish tint develops in the solution. Then add sufficient 60% alcohol to make 100 ml.

8-Hydroxyquinoline TS Dissolve 5 g of 8-hydroxyquinoline (oxine) in sufficient alcohol to make 100 ml.

Indigo Carmine TS (*Sodium Indigotindisulfonate TS*) Dissolve a quantity of sodium indigotindisulfonate, equivalent to 180 mg of $C_{16}H_8N_2O_2(SO_3Na)_2$, in sufficient water to make 100 ml. Use within 60 days.

Iodine TS Dissolve 14 g of iodine, I_2, in a solution of 36 g of potassium iodide, KI, in 100 ml of water, add 3 drops of hydrochloric acid, dilute with water to 1000 ml, and mix.

Isopropanol [*Isopropyl Alcohol; 2-Propanol; $(CH_3)_2CHOH$*]

Use ACS reagent-grade *Isopropyl Alcohol*. (NOTE: For use in assays and tests involving ultraviolet spectrophotometry, use ACS reagent-grade *Isopropyl Alcohol Suitable for Use in Ultraviolet Spectrophotometry*.)

Isopropanol, Anhydrous (*Dehydrated Isopropanol*) Use *Isopropanol* that has been previously dried by shaking with anhydrous calcium chloride, followed by filtering.

Lead Acetate TS Dissolve 9.5 g of clear, transparent crystals of lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$, in sufficient recently boiled water to make 100 ml. Store in well-stoppered bottles.

Lead Subacetate TS Triturate 14 g of lead monoxide, PbO, to a smooth paste with 10 ml of water, and transfer the mixture to a bottle, using an additional 10 ml of water for rinsing. Dissolve 22 g of lead acetate, $Pb(C_2H_3O_2)_2.3H_2O$, in 70 ml of water, and add the solution to the lead oxide mixture. Shake it vigorously for 5 min, then set it aside, shaking it frequently during 7 days. Finally filter, and add enough recently boiled water through the filter to make 100 ml.

Lead Subacetate TS, Diluted Dilute 3.25 ml of lead subacetate TS with sufficient water, recently boiled and cooled, to make 100 ml. Store in small, well-fitted, tight containers.

Litmus TS Digest 25 g of powdered litmus with three successive 100-ml portions of boiling alcohol, continuing each extraction for about 1 h. Filter, wash with alcohol, and discard the alcohol filtrate. Macerate the residue with about 25 ml of cold water for 4 h, filter, and discard the filtrate. Finally, digest the residue with 125 ml of boiling water for 1 h, cool, and filter.

Magnesia Mixture TS Dissolve 5.5 g of magnesium chloride, $MgCl_2.6H_2O$, and 7 g of ammonium chloride, NH_4Cl, in 65 ml of water, add 35 ml of ammonia TS, set the mixture aside for a few days in a well-stoppered bottle, and filter. If the solution is not perfectly clear, filter it before using.

Magnesium Sulfate TS Dissolve 12 g of crystals of magnesium sulfate, $MgSO_4.7H_2O$, selected for freedom from efflorescence, in water to make 100 ml.

Malachite Green TS Dissolve 1 g of malachite green oxalate in 100 ml of glacial acetic acid.

Mayer's Reagent See *Mercuric-Potassium Iodide TS*.

Mercuric Acetate TS Dissolve 6 g of mercuric acetate, $Hg(C_2H_3O_2)_2$, in sufficient glacial acetic acid to make 100 ml. Store in tight containers protected from direct sunlight.

Mercuric Chloride TS Dissolve 6.5 g of mercuric chloride, $HgCl_2$, in water to make 100 ml.

Mercuric-Potassium Iodide TS (*Mayer's Reagent*) Dissolve 1.358 g of mercuric chloride, $HgCl_2$, in 60 ml of water. Dissolve 5 g of potassium iodide, KI, in 10 ml of water. Mix the two solutions, and add water to make 100 ml.

Mercuric-Potassium Iodide TS, Alkaline (*Nessler's Reagent*) Dissolve 10 g of potassium iodide, KI, in 10 ml of water, and add slowly, with stirring, a saturated solution of mercuric chloride until a slight red precipitate remains undissolved. To this mixture add an ice-cold solution of 30 g of potassium hydroxide, KOH, in 60 ml of water, then add 1 ml more of the saturated solution of mercuric chloride. Dilute with water to 200 ml. Allow the precipitate to settle, and draw off the clear liquid. A 2-ml portion of this reagent, when added to 100 ml of a 1 in 300,000 solution of ammonium chloride in ammonia-free water, produces at once a yellowish brown color.

Mercuric Sulfate TS (*Denigès' Reagent*) Mix 5 g of yellow mercuric oxide, HgO, with 40 ml of water, and while stirring slowly add 20 ml of sulfuric acid, then add another 40 ml of water, and stir until completely dissolved.

Methanol (*Methyl Alcohol*) Use ACS reagent-grade *Methanol*.

Methanol, Anhydrous (*Dehydrated Methanol*) Use *Methanol*.

***p*-Methylaminophenol Sulfate TS** Dissolve 2 g of *p*-methylaminophenol sulfate, $(HOC_6H_4NHCH_3)_2.H_2SO_4$, in 100 ml of water. To 10 ml of this solution add 90 ml of water and 20 g of sodium bisulfite. Confirm the suitability of this solution by the following test: Add 1 ml of the solution to each of four tubes containing 25 ml of 0.5 *N* sulfuric acid and 1 ml of ammonium molybdate TS. Add 5 µg of phosphate (PO_4) to one tube, 10 µg to a second, and 20 µg to a third, using 0.5, 1.0, and 2.0 ml, respectively, of *Phosphate Standard Solution*, and allow to stand for 2 h. The solutions in the three tubes should show readily perceptible differences in blue color corresponding to the relative amounts of phosphate added, and the one to which 5 µg of phosphate was added should be perceptibly bluer than the blank.

Methylene Blue TS Dissolve 125 mg of methylene blue in 100 ml of alcohol, and dilute with alcohol to 250 ml.

Methyl Orange TS Dissolve 100 mg of methyl orange in 100 ml of water, and filter if necessary.

Methyl Red TS Dissolve 100 mg of methyl red in 100 ml of alcohol, and filter if necessary.

Methyl Red–Methylene Blue TS Add 10 ml of methyl red TS to 10 ml of methylene blue TS, and mix.

Methylrosaniline Chloride TS See *Crystal Violet TS*.

Methyl Violet TS See *Crystal Violet TS*.

Millon's Reagent To 2 ml of mercury in an Erlenmeyer flask add 20 ml of nitric acid. Shake the flask under a hood to break up the mercury into small globules. After about 10 min add 35 ml of water, and, if a precipitate or crystals appear, add sufficient dilute nitric acid (1 in 5, prepared from nitric acid from which the oxides have been removed by blowing air through it until it is colorless) to dissolve the separated solid. Add sodium hydroxide solution (1 in 10), dropwise, with thorough mixing, until the curdy precipitate that forms after the addition of each drop no longer redissolves but is dispersed to form a suspension. Add 5 ml more of the dilute nitric acid, and mix well. Prepare this solution fresh.

Naphthol Green TS Dissolve 500 mg of naphthol green B in water to make 1000 ml.

Nessler's Reagent See *Mercuric-Potassium Iodide TS, Alkaline*.

Neutral Red TS Dissolve 100 mg of neutral red in 100 ml of 50% alcohol.

Ninhydrin TS See *Triketohydrindene Hydrate TS*.

Nitric Acid Use ACS reagent-grade *Nitric Acid* (69.0% to 71.0% of HNO_3).

Nitric Acid TS, Diluted A solution containing about 10% (w/v) of HNO_3. Prepare by diluting 105 ml of nitric acid (70%) with water to make 1000 ml.

Orthophenanthroline TS Dissolve 150 mg of orthophenanthroline, $C_{12}H_8N_2.H_2O$, in 10 ml of a solution of ferrous sulfate, prepared by dissolving 700 mg of clear crystals of ferrous sulfate, $FeSO_4.7H_2O$, in 100 ml of water. The ferrous sulfate solution must be prepared immediately before dissolving the orthophenanthroline. Store the solution in well-closed containers.

Oxalic Acid TS Dissolve 6.3 g of oxalic acid, $H_2C_2O_4.2H_2O$, in water to make 100 ml.

Phenol Red TS (*Phenolsulfonphthalein TS*) Dissolve 100 mg of phenolsulfonphthalein in 100 ml of alcohol, and filter if necessary.

Phenolphthalein TS Dissolve 1 g of phenolphthalein in 100 ml of alcohol.

Phenolsulfonphthalein TS See *Phenol Red TS*.

***p*-Phenylphenol TS** On the day of use, dissolve 750 mg of *p*-phenylphenol in 50 ml of sodium hydroxide TS.

Phosphoric Acid Use ACS reagent-grade *Phosphoric Acid* (not less than 85.0% of H_3PO_4).

Phosphotungstic Acid TS Dissolve 1 g of phosphotungstic acid (approximately $24WO_3.2H_3PO_4.48H_2O$) in water to make 100 ml.

Picric Acid TS See *Trinitrophenol TS*.

Potassium Acetate TS Dissolve 10 g of potassium acetate, $KC_2H_3O_2$, in water to make 100 ml.

Potassium Chromate TS Dissolve 10 g of potassium chromate, K_2CrO_4, in water to make 100 ml.

Potassium Dichromate TS Dissolve 7.5 g of potassium dichromate, $K_2Cr_2O_7$, in water to make 100 ml.

Potassium Ferricyanide TS Dissolve 1 g of potassium ferricyanide, $K_3Fe(CN)_6$, in 10 ml of water. Prepare this solution fresh.

Potassium Ferrocyanide TS Dissolve 1 g of potassium ferrocyanide, $K_4Fe(CN)_6.3H_2O$, in 10 ml of water. Prepare this solution fresh.

Potassium Hydroxide TS Dissolve 6.5 g of potassium hydroxide, KOH, in water to make 100 ml.

Potassium Hydroxide TS, Alcoholic Use *0.5 N Alcoholic Potassium Hydroxide*, page 566.

Potassium Iodide TS Dissolve 16.5 g of potassium iodide, KI, in water to make 100 ml. Store in light-resistant containers.

Potassium Permanganate TS Use *0.1 N Potassium Permanganate*, page 566.

Potassium Sulfate TS Dissolve 1 g of potassium sulfate, K_2SO_4, in sufficient water to make 100 ml.

Quimociac TS Dissolve 70 g of sodium molybdate ($Na_2MoO_4.2H_2O$) in 150 ml of water (*Solution A*). Dissolve 60 g of citric acid in a mixture of 85 ml of nitric acid and 150 ml of water, and cool (*Solution B*). Gradually add *Solution A* to *Solution B*, with stirring, to produce *Solution C*. Dissolve 5.0 ml of synthetic quinoline in a mixture of 35 ml of nitric acid and 100 ml of water (*Solution D*). Gradually add *Solution D* to *Solution C*, mix well, and allow to stand overnight. Filter the mixture, add 280 ml of acetone to the filtrate, dilute to 1000 ml with water, and mix. Store in a polyethylene bottle.

> *Caution:* This reagent contains acetone. Do not use it near an open flame. Operations involving heating or boiling should be conducted in a well-ventilated hood.

Quinaldine Red TS Dissolve 100 mg of quinaldine red in 100 ml of glacial acetic acid.

Schiff's Reagent, Modified Dissolve 200 mg of rosaniline hydrochloride, $C_{20}H_{20}ClN_3$, in 120 ml of hot water. Cool, add 2 g of sodium bisulfite, $NaHSO_3$, followed by 2 ml of hydrochloric acid, and dilute to 200 ml with water. Store in a brown bottle at 15° or lower.

Silver Nitrate TS Use *0.1 N Silver Nitrate*, page 567.

Sodium Bisulfite TS Dissolve 10 g of sodium bisulfite, $NaHSO_3$, in water to make 30 ml. Prepare this solution fresh.

Sodium Bitartrate TS Dissolve 1 g of sodium bitartrate, $NaHC_4H_4O_6.H_2O$, in water to make 10 ml. Prepare this solution fresh.

Sodium Borate TS Dissolve 2 g of sodium borate, $Na_2B_4O_7.10H_2O$, in water to make 100 ml.

Sodium Carbonate TS Dissolve 10.6 g of anhydrous sodium carbonate, Na_2CO_3, in water to make 100 ml.

Sodium Cobaltinitrite TS Dissolve 10 g of sodium cobaltinitrite, $Na_3Co(NO_2)_6$, in water to make 50 ml, and filter if necessary.

Sodium Fluoride TS Dry about 500 mg of sodium fluoride, NaF, at 200° for 4 h. Weigh accurately 222 mg of the dried sodium fluoride, and dissolve it in sufficient water to make exactly 100 ml. Transfer 10.0 ml of this solution into a 1000-ml volumetric flask, dilute to volume with water, and mix. Each ml of this final solution corresponds to 10 µg of fluorine (F).

Sodium Hydroxide TS Dissolve 4.3 g of sodium hydroxide, NaOH, in water to make 100 ml.

Sodium Indigotindisulfonate TS See *Indigo Carmine TS*.

Sodium Nitroferricyanide TS Dissolve 1 g of sodium nitroferricyanide, $Na_2Fe(NO)(CN)_5.2H_2O$, in water to make 20 ml. Prepare this solution fresh.

Sodium Phosphate TS Dissolve 12 g of clear crystals of dibasic sodium phosphate, $Na_2HPO_4.7H_2O$, in water to make 100 ml.

Sodium Sulfide TS Dissolve 1 g of sodium sulfide, $Na_2S.9H_2O$, in water to make 10 ml. Prepare this solution fresh.

Sodium Thiosulfate TS Use *0.1 N Sodium Thiosulfate*, page 567.

Stannous Chloride TS Dissolve 40 g of reagent-grade stannous chloride dihydrate, $SnCl_2.2H_2O$, in 100 ml of hydrochloric acid.

Starch TS Mix 1 g of a suitable starch with 10 mg of red mercuric oxide and sufficient cold water to make a thin paste. Add 20 ml of boiling water, boil for 1 min with continuous stirring, and cool. Use only the clear solution.

Starch Iodide Paste TS Heat 100 mg of water in a 250-ml beaker to boiling, add a solution of 750 mg of potassium iodide, KI, in 5 ml of water, then add 2 g of zinc chloride, $ZnCl_2$, dissolved in 10 ml of water, and, while the solution is boiling, add with stirring a smooth suspension of 5 g of potato starch in 30 ml of cold water. Continue to boil for 2 min, then cool. Store in well-closed containers in a cool place. This mixture must show a definite blue streak when a glass rod dipped in a mixture of 1 ml of 0.1 *M* sodium nitrite, 500 ml of water, and 10 ml of hydrochloric acid is streaked on a smear of the paste.

Sulfanilic Acid TS Dissolve 800 mg of sulfanilic acid, *p*-$NH_2C_6H_4SO_3H.H_2O$, in 100 ml of acetic acid. Store in tight containers.

Sulfuric Acid Use ACS reagent-grade *Sulfuric Acid* (95.0% to 98.0% of H_2SO_4).

Sulfuric Acid TS See page 532.

Sulfuric Acid TS, Diluted A solution containing 10% (w/v) of H_2SO_4. Prepare by cautiously adding 57 ml of sulfuric acid (95% to 98%) or sulfuric acid TS to about 100 ml of water, then cool to room temperature, and dilute with water to 1000 ml.

Tannic Acid TS Dissolve 1 g of tannic acid (tannin) in 1 ml of alcohol, and add water to make 10 ml. Prepare this solution fresh.

Thymol Blue TS Dissolve 100 mg of thymol blue in 100 ml of alcohol, and filter if necessary.

Thymolphthalein TS Dissolve 100 mg of thymolphthalein in 100 ml of alcohol, and filter if necessary.

Triketohydrindene Hydrate TS (*Ninhydrin TS*) Dissolve 200 mg of triketohydrindene hydrate, $C_9H_4O_3.H_2O$, in water to make 100 ml. Prepare this solution fresh.

Trinitrophenol TS (*Picric Acid TS*) Dissolve the equivalent of 1 g of anhydrous trinitrophenol in 100 ml of hot water. Cool the solution, and filter if necessary.

Xylenol Orange TS Dissolve 100 mg of xylenol orange in 100 ml of alcohol.

Volumetric Solutions

Normal Solutions A normal solution contains 1 g equivalent weight of the solute per liter of solution. The normalities of solutions used in volumetric determinations are designated as 1 *N*, 0.1 *N*, 0.05 *N*, etc., in this Codex.

Molar Solutions A molar solution contains 1 g molecular weight of the solute per liter of solution. The molarities of such solutions are designated as 1 *M*, 0.1 *M* , 0.05 *M*, etc., in this Codex.

Preparation and Methods of Standardization

The details for the preparation and standardization of solutions used in several normalities are usually given only for the one most frequently required. Solutions of other normalities are prepared and standardized in the same general manner as described. Solutions of lower normalities may be prepared accurately by making an exact dilution of a stronger solution, but solutions prepared in this way should be restandardized before use.

Dilute solutions that are not stable, such as 0.01 *N* potassium permanganate and sodium thiosulfate, are preferably prepared by diluting exactly the higher normality with thoroughly boiled and cooled water on the same day they are to be used.

All volumetric solutions should be prepared, standardized, and used at the standard temperature of 25°, if practicable. When a titration must be carried out at a markedly different temperature, the volumetric solution should be standardized at that same temperature, or a suitable temperature correction should be made. Since the strength of a standard solution may change upon standing, the normality or molarity factor should be redetermined frequently.

Although the directions provide only one method of standardization, other methods of equal or greater accuracy may be used. For substances available as certified primary standards, or of comparable quality, the final standard solution may be prepared by weighing accurately a suitable quantity of the substance and dissolving it to produce a specific volume solution of known concentration. Hydrochloric and sulfuric acids may be standardized against a certified primary standard.

In volumetric assays described in this Codex, the number of mg of the test substance equivalent to 1 ml of the primary volumetric solution is given. In general, these equivalents may be derived by simple calculation (see also *Solutions*, page 3).

Ammonium Thiocyanate, 0.1 N (7.612 g NH_4SCN per 1000 ml) Dissolve about 8 g of ammonium thiocyanate, NH_4SCN, in 1000 ml of water, and standardize by titrating the solution against 0.1 *N* silver nitrate as follows: Transfer about 30 ml of 0.1 *N* silver nitrate, accurately measured, into a glass-stoppered flask. Dilute with 50 ml of water, then add 2 ml of ferric ammonium sulfate TS and 2 ml of nitric acid, and titrate with the ammonium thiocyanate solution to the first appearance of a red brown color. Calculate the normality, and, if desired, adjust the solution to exactly 0.1 *N*. If desired, 0.1 *N* ammonium thiocyanate may be replaced by 0.1 *N* potassium thiocyanate where the former is directed in various tests and assays.

Bromine, 0.1 N (7.990 g Br per 1000 ml) Dissolve 3 g of potassium bromate, $KBrO_3$, and 15 g of potassium bromide, KBr, in sufficient water to make 1000 ml, and standardize the solution as follows: Transfer about 25 ml of the solution, accurately measured, into a 500-ml iodine flask, and dilute with 120 ml of water. Add 5 ml of hydrochloric acid, stopper the flask, and shake it gently. Then add 5 ml of potassium iodide TS, restopper, shake the mixture, allow it to stand for 5 min, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS near the end of the titration. Calculate the normality. Store this solution in dark amber–colored, glass-stoppered bottles.

Ceric Sulfate, 0.1 N [33.22 g $Ce(SO_4)_2$ per 1000 ml] Transfer 59 g of ceric ammonium nitrate, $Ce(NO_3)_4.2NH_4NO_3.2H_2O$, to a beaker, add 31 ml of sulfuric acid, mix, and cautiously add water, in 20-ml portions, until solution is complete. Cover the

beaker, let stand overnight, filter through a sintered-glass crucible of fine porosity, add water to make 1000 ml, and mix. Standardize the solution as follows: Weigh accurately 200 mg of primary standard arsenic trioxide, As_2O_3, previously dried at 100° for 1 h, and transfer to a 500-ml Erlenmeyer flask. Wash down the inner walls of the flask with 25 ml of sodium hydroxide solution (2 in 25), swirl to dissolve the sample, and when solution is complete add 100 ml of water, and mix. Add 10 ml of dilute sulfuric acid (1 in 3) and 2 drops each of orthophenanthroline TS and a solution of osmium tetroxide in 0.1 *N* sulfuric acid (1 in 400), and slowly titrate with the ceric sulfate solution until the pink color is changed to a very pale blue. Calculate the normality. Each 4.946 mg of As_2O_3 is equivalent to 1 ml of 0.1 *N* ceric sulfate.

Ceric Sulfate, 0.01 N [3.322 g $Ce(SO_4)_2$ per 1000 ml] Dissolve 4.2 g of ceric sulfate, $Ce(SO_4)_2.4H_2O$, or 5.5 g of the acid sulfate, $Ce(HSO_4)_4$, in about 500 ml of water containing 28 ml of sulfuric acid, and dilute to 1000 ml. Allow the solution to stand overnight, and filter. Standardize this solution daily as follows: Weigh accurately about 275 mg of hydroquinone, $C_6H_6O_2$, dissolve it in sufficient 0.5 *N* alcoholic sulfuric acid to make 500.0 ml, and mix. To 25.0 ml of this solution add 75 ml of 0.5 *N* sulfuric acid, 20 ml of water, and 2 drops of diphenylamine TS. Titrate with the ceric sulfate solution at a rate of about 25 drops per 10 s until an endpoint is reached that persists for 10 s. Perform a blank determination using 100 ml of 0.5 *N* alcoholic sulfuric acid, 20 ml of water, and 2 drops of diphenylamine TS, and make any necessary correction. Calculate the normality of the ceric sulfate solution by the formula $0.05W/55.057V$, in which W is the weight, in mg, of the hydroquinone sample taken, and V is the volume, in ml, of the ceric sulfate solution consumed in the titration.

Disodium EDTA, 0.05 M (16.81 g $C_{10}H_{14}N_2Na_2O_8$ per 1000 ml) Dissolve 18.6 g of disodium ethylenediaminetetraacetate, $C_{10}H_{14}N_2Na_2O_8.2H_2O$, in sufficient water to make 1000 ml, and standardize the solution as follows: Weigh accurately about 200 mg of chelometric standard calcium carbonate, $CaCO_3$, transfer to a 400-ml beaker, add 10 ml of water, and swirl to form a slurry. Cover the beaker with a watch glass, and introduce 2 ml of diluted hydrochloric acid TS from a pipet inserted between the lip of the beaker and the edge of the watch glass. Swirl the contents of the beaker to dissolve the calcium carbonate. Wash down the sides of the beaker, the outer surface of the pipet, and the watch glass, and dilute to about 100 ml with water. While stirring, preferably with a magnetic stirrer, add about 30 ml of the disodium EDTA solution from a 50-ml buret, then add 15 ml of sodium hydroxide TS and 300 mg of hydroxy naphthol blue indicator, and continue the titration to a blue endpoint. Calculate the molarity by the formula $W/100.09V$, in which W is the weight, in mg, of $CaCO_3$ in the sample of calcium carbonate taken, and V is the volume, in ml, of disodium EDTA solution consumed. Each 5.004 mg of $CaCO_3$ is equivalent to 1 ml of 0.05 *M* disodium EDTA.

For the determination of aluminum in its salts, use 0.05 *M* disodium EDTA standardized as follows: Transfer 2 g, accurately weighed, of aluminum wire to a 1000-ml volumetric flask, and add 50 ml of a 1:1 hydrochloric acid–water mixture. Swirl the flask to ensure complete wetting of the wire, and allow the reaction to proceed. When dissolution is complete, dilute with water to volume, and mix. Transfer 10.0 ml of this solution to a 250-ml beaker, add 25.0 ml of the disodium EDTA solution, boil gently for 5 min, and cool. Add in the order given, and with continuous stirring, 20 ml of pH 4.5 buffer solution (77.1 g of ammonium acetate and 57 ml of glacial acetic acid in 1000 ml of solution), 50 ml of alcohol, and 2 ml of dithizone TS. Titrate with 0.05 *M* zinc sulfate to a bright rose pink color, and perform a blank determination, substituting 10 ml of water for the 10.0 ml of aluminum solution. Each ml of disodium EDTA solution is equivalent to 1.349 mg of aluminum (Al).

Ferrous Ammonium Sulfate, 0.1 N [39.21 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ per 1000 ml] Dissolve 40 g of ferrous ammonium sulfate hexahydrate in a previously cooled mixture of 40 ml of sulfuric acid and 200 ml of water, dilute to 1000 ml with water, and mix. On the day of use, standardize the solution as follows: Transfer from 25 to 30 ml of the solution, accurately measured, into a flask, add 2 drops of orthophenanthroline TS, and titrate with 0.1 *N* ceric sulfate until the red color is changed to pale blue. From the volume of 0.1 *N* ceric sulfate consumed, calculate the normality.

Hydrochloric Acid, 1 N (36.46 g HCl per 1000 ml) Dilute 85 ml of hydrochloric acid with water to make 1000 ml, and standardize the solution as follows: Accurately weigh about 1.5 g of primary standard anhydrous sodium carbonate, Na_2CO_3, that has been heated at a temperature of about 270° for 1 h. Dissolve it in 100 ml of water, and add 2 drops of methyl red TS. Add the acid slowly from a buret, with constant stirring, until the solution becomes faintly pink. Heat the solution to boiling, and continue the titration until the faint pink color is no longer affected by continued boiling. Calculate the normality. Each 52.99 mg of Na_2CO_3 is equivalent to 1 ml of 1 *N* hydrochloric acid.

Hydroxylamine Hydrochloride, 0.5 N (35 g $NH_2OH.HCl$ per 1000 ml) Dissolve 35 g of hydroxylamine hydrochloride in 150 ml of water, and dilute to 1000 ml with anhydrous methanol. To 500 ml of this solution add 15 ml of a 0.04% solution of bromophenol blue in alcohol, and titrate with 0.5 *N* triethanolamine until the solution appears greenish blue by transmitted light. *Prepare this solution fresh before each series of analyses.*

Iodine, 0.1 N (12.69 g I per 1000 ml) Dissolve about 14 g of iodine, I, in a solution of 36 g of potassium iodide, KI, in 100 ml of water, add 3 drops of hydrochloric acid, dilute with water to 1000 ml, and standardize as follows: Weigh accurately about 150 mg of primary standard arsenic trioxide, As_2O_3, previously dried at 105° for 1 h, and dissolve it in 20 ml of 1 *N* sodium hydroxide by warming if necessary. Dilute with 40 ml of water, add 2 drops of methyl orange TS, and follow with diluted hydrochloric acid TS until the yellow color is changed to pink. Then add 2 g of sodium bicarbonate, $NaHCO_3$, dilute with 50 ml of water, add 3 ml of starch TS, and slowly add the iodine solution from a buret until a permanent blue color is produced. Calculate the normality. Each 4.946 mg of As_2O_3 is equivalent

to 1 ml of 0.1 *N* iodine. Store this solution in glass-stoppered bottles.

Lithium Methoxide, 0.1 N (3.797 g CH_3OLi per 1000 ml) Dissolve 600 mg of freshly cut lithium metal in a mixture of 150 ml of anhydrous methanol and 850 ml of benzene. Filter the resulting solution if it is cloudy, and standardize it as follows: Dissolve about 80 mg of benzoic acid (National Bureau of Standards primary standard), accurately weighed, in 35 ml of dimethylformamide, add 5 drops of thymol blue TS, and titrate with the lithium methoxide solution to a dark blue endpoint.

> *Caution:* Protect the solution from absorption of carbon dioxide and moisture by covering the titration vessel with aluminum foil while dissolving the benzoic acid sample and during the titration.

Each ml of 0.1 *N* lithium methoxide is equivalent to 12.21 mg of benzoic acid.

Mercuric Nitrate, 0.1 M [32.46 g $Hg(NO_3)_2$ per 1000 ml] Dissolve about 35 g of mercuric nitrate, $Hg(NO_3)_2{\cdot}H_2O$, in a mixture of 5 ml of nitric acid and 500 ml of water, and dilute with water to 1000 ml. Standardize the solution as follows: Transfer an accurately measured volume of about 20 ml of the solution into an Erlenmeyer flask, and add 2 ml of nitric acid and 2 ml of ferric ammonium sulfate TS. Cool to below 20°, and titrate with 0.1 *N* ammonium thiocyanate to the first appearance of a permanent brownish color. Calculate the molarity.

Oxalic Acid, 0.1 N (4.502 g $H_2C_2O_4$ per 1000 ml) Dissolve 6.45 g of oxalic acid, $H_2C_2O_4{\cdot}2H_2O$, in sufficient water to make 1000 ml. Standardize by titration against freshly standardized 0.1 *N* potassium permanganate as directed under *Potassium Permanganate, 0.1 N*. Store this solution in glass-stoppered bottles, protected from light.

Perchloric Acid, 0.1 N (10.046 g $HClO_4$ per 1000 ml) Mix 8.5 ml of perchloric acid (70%) with 500 ml of glacial acetic acid and 30 ml of acetic anhydride. Cool, and add glacial acetic acid to make 1000 ml. Allow the prepared solution to stand for 1 day for the excess acetic anhydride to be combined, and determine the water content by the *Karl Fischer Titrimetric Method*, page 552. If the water content exceeds 0.05%, add more acetic anhydride, but if the solution contains no titratable water, add sufficient water to make the content between 0.02% and 0.05% of water. Allow to stand for 1 day, and again determine the water content by titration. Standardize the solution as follows: Weigh accurately about 700 mg of primary standard potassium biphthalate, $KHC_6H_4(COO)_2$, previously dried at 105° for 2 h, and dissolve it in 50 ml of glacial acetic acid in a 250-ml flask. Add 2 drops of crystal violet TS, and titrate with the perchloric acid solution until the violet color changes to emerald green. Deduct the volume of the perchloric acid consumed by 50 ml of the glacial acetic acid, and calculate the normality. Each 20.42 mg of $KHC_6H_4(COO)_2$ is equivalent to 1 ml of 0.1 *N* perchloric acid.

Perchloric Acid, 0.1 N, in Dioxane Mix 8.5 ml of perchloric acid (70%) with sufficient dioxane, which has been especially purified by adsorption, to make 1000 ml. Standardize the solution as follows: Weigh accurately about 700 mg of primary standard potassium biphthalate, $KHC_6H_4(COO)_2$, previously dried at 105° for 2 h, and dissolve in 50 ml of glacial acetic acid in a 250-ml flask. Add 2 drops of crystal violet TS, and titrate with the perchloric acid solution until the violet color changes to bluish green. Deduct the volume of the perchloric acid consumed by 50 ml of the glacial acetic acid, and calculate the normality. Each 20.42 mg of $KHC_6H_4(COO)_2$ is equivalent to 1 ml of 0.1 *N* perchloric acid.

Potassium Acid Phthalate, 0.1 N [20.42 g $KHC_6H_4(COO)_2$ per 1000 ml] Dissolve 20.42 g of primary standard potassium biphthalate, $KHC_6H_4(COO)_2$, previously dried at 105° for 2 h, in glacial acetic acid in a 1000-ml volumetric flask, warming on a steam bath if necessary to effect solution and protecting the solution from contamination by moisture. Cool to room temperature, dilute to volume with glacial acetic acid, and mix.

Potassium Dichromate, 0.1 N (4.903 g $K_2Cr_2O_7$ per 1000 ml) Dissolve about 5 g of potassium dichromate, $K_2Cr_2O_7$, in 1000 ml of water, transfer quantitatively 25 ml of this solution to a 500-ml glass-stoppered flask, add 2 g of potassium iodide (free from iodate), KI, dilute with 200 ml of water, add 5 ml of hydrochloric acid, and mix. Allow to stand for 10 min in a dark place, and titrate the liberated iodine with 0.1 *N* sodium thiosulfate, adding starch TS as the endpoint is approached. Correct for a blank run on the same quantities of the same reagents, and calculate the normality.

Potassium Hydroxide, 1 N (56.11 g KOH per 1000 ml) Prepare and standardize 1 *N* potassium hydroxide by the procedure set forth for *Sodium Hydroxide, 1 N*, using 74 g of the potassium hydroxide, KOH, to prepare the solution. Each 204.2 mg of $KHC_6H_4(COO)_2$ is equivalent to 1 ml of 1 *N* potassium hydroxide.

Potassium Hydroxide, 0.5 N, Alcoholic Dissolve about 35 g of potassium hydroxide, KOH, in 20 ml of water, and add sufficient aldehyde-free alcohol to make 1000 ml. Allow the solution to stand in a tightly stoppered bottle for 24 h. Then quickly decant the clear supernatant liquid into a suitable, tight container, and standardize as follows: Transfer quantitatively 25 ml of 0.5 *N* hydrochloric acid into a flask, dilute with 50 ml of water, add 2 drops of phenolphthalein TS, and titrate with the alcoholic potassium hydroxide solution until a permanent, pale pink color is produced. Calculate the normality. Store this solution in tightly stoppered bottles protected from light.

Potassium Iodate, 0.05 M (10.70 g KIO_3 per 1000 ml) Dissolve 10.700 g of potassium iodate of primary standard quality, KIO_3, previously dried at 110° to constant weight, in sufficient water to make 1000.0 ml.

Potassium Permanganate, 0.1 N (3.161 g $KMNO_4$ per 1000 ml) Dissolve about 3.3 g of potassium permanganate,

$KMnO_4$, in 1000 ml of water in a flask, and boil the solution for about 15 min. Stopper the flask, allow it to stand for at least 2 days, and filter through a fine-porosity sintered-glass crucible. If necessary, the bottom of the crucible may be lined with a pledget of glass wool. Standardize the solution as follows: Weigh accurately about 200 mg of sodium oxalate of primary standard quality, $Na_2C_2O_4$, previously dried at 100° to constant weight, and dissolve it in 250 ml of water. Add 7 ml of sulfuric acid, heat to about 70°, and then slowly add the permanganate solution from a buret, with constant stirring, until a pale pink color that persists for 15 s is produced. The temperature at the conclusion of the titration should be not less than 60°. Calculate the normality. Each 6.700 mg of $Na_2C_2O_4$ is equivalent to 1 ml of 0.1 *N* potassium permanganate. Potassium permanganate is reduced on contact with organic substances such as rubber; therefore, the solution must be handled in apparatus entirely of glass or other suitably inert material. Store it in glass-stoppered, amber-colored bottles, and restandardize frequently.

Silver Nitrate, 0.1 N (16.99 g $AgNO_3$ per 1000 ml) Dissolve about 17.5 g of silver nitrate, $AgNO_3$, in 1000 ml of water, and standardize the solution as follows: Weigh accurately 100 mg of primary standard sodium chloride, previously dried at 120° for 16 h, into a 150-ml beaker, and dissolve it in 5 ml of water. Add 5 ml of acetic acid, 50 ml of methanol, and 2 or 3 drops of eosin Y TS, and titrate with the silver nitrate solution to the endpoint. Calculate the normality.

Sodium Arsenite, 0.05 N (3.248 g $NaAsO_2$ per 1000 ml) Transfer 2.4725 g of arsenic trioxide, which has been pulverized and dried at 100° to constant weight, to a 1000-ml volumetric flask, dissolve it in 20 ml of 1 *N* sodium hydroxide, and add 1 *N* sulfuric acid or 1 *N* hydrochloric acid until the solution is neutral or only slightly acid to litmus. Add 15 g of sodium bicarbonate, dilute to volume with water, and mix.

Sodium Hydroxide, 1 N (40.00 g NaOH per 1000 ml) Dissolve about 45 g of sodium hydroxide, NaOH, in about 950 ml of water, and add a freshly prepared saturated solution of barium hydroxide until no more precipitate forms. Shake the mixture thoroughly, and allow it to stand overnight in a stoppered bottle. Decant or filter the solution, and standardize the clear liquid as follows: Transfer about 5 g of primary standard potassium biphthalate, $KHC_8H_4(COO)_2$, previously dried at 105° for 2 h and accurately weighed, to a flask, and dissolve it in 75 ml of carbon dioxide–free water. If the potassium biphthalate is in the form of large crystals, it should be crushed before drying. To the flask add 2 drops of phenolphthalein TS, and titrate with the sodium hydroxide solution to a permanent pink color. Calculate the normality. Each 204.2 mg of potassium biphthalate is equivalent to 1 ml of 1 *N* sodium hydroxide.

NOTE: Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. They should therefore be stored in bottles with well-fitted, suitable stoppers, provided with a tube filled with a mixture of sodium hydroxide and lime so that air entering the container must pass through this tube, which will absorb the carbon dioxide. Standard solutions of sodium hydroxide should be restandardized frequently.

Sodium Methoxide, 0.1 N, in Pyridine (5.40 g CH_3ONa per 1000 ml) Weigh 14 g of freshly cut sodium metal, and cut into small cubes. Place about 0.5 ml of anhydrous methanol in a round-bottom 120-ml flask equipped with a ground-glass joint, add 1 cube of the sodium metal, and, when the reaction subsides, add the remaining sodium metal to the flask. Connect a water-cooled condenser to the flask, and slowly add 100 ml of anhydrous methanol, in small portions, through the top of the condenser. Regulate the addition of the methanol so that the vapors are condensed and do not escape through the top of the condenser. After addition of the methanol is complete, connect a drying tube to the top of the condenser, and allow the solution to cool. Transfer 17.5 ml of this solution (approximately 6 *N*) into a 1000-ml volumetric flask containing 70 ml of anhydrous methanol, and dilute to volume with freshly distilled pyridine. Store preferably in the reservoir of an automatic buret suitably protected from carbon dioxide and moisture. Standardize the solution as follows: Weigh accurately about 400 mg of primary standard benzoic acid, transfer it into a 250-ml wide-mouth Erlenmeyer flask, and dissolve it in 50 ml of freshly distilled pyridine. Add a few drops of thymolphthalein TS, and titrate immediately with the sodium methoxide solution to a blue endpoint. During the titration, direct a gentle stream of nitrogen into the flask through a short piece of 6-mm glass tubing fastened near the tip of the buret. Perform a blank determination (see page 2), correct for the volume of sodium methoxide solution consumed by the blank, and calculate the normality. Each 12.21 mg of benzoic acid is equivalent to 1 ml of 0.1 *N* sodium methoxide in pyridine.

Sodium Methoxide, 0.02 N, in Toluene (1.08 g CH_3ONa per 1000 ml) Weigh 2.5 g of freshly cut sodium metal, and cut into small cubes. Place about 200 ml of anhydrous methanol in a 1000-ml volumetric flask, chill in an ice bath, and add the cubes one at a time to the methanol. When the last cube is dissolved, dilute to the mark with toluene, and mix. Standardize the solution as follows: Weigh accurately about 20 mg of primary standard benzoic acid, transfer it into a 50-ml conical flask, and dissolve it in 25 ml of dimethylformamide. Add 2 drops of a solution of 100 mg of thymol blue in 10 ml of dimethylformamide, and titrate immediately with the sodium methoxide solution to a blue endpoint. Titrate a blank solution of dimethylformamide in the same manner, correct the volume of sodium methoxide solution consumed by the blank, and calculate the normality. Each 2.442 mg of benzoic acid is equivalent to 1 ml of 0.02 *N* sodium methoxide in toluene.

Sodium Thiosulfate, 0.1 N (15.81 g $Na_2S_2O_3$ per 1000 ml) Dissolve about 26 g of sodium thiosulfate, $Na_2S_2O_3.5H_2O$, and 200 mg of sodium carbonate, Na_2CO_3, in 1000 ml of recently boiled and cooled water. Standardize the solution as follows: Weigh accurately about 210 mg of primary standard potassium dichromate, previously pulverized and dried at 120° for 4 h, and dissolve in 100 ml of water in a 500-ml glass-stoppered flask. Swirl to dissolve the sample, remove the

stopper, and quickly add 3 g of potassium iodide, KI, and 5 ml of hydrochloric acid. Stopper the flask, swirl to mix, and let stand in the dark for 10 min. Rinse the stopper and inner walls of the flask with water, and titrate the liberated iodine with the sodium thiosulfate solution until the solution is only faint yellow in color. Add starch TS, and continue the titration to the discharge of the blue color. Calculate the normality.

Sulfuric Acid, 1 N (49.04 g H_2SO_4 per 1000 ml) Add slowly, with stirring, 30 ml of sulfuric acid to about 1020 ml of water, allow to cool to 25°, and standardize by titration against primary standard sodium carbonate, Na_2CO_3, as directed under *Hydrochloric Acid, 1 N*. Each 52.99 mg of Na_2CO_3 is equivalent to 1 ml of 1 *N* sulfuric acid.

Sulfuric Acid, Alcoholic, 5 N (245.2 g H_2SO_4 per 1000 ml) Add cautiously, with stirring, 139 ml of sulfuric acid to a sufficient quantity of absolute alcohol to make 1000.0 ml.

Sulfuric Acid, Alcoholic, 0.5 N Add cautiously, with stirring, 13.9 ml of sulfuric acid to a sufficient quantity of absolute alcohol to make 1000.0 ml. Alternatively, this solution may be prepared by diluting 100.0 ml of 5 *N* sulfuric acid with absolute alcohol to make 1000.0 ml.

Thorium Nitrate, 0.1 M [48.01 g $Th(NO_3)_4$ per 1000 ml] Weigh accurately 55.21 g of thorium nitrate, $Th(NO_3)_4{\cdot}4H_2O$, dissolve it in water, dilute to 1000.0 ml, and mix. Standardize the solution as follows: Transfer 50.0 ml into a 500-ml volumetric flask, dilute to volume with water, and mix. Transfer 50.0 ml of the diluted solution into a 400-ml beaker, add 150 ml of water and 5 ml of hydrochloric acid, and heat to boiling. While stirring, add 25 ml of a saturated solution of oxalic acid, then digest the mixture for 1 h just below the boiling point, and allow to stand overnight. Decant through Whatman No. 42, or equivalent, filter paper, and transfer the precipitate to the filter using about 100 ml of a wash solution consisting of 70 ml of the saturated oxalic acid solution, 430 ml of water, and 5 ml of hydrochloric acid. Transfer the precipitate and filter paper to a tared tall-form porcelain crucible, dry, char the paper, and ignite at 950° for 1.5 h or to constant weight. Cool in a desiccator, weigh, and calculate the molarity of the solution by the formula $200W/264.04$, in which W is the weight, in g, of thorium oxide obtained.

Triethanolamine, 0.5 N [74 g $N(CH_2CH_2OH)_3$ per 1000 ml] Transfer 65 ml (74 g) of 98% triethanolamine into a 1000-ml volumetric flask, dilute to volume with water, stopper the flask, and mix thoroughly.

Zinc Sulfate, 0.05 M (8.072 g $ZnSO_4$ per 1000 ml) Dissolve about 15 g of zinc sulfate, $ZnSO_4{\cdot}7H_2O$, in sufficient water to make 1000 ml, and standardize the solution as follows: Dilute about 35 ml, accurately measured, with 75 ml of water, add 5 ml of ammonia-ammonium chloride buffer TS and 0.1 ml of eriochrome black TS, and titrate with 0.05 *M* disodium EDTA until the solution is deep blue in color. Calculate the molarity.

Indicators

The necessary solutions of indicators may be prepared as directed under *Test Solutions (TS) and Other Reagents*, page 558. The sodium salts of many indicators are commercially available and may be used interchangeably in water solutions with the alcohol solutions specified for the free indicators.

Useful pH indicators, listed in ascending order of the lower limit of their range, are: methyl yellow (pH 2.9–4.0), bromophenol blue (pH 3.0–4.6), bromocresol green (pH 4.0–5.4), methyl red (pH 4.2–6.2), bromocresol purple (pH 5.2–6.8), bromothymol blue (pH 6.0–7.6), phenol red (pH 6.8–8.2), thymol blue (pH 8.0–9.2), and thymolphthalein (pH 9.3–10.5).

Alphazurine 2G Use a suitable grade.

Azo Violet [*4-(p-Nitrophenylazo) Resorcinol*] A red powder, melting at about 193° with decomposition.

Bromocresol Blue Use *Bromocresol Green*.

Bromocresol Green (*Bromocresol Blue; Tetrabromo-m-cresolsulfonphthalein*) A white or pale buff–colored powder; slightly soluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 3.8 (yellow) to 5.4 (blue).

Bromocresol Purple (*Dibromo-o-cresolsulfonphthalein*) A white to pink, crystalline powder; insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 5.2 (yellow) to 6.8 (purple).

Bromophenol Blue (*Tetrabromophenolsulfonphthalein*) Pinkish crystals, soluble in alcohol. Insoluble in water; soluble in solutions of alkali hydroxides. Transition interval: from pH 3.0 (yellow) to 4.6 (blue).

Bromothymol Blue (*Dibromothymolsulfonphthalein*) A rose red powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 6.0 (yellow) to 7.6 (blue).

Cresol Red (*o-Cresolsulfonphthalein*) A red brown powder. Slightly soluble in water; soluble in alcohol and in dilute solutions of alkali hydroxides. Transition interval: from pH 7.2 (yellow) to 8.8 (blue).

Crystal Violet (*Hexamethyl-p-rosaniline Chloride*) Dark green crystals. Slightly soluble in water; sparingly soluble in alcohol and in glacial acetic acid. Its solutions are deep violet in color.

Sensitiveness Dissolve 100 mg in 100 ml of glacial acetic acid, and mix. Pipet 1 ml of the solution into a 100-ml volumetric flask, and dilute with glacial acetic acid to volume. The solution is violet blue in color and does not show a reddish tint. Pipet 20 ml of the diluted solution into a beaker, and titrate with 0.1 *N* perchloric acid, adding the perchloric acid slowly

from a microburet. Not more than 0.1 ml of 0.1 *N* perchloric acid is required to produce an emerald green color.

Dithizone (*Diphenylthiocarbazone*) A bluish black powder. Insoluble in water; soluble in alcohol, in chloroform, and in carbon tetrachloride, yielding intensely green solutions even in high dilutions.

Eriochrome Black T [*Sodium 1-(1-Hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonate*] A brownish black powder having a faint metallic sheen. Soluble in alcohol, in methanol, and in hot water.

Sensitiveness To 10 ml of a 1 in 200,000 solution in a mixture of equal parts of methanol and water add sodium hydroxide solution (1 in 100) until the pH is 10. The solution is pure blue in color and free from cloudiness. Add 0.2 ml of *Magnesium Standard Solution* (10 µg Mg ion). The color of the solution changes to red violet, and with the continued addition of magnesium ion it becomes wine red in color.

***p*-Ethoxychrysoidin Monohydrochloride** [*4-(p-Ethoxyphenylazo)-m-phenylenediamine Monohydrochloride; 4'-Ethoxy-2,4-diaminoazobenzene Monohydrochloride*] A reddish powder, insoluble in water. Transition interval: from pH 3.5 (red) to 5.5 (yellow).

Hydroxy Naphthol Blue The disodium salt of 1-(2-naphtholazo-3,6-disulfonic acid)-2-naphthol-4-sulfonic acid deposited on crystals of sodium chloride. Small blue crystals, freely soluble in water. In the pH range between 12 and 13 its solution is reddish pink in the presence of calcium ion and deep blue in the presence of excess disodium EDTA.

Suitability for Calcium Determinations Dissolve 300 mg in 100 ml of water, add 10 ml of sodium hydroxide TS and 1.0 ml of calcium chloride solution (1 in 200), and dilute with water to 165 ml. The solution is reddish pink in color. Add 1.0 ml of 0.05 *M* disodium EDTA. The solution becomes deep blue in color.

Litmus A blue powder, cubes, or pieces. Partly soluble in water and in alcohol. Transition interval: from approximately pH 4.5 (red) to 8 (blue). Litmus is unsuitable for determining the pH of solutions of carbonates or bicarbonates.

Methylene Blue [*3,7-Bis(dimethylamino)phenazathionium Chloride*] Dark green crystals or a crystalline powder having a bronzelike luster. Soluble in water and in chloroform; sparingly soluble in alcohol.

Methyl Orange (*Helianthin; Tropaeolin D; 4'-Dimethylaminoazobenzene-4-sodium Sulfonate*) An orange yellow powder or crystalline scales. Slightly soluble in cold water; readily soluble in hot water; insoluble in alcohol. Transition interval: from pH 3.2 (pink) to 4.4 (yellow).

Methyl Red (*o-Carboxybenzeneazodimethylaniline Hydrochloride*) A dark red powder or violet crystals. Sparingly soluble in water; soluble in alcohol. Transition interval: from pH 4.2 (red) to 6.2 (yellow).

Methyl Red Sodium The sodium salt of *o*-carboxybenzeneazodimethylaniline. An orange brown powder. Freely soluble in cold water and in alcohol. Transition interval: from pH 4.2 (red) to 6.2 (yellow).

Methyl Yellow (*p-Dimethylaminoazobenzene*) Yellow crystals, melting between 114° and 117°. Insoluble in water; soluble in alcohol, in benzene, in chloroform, in ether, in dilute mineral acids, and in oils. Transition interval: from pH 2.9 (red) to 4.0 (yellow).

Murexide Indicator Preparation Add 400 mg of murexide to 40 g of powdered potassium sulfate, K_2SO_4, and grind in a glass mortar to a homogeneous mixture. Alternatively, tablets containing 0.4 mg of murexide admixed with potassium sulfate or potassium chloride, available commercially, may be used.

Naphthol Green B The ferric salt of 6-sodium sulfo-1-isonitroso-1,2-naphthoquinone. A dark green powder, insoluble in water.

Neutral Red (*3-Amino-7-dimethylamino-2-methylphenazine Chloride*) A coarse, reddish to olive green powder. Sparingly soluble in water and in alcohol. Transition interval: from pH 6.8 (red) to 8.0 (orange).

Phenol Red (*Phenolsulfonphthalein*) A bright to dark red crystalline powder, very slightly soluble in water; sparingly soluble in alcohol; soluble in solutions of alkali hydroxides. Transition interval: from pH 6.8 (yellow) to 8.2 (red).

Phenolphthalein White or yellowish white crystals; practically insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 8.0 (colorless) to 10.0 (red).

Quinaldine Red (*5-Dimethylamino-2-strylethylquinolinium Iodide*) A dark, blue black powder, melting at about 260° with decomposition. Sparingly soluble in water; freely soluble in alcohol. Transition interval: from pH 1.4 (colorless) to 3.2 (red).

Thymol Blue (*Thymolsulfonphthalein*) A dark, brownish green, crystalline powder. Slightly soluble in water; soluble in alcohol and in dilute alkali solutions. Acid transition interval: from pH 1.2 (red) to 2.8 (yellow). Alkaline transition interval: from pH 8.0 (yellow) to 9.2 (blue).

Thymolphthalein A white to slightly yellow, crystalline powder. Insoluble in water; soluble in alcohol and in solutions of alkali hydroxides. Transition interval: from pH 9.3 (colorless) to 10.5 (blue).

Xylenol Orange [*3,3'-Bis-di(carboxymethyl)aminomethyl-o-cresolsulfonphthalein*] An orange powder. Soluble in water and in alcohol. In acid solution it is colored lemon yellow and its metal complexes intensely red. It gives a distinct endpoint in the direct EDTA titration of metals such as bismuth, thorium, scandium, lead, zinc, lanthanum, cadmium, and mercury.

Indicator Papers and Test Papers

Indicator papers and test papers are strips of paper of suitable dimension and grade (usually Swedish O filter paper or other makes of like surface, quality, and ash) impregnated with a sufficiently stable indicator solution or reagent.

Treat strong, white filter paper with hydrochloric acid, and wash with water until the last washing shows no acid reaction to methyl red TS. Then treat with ammonia TS, wash again with water until the last washing is not alkaline toward phenolphthalein TS, and dry thoroughly. Saturate the dry paper with the appropriate indicator solution prepared as directed below, and dry carefully by suspending from glass rods or other inert material in still air free from acid, alkali, and other fumes. Cut the paper into strips of convenient size, and store in well-closed containers protected from light and moisture.

Indicator papers and test papers that are available commercially may be used, if desired.

Acetaldehyde Test Paper Use a solution prepared by mixing equal volumes of a 20% solution of morpholine and a 5% solution of sodium nitroferricyanide. Saturate the prepared filter paper in the mixture, and use the moistened paper without drying.

Cupric Sulfate Test Paper Use *Cupric Sulfate TS*.

Lead Acetate Test Paper Usually about 6 × 80 mm in size. Use *Lead Acetate TS*, and dry the paper at 100°, avoiding contact with metal.

Litmus Paper, Blue Usually about 6 × 50 mm in size. It meets the requirements of the following tests.

Phosphate Place 10 strips in 10 ml of water to which have been added 1 ml of nitric acid and 0.5 ml of ammonia TS. Allow to stand for 10 min, then decant the solution, warm, and add 5 ml of ammonium molybdate TS. Shake at about 40° for 5 min. No precipitate of phosphomolybdate is formed.

Residue on Ignition Ignite carefully 10 strips of the paper to constant weight. The weight of the residue corresponds to not more than 400 μg per strip of about 3 cm^2.

Rosins, Acids, Etc. Immerse a strip of the blue paper in a solution of 100 mg of silver nitrate, $AgNO_3$, in 50 ml of water. The color of the paper does not change in 30 s.

Sensitiveness Drop a 10- to 12-mm strip in 100 ml of 0.0005 *N* hydrochloric acid contained in a beaker, and stir continuously. The color of the paper is changed within 45 s.

Litmus Paper, Red Usually about 6 × 50 mm in size. Red litmus meets the requirements for *Phosphate*, *Residue on Ignition*, and *Rosin Acids, Etc.*, under *Litmus Paper, Blue*.

Sensitiveness Drop a 10- × 12-mm strip into 100 ml of 0.0005 *N* sodium hydroxide contained in a beaker, and stir continuously. The color of the paper changes within 30 s.

Phenolphthalein Paper Use a 1 in 1000 solution of phenolphthalein in dilute alcohol (1 in 2).

Starch Iodate Paper Use a mixture of equal volumes of *Starch TS* and potassium iodate solution (1 in 20).

Starch Iodide Paper Use a solution of 500 mg of potassium iodide, KI, in 100 ml of freshly prepared *Starch TS*.

8 / *General Information*

Operating Procedures of the *Food Chemicals Codex*

ORGANIZATION

The *Food Chemicals Codex* project is an activity of the Food and Nutrition Board, under the Division of Biological Sciences, Assembly of Life Sciences, National Academy of Sciences–National Research Council. The immediate responsibility for developing the *Food Chemicals Codex* lies with the Board's Committee on Codex Specifications. The Committee consists of 12 to 15 members who are appointed, upon recommendation of the Food and Nutrition Board, by the Chairman of the National Research Council. Members are paid no consulting fees or honoraria and are reimbursed only for expenses incurred in attending meetings and other activities of the Committee.

FUNCTIONS OF THE COMMITTEE ON CODEX SPECIFICATIONS

The principal functions of the Committee on Codex Specifications are as follows.

- To establish the general policies and guidelines by which FCC specifications are prepared.
- To evaluate comments submitted by interested parties on any aspect of the specifications and test procedures.
- To propose means by which the specifications may be kept current in reflecting food-grade quality based on safety and good manufacturing practice.
- To provide information, within each member's respective area of expertise, on issues dealing with specifications for particular substances or analytical test procedures.
- To seek the advice of such specialists as toxicologists and microbiologists when expert opinion is needed in making decisions regarding safety of specifications.
- To consider and act upon any other matter concerning the development and publication of specifications and test procedures, through the application of the principles of safety and good manufacturing practice, for food-grade ingredients.
- To approve the final manuscript before the publication of any edition of the *Food Chemicals Codex* or its supplements.

The business of the Committee is conducted through a central office at the National Academy of Sciences in Washington, D.C. The Academy's staff officer (project director) is responsible for coordinating all of the Committee's activities.

The Committee meets in regular session usually twice a year. Ad hoc meetings on short-term projects are held as needed and are conducted by one or more members of the Committee or by the project staff officer. Workshops and symposia are organized as appropriate to exchange information with interested parties on key issues, whether of broad or limited scope. (NOTE: Further information concerning the operation of committees of the National Research Council is contained in two pamphlets, single copies of which are available upon request to the Codex office: *Of Questions and Committees: How the National Research Council Does Its Work* and *General Information for Members of Committees of the National Research Council.*)

REQUIREMENTS FOR LISTING SUBSTANCES IN THE *FOOD CHEMICALS CODEX*

The Committee on Codex Specifications does not have a rigid screening procedure for the admission of substances to the *Food*

Chemicals Codex. The only requirements are that (a) the substance is permitted for use in foods or in food processing by the U.S. Food and Drug Administration (or, in certain cases, by other countries in which *Food Chemicals Codex* specifications are recognized); (b) the substance is in use and is commercially available; and (c) suitable specifications, complete with analytical test procedures, can be prepared.

CRITERIA FOR *FOOD CHEMICALS CODEX* GRADE

The specifications of the *Food Chemicals Codex* are based primarily on the criteria of safety and good manufacturing practice (GMP). An FCC-grade substance is one that is prepared by GMP (see page 573) and is of such purity as to ensure that harmful or objectionable contaminants are absent or are present at such levels as to be harmless to the consumer of the foods in which the substance is used. Thus, *Food Chemicals Codex* specifications define substances of a quality sufficiently high to ensure their safety under customary conditions of intentional use in foods or in food processing. The specifications generally represent acceptable levels of quality and purity of food-grade substances available in the United States and in other countries in which *Food Chemicals Codex* specifications are recognized.

Because the different types of ingredients used in foods are diverse and complex, few general criteria can be established that will apply to all substances for which *Food Chemicals Codex* specifications are prepared. The Committee has, however, set general limits for certain impurities (e.g., arsenic, heavy metals, lead, fluoride), as discussed under *Limits of Impurities* on page xx of the *Preface to the Third Edition*. At the same time, the Committee recognizes that limits and tests cannot be provided to cover all possible unusual or unexpected impurities, the presence of which would be inconsistent with good manufacturing practice. This matter is discussed further under *Trace Impurities*, on page 3 of the *General Provisions*, and in *General Good Manufacturing Practice Guidelines for Food Chemicals*, commencing on page 573 in this section.

In addition to impurity limits, proposed specifications should consist, at the minimum, of the following (where applicable): empirical formula, structural formula, and molecular weight; description of the substance, including physical form, odor, and solubility (see the descriptive terms for solubility on page 4 of the *General Provisions*); identification; assay (or a quantitative test to serve as an assay); such physicochemical characteristics as specific rotation, melting range or solidification point, viscosity, specific gravity, refractive index, pH, etc.; loss on drying or water content; limits for mycotoxins and microbiological contaminants; and limits for by-products and other adventitious constituents usually occurring in, or arising from the manufacture of, the substance. Furthermore, the data provided, taken together, should represent a complete compositional analysis of the substance. Information should also be provided on how the substance should be packaged and stored to maintain its integrity and on its functional use(s) in foods. If the ingredient contains an "added substance," mention should be made of this fact to enable the Committee to judge whether or not the specifications should provide for it (see *Added Substances* on page 5 of the *General Provisions*).

PROCEDURES FOR SUBMISSION AND DEVELOPMENT OF SPECIFICATIONS

The Committee will accept and consider proposed specifications submitted by any interested party, including food ingredient manufacturers and suppliers, food processors, and industry associations. Proposed specifications should be submitted, preferably in duplicate, to Food Chemicals Codex, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

Proposed specifications are examined critically and are often expanded to meet the general criteria required by the Committee. A draft monograph is then sent to the originator for comment (and to any other manufacturers that can be identified). After the draft has gone through this process and all necessary revisions have been made, it is sent to the Committee for review. If the Committee finds deficiencies, or if any questions are raised, the proposal is returned to the originator and other interested parties with the Committee's comments and recommendations for improvement. Eventually, the approved monograph is published in the next edition of the *Food Chemicals Codex* or in a supplement.

PROCEDURE FOR REVISING SPECIFICATIONS

The specifications of the *Food Chemicals Codex* are subject to revision at any time. Proposals for revision may be initiated by manufacturers, suppliers, or users of the ingredients; by the Committee itself; or by any other interested party. All proposals for revision should be accompanied by supporting data.

In the case of revisions of test procedures and analytical methods, comparative data from both the existing and proposed procedures should be submitted, including replicate results on a single sample as well as results on representative samples currently being produced.

Where changes in limits or other tolerances are proposed, data should be presented on samples from more than a single source, provided the ingredient may be obtained from more than one source. Proposals for raising the limits of certain impurities (e.g., arsenic, heavy metals, lead, fluoride, mercury) in the specifications for a particular additive may require, in addition, the submission of safety data and information concerning the daily intake of the impurity by people consuming foods containing the additive.

All proposals for revision, together with the supporting data, are reviewed by the Committee on Codex Specifications. If other manufacturers are involved (and have been identified), they are also asked to comment. If the Committee finds deficiencies, or if any questions arise, the proposal is returned to the originator (and other manufacturers, where appropriate) with the Committee's comments or questions. If agreement cannot be reached at this point between the Committee and the originator, or among manufacturers and other interested parties, a special meeting may be held to discuss the matter, or the parties involved may be invited to one of the Committee's regular meetings to examine the question in depth. Eventually, approved revisions are published in either the next edition of the *Food Chemicals Codex* or in a supplement.

PUBLIC COMMENT ON PROPOSED NEW OR REVISED SPECIFICATIONS

Although the Committee attempts to obtain comments on proposed new specifications and on revisions of existing specifications from all of the manufacturers of the substance in question, some manufacturers may be inadvertently overlooked. Furthermore, it is not possible for the Committee to identify and seek comments from all of the possible users of any particular additive. For these reasons, the Committee has attempted to maximize its contact with affected parties through notices in trade journals, in newsletters, and, with the assistance of the Food and Drug Administration, in the *Federal Register*.

FURTHER INFORMATION

Users of the *Food Chemicals Codex* should become thoroughly familiar with the *General Provisions* pertaining to this edition (see pages 1–5). Additional information concerning the operation of the project and the revision process will be found in the *Preface to the Third Edition*, beginning on page xvii. Inquiries regarding any aspect of the operation of the *Food Chemicals Codex* project may be directed to Food Chemicals Codex, National Academy of Sciences, 2101 Constitution Avenue, N.W., Washington, D.C. 20418.

General Good Manufacturing Practice Guidelines for Food Chemicals*

Food chemicals and other substances employed as adjuncts in foods and as aids in food processing must meet recognized standards of performance and quality for their intended uses and applications. The requirements contained in the monographs of the *Food Chemicals Codex* pertain to the characteristics of food chemical articles throughout their useful lives.

It is not sufficient, however, for an end product merely to meet the Codex requirements. A product must be made and handled in a sanitary manner, in a way designed to preclude the formation of undesirable by-products, as well as contamination, deterioration, mix-up, and mislabeling, and in a way that avoids the introduction of unusual or unexpected impurities.

Food chemicals are subject to applicable regulations promulgated by the responsible government agencies in countries in which Codex specifications are recognized. In the United States, for example, the pertinent regulations, which deal primarily with sanitation, are "Current Manufacturing Practice in Manufacturing, Processing, Packing, or Holding Human Food."†

Beyond requirements related to sanitation, however, manufacturers, processors, packers, and distributors should establish and exercise other appropriate systems of controls throughout their operations. These controls, together with the regulations cited above, constitute "good manufacturing practice." While the details of the application of the principles of good manufacturing practice to the manufacturing, processing, packing, and distribution of food chemical articles will vary, the fundamental relevance of such principles at all stages of an operation should be recognized.

The principles of good manufacturing practice encompass such considerations as:

- Systems of quality control and assurance
- Qualification of personnel
- Design, operation, and maintenance of building and equipment
- Procedures and records that permit tracing the lot history of the manufacture, packaging, and distribution of the product
- Handling and control of raw materials, process aids, intermediates, and finished products
- Product containers, closures, and labeling
- Master manufacturing, batch production, packaging, and distribution records
- Laboratory and inspection controls, including the effect of process changes
- Product stability
- Systems for holding and disposing of returned materials and rejected products
- Procedures for investigating complaints and taking appropriate corrective actions

It should be recognized that the manufacture of food chemicals, whether it involves chemical synthesis and purification, or recovery from natural materials, has a number of characteristics that must be taken into account in establishing a system of good manufacturing practice. For example, in the production of many chemicals, recycle of process liquors and recovery from waste streams are necessary for reasons of quality, economics, and environmental protection. In addition, the production of some food chemicals involves processes in which chemical and biochemical mechanisms have not been fully elucidated, and thus the methods and procedures for materials accountability usually will differ from those applicable to the manufacture of other classes of materials.

Another aspect of good manufacturing practice for food chemicals relates to the possible presence of objectionable impurities. It obviously is impossible to provide limits and tests in each Codex monograph for the detection of all possible impurities, since these may vary with the raw materials and with the method of processing used in making the chemical. The limits and tests provided in the Codex are consistent with information available to the Codex concerning current methods of manufacture and common impurities that may be present. In order to evaluate whether other undesirable impurities may be

*As indicated in the *Preface to the Third Edition* (see page xvii), these guidelines are presented for information only and are not intended to be mandatory in any sense as regards compliance with *Food Chemicals Codex* specifications.

†Code of Federal Regulations, Title 21, Part 110, which may be obtained from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

present, the manufacturer should understand, to the best degree possible, for the process he uses, the factors that contribute to the presence of impurities. Impurities in the raw materials, solvents, and other processing aids that might carry into the final product should be considered. In synthetic processes, it is necessary also to consider intermediates and the products of side reactions that might carry into the final product. Another factor in synthetic processes is the possible formation of isomeric compounds, including epimers and enantiomorphs.

If objectionable impurities other than those covered by the Codex specifications are suspected to be present, good manufacturing practice requires that additional tests and limits be applied by the manufacturer to ensure that the substance is suitable for its intended applications as a food chemical.

Former and Current Titles of *Food Chemicals Codex* Substances

Second Edition Title	*Third Edition Title*
Amyris Oil	Amyris Oil, West Indian Type
Basil Oil	Basil Oil, Comores Type
Bergamot Oil, Expressed	Bergamot Oil, Coldpressed
tert-Butylhydroquinone	TBHQ
Calcium Pantothenate, Racemic, Calcium Chloride Double Salt	Calcium Pantothenate, Calcium Chloride Double Salt
Calcium Stearoyl-2-Lactylate	Calcium Stearoyl Lactylate
Chamomile Oil, English	Chamomile Oil, English Type
Chamomile Oil, German	Chamomile Oil, German Type
Cinnamon Bark Oil, Ceylon	Cinnamon Bark Oil, Ceylon Type
Diatomaceous Silica	Diatomaceous Earth
Dill Seed Oil, European	Dill Seed Oil, European Type
Dillweed Oil, American	Dillweed Oil, American Type
Ethylcellulose	Ethyl Cellulose
Fir Needle Oil, Canadian	Fir Needle Oil, Canadian Type
Fir Needle Oil, Siberian	Fir Needle Oil, Siberian Type
Geranium Oil, Algerian	Geranium Oil, Algerian Type
Glutamic Acid Hydrochloride	L-Glutamic Acid Hydrochloride
Grapefruit Oil, Expressed	Grapefruit Oil, Coldpressed
Lavandin Oil, Abrial	Lavandin Oil, Abrial Type
Lemon Oil	Lemon Oil, Coldpressed
Locust Bean Gum	Locust (Carob) Bean Gum
Mandarin Oil, Expressed	Mandarin Oil, Coldpressed
Marjoram Oil, Spanish	Marjoram Oil, Spanish Type
Mentha Arvensis Oil, Dementholized	Mentha Arvensis Oil, Partially Dementholized
α-Methylbenzyl Acetate	Methylbenzyl Acetate*
Methylethylcellulose	Methyl Ethyl Cellulose
Orange Oil	Orange Oil, Coldpressed
Orange Oil, Bitter	Orange Oil, Bitter, Coldpressed
Origanum Oil, Spanish	Origanum Oil, Spanish Type
Papain, Pepsin	Subsumed by new monograph on Enzyme Preparations
Petitgrain Oil, Paraguay	Petitgrain Oil, Paraguay Type
Pine Needle Oil, Scotch	Pine Needle Oil, Scotch Type
Propylene Glycol Monostearate	Propylene Glycol Mono- and Diesters
Sage Oil, Dalmatian	Sage Oil, Dalmatian Type
Sage Oil, Spanish	Sage Oil, Spanish Type
Sandalwood Oil, East Indian	Sandalwood Oil, East Indian Type
Sodium Metaphosphate	Sodium Metaphosphate, Insoluble; Sodium Polyphosphates, Glassy; Sodium Trimetaphosphate
Sodium Silicoaluminate	Sodium Aluminosilicate
Sodium Stearoyl-2-Lactylate	Sodium Stearoyl Lactylate
Tangerine Oil, Expressed	Tangerine Oil, Coldpressed
Thiamine Hydrochloride	Thiamin Hydrochloride
Thiamine Mononitrate	Thiamin Mononitrate
dl-Alpha Tocopherol	*dl*-α-Tocopherol
d-Alpha Tocopheryl Acetate	*d*-α-Tocopheryl Acetate
dl-Alpha Tocopheryl Acetate	*dl*-α-Tocopheryl Acetate
d-Alpha Tocopheryl Acetate Concentrate	*d*-α-Tocopheryl Acetate Concentrate
d-Alpha Tocopheryl Acid Succinate	*d*-α-Tocopheryl Acid Succinate

*See footnotes on pages 398 and 402 in *Section 3* concerning this substance and Methyl Phenylcarbinyl Acetate.

Food Chemicals Codex Substances Listed by Functional Use in Foods

Acids, Acidifiers
- Acetic Acid, Glacial
- Citric Acid
- Fumaric Acid
- Glucono Delta-Lactone
- Hydrochloric Acid
- Lactic Acid
- Malic Acid
- Phosphoric Acid
- Potassium Acid Tartrate
- Sodium Bisulfate
- Sulfuric Acid
- Tartaric Acid

Alkalies
- Ammonium Bicarbonate
- Ammonium Hydroxide
- Calcium Carbonate
- Calcium Oxide
- Magnesium Carbonate
- Magnesium Hydroxide
- Magnesium Oxide
- Potassium Bicarbonate
- Potassium Carbonate
- Potassium Carbonate Solution
- Potassium Hydroxide
- Potassium Hydroxide Solution
- Sodium Bicarbonate
- Sodium Carbonate
- Sodium Hydroxide

Sodium Hydroxide Solution
Sodium Sesquicarbonate

Anticaking Agents, Drying Agents
Calcium Phosphate, Tribasic
Calcium Silicate
Calcium Stearate
Cellulose, Microcrystalline
Cellulose, Powdered
Ferrous Ammonium Citrate, Green
Kaolin
Magnesium Carbonate
Magnesium Hydroxide
Magnesium Silicate
Magnesium Stearate
Silicon Dioxide
Sodium Aluminosilicate
Sodium Ferrocyanide
Talc

Antimicrobial Agents
Benzoic Acid
Chlorine
Heptylparaben
Methylparaben
Potassium Nitrate
Potassium Nitrite
Propylparaben
Sodium Benzoate
Sodium Nitrate
Sodium Nitrite

Antioxidants
Ascorbic Acid
Ascorbyl Palmitate
BHA
BHT
Butylated Hydroxymethylphenol
Calcium Ascorbate
Dilauryl Thiodipropionate
Erythorbic Acid
Ethoxyquin
Gum Guaiac
Lecithin
Potassium Metabisulfite
Potassium Sulfite
Propyl Gallate
Sodium Ascorbate
Sodium Erythorbate
Sodium Hypophosphite
Sodium Metabisulfite
Sodium Sulfite
Sodium Thiosulfate
Stannous Chloride
TBHQ
dl-α-Tocopherol
d-α-Tocopherol Concentrate
Tocopherols Concentrate, Mixed

Binders, Fillers, Plasticizers
Calcium Stearate
Cellulose, Microcrystalline
Cellulose, Powdered
Dextrin
Ethyl Cellulose
Food Starch, Modified
Glycerin
Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol
Magnesium Stearate
Methylcellulose
Mineral Oil, White
Oleic Acid
Polyethylene Glycols

Bleaching, Oxidizing Agents
Acetone Peroxides
Benzoyl Peroxide
Calcium Peroxide
Chlorine
Hydrogen Peroxide
Sulfur Dioxide

Bodying, Bulking Agents
Cellulose, Powdered
Glycerin
Methylcellulose
PVP
Xanthan Gum

Buffers, Neutralizing Agents
Adipic Acid
Aluminum Ammonium Sulfate
Aluminum Potassium Sulfate
Aluminum Sodium Sulfate
Ammonium Carbonate
Ammonium Phosphate, Dibasic
Ammonium Phosphate, Monobasic
Calcium Citrate
Calcium Gluconate
Calcium Hydroxide
Calcium Lactate
Calcium Phosphate, Monobasic
Calcium Phosphate, Tribasic
Calcium Pyrophosphate
Magnesium Oxide
Potassium Acid Tartrate
Potassium Citrate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acetate
Sodium Acetate, Anhydrous
Sodium Acid Pyrophosphate
Sodium Citrate
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Sodium Phosphate, Tribasic
Sodium Potassium Tartrate
Sodium Pyrophosphate
Sodium Sesquicarbonate
Succinic Acid

Carriers, Formulation Aids, Disintegrating Agents, Dispersing Agents, Tableting Aids
Cellulose, Microcrystalline
Cellulose, Powdered
Citric Acid
Dextrose
Fructose
Magnesium Carbonate
Polyethylene Glycols
PVP
Silicon Dioxide

Chewing Gum Base Components
Butadiene-Styrene 75/25 Rubber
Butadiene-Styrene 50/50 Rubber
Candelilla Wax
Glycerol Ester of Partially Dimerized Rosin
Glycerol Ester of Partially Hydrogenated Wood Rosin
Glycerol Ester of Polymerized Rosin
Glyercol Ester of Tall Oil Rosin
Glycerol Ester of Wood Rosin
Isobutylene-Isoprene Copolymer
Lanolin, Anhydrous
Limestone, Ground
Masticatory Substances, Natural
Methyl Ester of Rosin, Partially Hydrogenated
Paraffin, Synthetic
Pentaerythritol Ester of Partially Hydrogenated Wood Rosin
Pentaerythritol Ester of Wood Rosin
Petroleum Wax
Petroleum Wax, Synthetic
Polyethylene
Polyisobutylene
Polyvinyl Acetate
Rice Bran Wax
Terpene Resin, Natural
Terpene Resin, Synthetic

Clarifying Agents
Polyvinylpolypyrrolidone
PVP
Tannic Acid

Colors
β-Apo-8′-Carotenal
Canthaxanthin
Caramel
Carmine
β-Carotene
Oleoresin Paprika (see Spice Oleoresins)
Oleoresin Turmeric (see Spice Oleoresins)
Titanium Dioxide

Color Fixatives, Adjuncts; Color-Retention Agents
Ferrous Gluconate
Magnesium Carbonate
Magnesium Chloride
Magnesium Hydroxide
Potassium Nitrite
Sodium Nitrite

Components in the Manufacture of Other Food-Grade Additives
Decanoic Acid
Lauric Acid
Myristic Acid
Octanoic Acid

Oleic Acid
Palmitic Acid
Sodium Sulfate
Stearic Acid

Defoaming Agents
Decanoic Acid
Dimethylpolysiloxane
Lauric Acid
Mineral Oil, White
Myristic Acid
Octanoic Acid
Oleic Acid
Oxystearin
Palmitic Acid
Petrolatum
Petroleum Wax
Petroleum Wax, Synthetic
Polypropylene Glycol
Silicon Dioxide
Sorbitan Monostearate
Stearic Acid

Dough Conditioners
Acetone Peroxides
Ammonium Chloride
Ammonium Phosphate, Dibasic
Ammonium Phosphate, Monobasic
Ammonium Sulfate
Calcium Bromate
Calcium Carbonate
Calcium Iodate
Calcium Lactate
Calcium Oxide
Calcium Peroxide
Calcium Phosphate, Dibasic
Calcium Phosphate, Monobasic
Calcium Stearoyl Lactylate
Calcium Sulfate
Ethoxylated Mono- and Diglycerides
Potassium Bromate
Potassium Iodate
Sodium Chloride
Sodium Stearoyl Lactylate
Sodium Stearyl Fumarate
Succinylated Monoglycerides

Emulsifiers; Foaming, Whipping Agents
Acacia
Acetylated Monoglycerides
Agar
Alginic Acid
Ammonium Alginate
Calcium Alginate
Calcium Stearate
Calcium Stearoyl Lactylate
Carrageenan
Cholic Acid
Desoxycholic Acid
Diacetyl Tartaric Acid Esters of Mono- and Diglycerides
Dioctyl Sodium Sulfosuccinate
Ethoxylated Mono- and Diglycerides
Guar Gum
Hydroxylated Lecithin
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose
Karaya Gum
Lactated Mono-Diglycerides
Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol
Lactylic Esters of Fatty Acids
Lecithin
Locust (Carob) Bean Gum
Magnesium Stearate
Methylcellulose
Methyl Ethyl Cellulose
Mono- and Diglycerides
Pectin
Polyglycerol Esters of Fatty Acids
Polysorbate 20
Polysorbate 60
Polysorbate 65
Polysorbate 80
Potassium Alginate
Potassium Phosphate, Tribasic
Potassium Polymetaphosphate
Potassium Pyrophosphate
Propylene Glycol Alginate
Propylene Glycol Mono- and Diesters
Sodium Alginate
Sodium Aluminum Phosphate, Basic
Sodium Metaphosphate, Insoluble
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Sodium Phosphate, Tribasic
Sodium Polyphosphates, Glassy
Sodium Pyrophosphate
Sodium Stearoyl Lactylate
Sorbitan Monostearate
Succinylated Monoglycerides
Tragacanth
Xanthan Gum

Enzymes
Enzyme Preparations
ANIMAL DERIVED
Catalase (bovine liver)
Lipase
Pepsin
Rennet
Rennet, Bovine
Trypsin
PLANT DERIVED
Bromelain
Ficin
Malt
Papain
MICROBIALLY DERIVED
Carbohydrase (*Aspergillus niger* var.)
Carbohydrase (*Aspergillus oryzae* var.)
Carbohydrase (*Rhizopus oryzae* var.)
Carbohydrase (*Saccharomyces* species)
Carbohydrase (*Trichoderma reesei* var.)
Carbohydrase and Protease, Mixed (*Bacillus licheniformis*)
Carbohydrase and Protease, Mixed (*Bacillus subtilis*)
Catalase (*Aspergillus niger* var.)
Catalase (*Micrococcus lysodeikticus*)
Glucose Isomerase (*Actinoplanes missouriensis*)
Glucose Oxidase (*Aspergillus niger* var.)
Lipase (*Aspergillus niger* var.)
Lipase (*Aspergillus oryzae* var.)
Protease (*Aspergillus niger* var.)
Protease (*Aspergillus oryzae* var.)
Rennet (*Endothia parasitica*)
Rennet (*Mucor* species)

Enzyme Activators
Gibberellic Acid
Potassium Gibberellate

Filter Aid
Calcium Silicate
Cellulose, Powdered
Diatomaceous Earth
Magnesium Silicate
Perlite

Firming Agents
Aluminum Potassium Sulfate
Aluminum Sodium Sulfate
Aluminum Sulfate
Beeswax, White
Beeswax, Yellow
Calcium Carbonate
Calcium Chloride
Calcium Chloride, Anhydrous
Calcium Chloride Solution
Calcium Citrate
Calcium Gluconate
Calcium Hydroxide
Calcium Lactobionate
Calcium Phosphate, Monobasic
Calcium Sulfate
Magnesium Chloride

Flavor Enhancers, Intensifiers
Aspartame
Disodium Guanylate
Disodium Inosinate
Monoammonium L-Glutamate
Monopotassium L-Glutamate
Monosodium L-Glutamate
Sodium Chloride

Flavoring Adjuncts and Adjuvants
Brominated Vegetable Oil
1,3-Butylene Glycol
Formic Acid
Poloxamer 331
Poloxamer 407
Polyethylene Glycols

Flavoring Agents
Acetaldehyde
Acetanisole
Acetic Acid, Glacial
Acetoin
Acetophenone
3-Acetyl-2,5-dimethyl Furan

3-Acetylpyridine
2-Acetylpyrrole
Allyl Cyclohexanepropionate
Allyl Hexanoate
Allyl α-Ionone
Allyl Isothiocyanate
Almond Oil, Bitter, FFPA
Ambrette Seed Oil
α-Amylcinnamaldehyde
Amyl Cinnamate
Amyl Octanoate
Amyl Propionate
Amyris Oil, West Indian Type
Anethole
Angelica Root Oil
Angelica Seed Oil
Anise Oil
Anisole
Anisyl Acetate
Anisyl Alcohol
Balsam Peru Oil
Basil Oil, Comoros Type
Basil Oil, European Type
Bay Oil
Benzaldehyde
Benzophenone
Benzyl Acetate
Benzyl Alcohol
Benzyl Benzoate
Benzyl Butyrate
Benzyl Cinnamate
Benzyl Isobutyrate
Benzyl Isovalerate
Benzyl Phenylacetate
Benzyl Propionate
Benzyl Salicylate
Bergamot Oil, Expressed
Birch Tar Oil, Rectified
Black Pepper Oil
Bois de Rose Oil
Bornyl Acetate
Brominated Vegetable Oil
2-Butanone
Butan-3-one-2-yl Butyrate
Butyl Acetate
Butyl Alcohol
Butyl Butyrate
Butyl Butyryllactate
Butyl Isobutyrate
Butyraldehyde
Butyric Acid
γ-Butyrolactone
Caffeine
Camphene
Cananga Oil
Caraway Oil
Cardamom Oil
Carrot Seed Oil
Carvacrol
d-Carvone
l-Carvone
β-Caryophyllene
Cascarilla Oil
Cassia Oil
Cedar Leaf Oil
Celery Seed Oil
Chamomile Oil, English Type
Chamomile Oil, German Type
Cinnamaldehyde
Cinnamic Acid
Cinnamon Bark Oil, Ceylon Type
Cinnamon Leaf Oil
Cinnamyl Acetate
Cinnamyl Alcohol, Synthetic
Cinnamyl Anthranilate
Cinnamyl Formate
Cinnamyl Isovalerate
Cinnamyl Propionate
Citral
Citric Acid
Citronellal
Citronellol
Citronellyl Acetate
Citronellyl Butyrate
Citronellyl Formate
Citronellyl Isobutyrate
Citronellyl Propionate
Clary Oil
Clove Leaf Oil
Clove Oil
Clove Stem Oil
Cognac Oil, Green
Copaiba Oil
Coriander Oil
Costus Root Oil
Cresyl Acetate
Cubeb Oil
Cuminic Aldehyde
Cumin Oil
Cyclamen Aldehyde
trans,trans-2,4-Decadienal
Δ-Decalactone
Decanal
Decanoic Acid
1-Decanol, Natural
trans-2-Decen-1-al
cis-4-Decen-1-al
Diacetyl
Diethyl Malonate
Diethyl Sebacate
Diethyl Succinate
Dihydrocarveol
d-Dihydrocarvone
Dill Seed Oil, European Type
Dill Seed Oil, Indian Type
Dillweed Oil, American Type
Dimethyl Anthranilate
Dimethyl Benzyl Carbinol
Dimethyl Benzyl Carbinyl Acetate
Dimethyl Benzyl Carbinyl Butyrate
3,7-Dimethyl-1-Octanol
2,3-Dimethylpyrazine
2,5-Dimethylpyrazine
2,6-Dimethylpyrazine
2,5-Dimethylpyrrole
Δ-Dodecalactone
trans-2-Dodecen-1-al
Estragole
Ethyl Acetate
Ethyl Acetoacetate
Ethyl Acrylate
Ethyl *p*-Anisate
Ethyl Anthranilate
Ethyl Benzoate
2-Ethylbutyraldehyde
Ethyl Butyrate
2-Ethylbutyric Acid
Ethyl Cinnamate
Ethyl Decanoate
2-Ethyl-3,5(6)-dimethylpyrazine
2-Ethyl Fenchol
Ethyl Formate
Ethyl Heptanoate
Ethyl Hexanoate
Ethyl Isovalerate
Ethyl Lactate
Ethyl Laurate
Ethyl Maltol
Ethyl 2-Methylbutyrate
Ethyl Methylphenylglycidate
2-Ethyl-3-methylpyrazine
Ethyl Nonanoate
Ethyl Octanoate
Ethyl Oxyhydrate
Ethyl Phenylacetate
Ethyl Phenylglycidate
Ethyl Propionate
Ethyl Salicylate
Ethyl Vanillin
Eucalyptol
Eucalyptus Oil
Eugenol
Eugenyl Acetate
Farnesol
Fennel Oil
Fir Needle Oil, Canadian Type
Fir Needle Oil, Siberian Type
Fumaric Acid
Furfural
Garlic Oil
Geraniol
Geranium Oil, Algerian Type
Geranyl Acetate
Geranyl Benzoate
Geranyl Butyrate
Geranyl Formate
Geranyl Phenylacetate
Geranyl Propionate
Ginger Oil
L-Glutamic Acid Hydrochloride
Grapefruit Oil, Coldpressed
trans,trans-2,4-Heptadienal
γ-Heptalactone
Heptanal
2-Heptanone
3-Heptanone
cis-4-Hepten-1-al
Heptyl Alcohol
Hexanal
Hexanoic Acid
trans-2-Hexen-1-al
trans-2-Hexen-1-ol
cis-3-Hexen-1-ol
cis-3-Hexenyl Isovalerate
cis-3-Hexenyl 2-Methylbutyrate

Hexyl Alcohol, Natural
Hexyl-2-butenoate
α-Hexylcinnamaldehyde
Hexyl Isovalerate
Hexyl 2-Methylbutyrate
Hops Oil
Hydroxycitronellal
Hydroxycitronellal Dimethyl Acetal
6-Hydroxy-3,7-Dimethyloctanoic Acid Lactone
Indole
α-Ionone
β-Ionone
Isoamyl Acetate
Isoamyl Butyrate
Isoamyl Formate
Isoamyl Hexanoate
Isoamyl Isovalerate
Isoamyl Salicylate
Isobornyl Acetate
Isobutyl Acetate
Isobutyl Alcohol
Isobutyl-2-butenoate
Isobutyl Butyrate
Isobutyl Cinnamate
Isobutyl Phenylacetate
Isobutyl Salicylate
Isobutyraldehyde
Isobutyric Acid
Isoeugenol
Isoeugenyl Acetate
Isopropyl Acetate
Isopulegol
Isovaleric Acid
Juniper Berries Oil
Labdanum Oil
Laurel Leaf Oil
Lauric Acid
Lauryl Alcohol, Natural
Lauryl Aldehyde
Lavandin Oil, Abrial Type
Lavender Oil
Lemongrass Oil
Lemon Oil, Coldpressed
Lemon Oil, Distilled
Lemon Oil, Desert Type, Coldpressed
Lime Oil, Coldpressed
Lime Oil, Distilled
d-Limonene
l-Limonene
Linaloe Wood Oil
Linalool
Linalyl Acetate
Linalyl Acetate, Synthetic
Linalyl Benzoate
Linalyl Formate
Linalyl Isobutyrate
Linalyl Propionate
Lovage Oil
Mace Oil
Malic Acid
Maltol
Mandarin Oil, Coldpressed
Marjoram Oil, Spanish Type
Marjoram Oil, Sweet
Mentha Arvensis Oil, Partially Dementholized
Menthol
l-Menthone
dl-Menthyl Acetate
l-Menthyl Acetate
p-Methoxybenzaldehyde
2-Methoxy-3(5)-methylpyrazine
2-Methoxypyrazine
4'-Methyl Acetophenone
p-Methyl Anisole
Methyl Anthranilate
Methyl Benzoate
Methylbenzyl Acetate
α-Methylbenzyl Alcohol
2-Methylbutyl Isovalerate
α-Methylcinnamaldehyde
Methyl Cinnamate
Methyl Cyclopentenolone
Methyl Eugenol
6-Methyl-5-hepten-2-one
Methyl Isoeugenol
5-Methyl-2-isopropyl-2-hexenal
Methyl 2-Methylbutyrate
Methyl β-Naphthyl Ketone
Methyl 2-Octynoate
4-Methyl-2-pentanone
Methyl Phenylacetate
Methyl Phenylcarbinyl Acetate
2-Methylpyrazine
Methyl Salicylate
2-Methylundecanal
Myrcene
Myristic Acid
Myrrh Oil
Nerol
Nerolidol
trans,trans-2,4-Nonadienal
trans,cis-2,6-Nonadienal
trans,cis-2,6-Nonadienol
γ-Nonalactone
Nonanal
trans-2-Nonenal
trans-2-Nonen-1-ol
cis-6-Nonen-1-ol
Nonyl Acetate
Nonyl Alcohol
Nutmeg Oil
γ-Octalactone
Octanal
Octanoic Acid
1-Octanol, Natural
3-Octanol
trans-2-Octen-1-al
cis-3-Octen-1-ol
1-Octen-3-yl Acetate
1-Octen-3-yl Butyrate
Octyl Acetate
3-Octyl Acetate
Octyl Formate
Olibanum Oil
Onion Oil
Orange Oil, Coldpressed
Orange Oil, Bitter, Coldpressed
Orange Oil, Distilled
Origanum Oil, Spanish Type
Orris Root Oil
Palmarosa Oil
Parsley Herb Oil
Parsley Seed Oil
Pennyroyal Oil
2,3-Pentanedione
2-Pentanone
Peppermint Oil
Petitgrain Oil, Paraguay Type
α-Phellandrene
Phenethyl Acetate
Phenethyl Alcohol
Phenethyl Isobutyrate
Phenethyl Isovalerate
2-Phenethyl-2-methylbutyrate
Phenethyl Phenylacetate
Phenethyl Salicylate
Phenoxyethyl Isobutyrate
Phenylacetaldehyde
Phenylacetaldehyde Dimethyl Acetal
Phenylacetic Acid
3-Phenyl-1-propanol
2-Phenylpropionaldehyde
3-Phenylpropionaldehyde
2-Phenylpropionaldehyde Dimethyl Acetal
3-Phenylpropyl Acetate
Pimenta Leaf Oil
Pimenta Oil
α-Pinene
β-Pinene
Pine Needle Oil, Dwarf
Pine Needle Oil, Scotch Type
Piperonal
Propenylguaethol
Propionaldehyde
Propionic Acid
p-Propyl Anisole
Pyrrole
Quinine Hydrochloride
Quinine Sulfate
Rhodinol
Rhodinyl Acetate
Rhodinyl Formate
Rosemary Oil
Rose Oil
Rue Oil
Sage Oil, Dalmatian Type
Sage Oil, Spanish Type
Sandalwood Oil, East Indian Type
Santalol
Santalyl Acetate
Savory Oil (Summer Variety)
Sodium Chloride
Spearmint Oil
Spice Oleoresins
  Oleoresin Black Pepper
  Oleoresin Capsicum
  Oleoresin Celery
  Oleoresin Ginger
  Oleoresin Paprika
  Oleoresin Turmeric
Spike Lavender Oil
Tangerine Oil, Coldpressed

Tarragon Oil
α-Terpinene
γ-Terpinene
Terpineol
Terpinyl Acetate
Terpinyl Propionate
Tetrahydrolinalool
2,3,5,6-Tetramethylpyrazine
Thyme Oil
p-Tolyl Isobutyrate
Tributyrin
2-Tridecenal
2,3,5-Trimethylpyrazine
2,4,5-Trimethylpyrazine Δ-3-Oxazoline
γ-Undecalactone
Undecanal
10-Undecenal
2-Undecenol
Undecyl Alcohol
Valeric Acid
γ-Valerolactone
Vanillin
Wintergreen Oil

Humectants, Moisture-Retaining Agents
Dextrose
Glycerin
Potassium Polymetaphosphate
Propylene Glycol
Sorbitol
Sorbitol Solution
Triacetin

Leavening Agents
Ammonium Bicarbonate
Ammonium Phosphate, Dibasic
Ammonium Phosphate, Monobasic
Calcium Phosphate, Monobasic
Glucono Delta-Lactone
Potassium Bicarbonate
Sodium Acid Pyrophosphate
Sodium Aluminum Phosphate, Acidic
Sodium Bicarbonate

Lubricants; Antisticking, Release Agents
Acetylated Monoglycerides
Castor Oil
Mineral Oil, White
Oleic Acid
Petrolatum
Polyethylene Glycols
Stearic Acid
Talc

Maturing Agents
Acetone Peroxides
Azodicarbonamide
Calcium Bromate
Calcium Iodate
Potassium Bromate
Potassium Iodate

Miscellaneous and General Purpose
Ammonium Carbonate
Ammonium Sulfate
Beeswax, White
Beeswax, Yellow
Calcium Chloride
Calcium Chloride, Anhydrous
Calcium Chloride Solution
Calcium Gluconate
Calcium Hydroxide
Potassium Citrate
Potassium Sulfate
Succinic Acid

Mold and Rope Inhibitors
Calcium Propionate
Propionic Acid
Sodium Diacetate
Sodium Propionate

Nonnutritive Sweeteners, Sugar Substitutes
Ammonium Saccharin
Aspartame
Calcium Saccharin
Saccharin
Sodium Saccharin

Nutrients, Dietary Supplements
N-Acetyl-L-Methionine
DL-Alanine
L-Alanine
L-Arginine
L-Arginine Monohydrochloride
Ascorbic Acid
L-Asparagine
DL-Aspartic Acid
L-Aspartic Acid
Biotin
Calcium Carbonate
Calcium Glycerophosphate
Calcium Oxide
Calcium Pantothenate
Calcium Pantothenate, Racemic
Calcium Pantothenate, Calcium Chloride Double Salt
Calcium Phosphate, Dibasic
Calcium Phosphate, Monobasic
Calcium Phosphate, Tribasic
Calcium Pyrophosphate
Calcium Sulfate
β-Carotene
Choline Bitartrate
Choline Chloride
Copper Gluconate
L-Cysteine Monohydrochloride
L-Cystine
Dexpanthenol
Ferrous Ammonium Citrate, Brown
Ferrous Ammonium Citrate, Green
Ferric Phosphate
Ferric Pyrophosphate
Ferrous Fumarate
Ferrous Gluconate
Ferrous Sulfate
Ferrous Sulfate, Dried
Folic Acid
L-Glutamic Acid
L-Glutamic Acid Hydrochloride
L-Glutamine
Glycine
L-Histidine
L-Histidine Monohydrochloride
Inositol
Iron, Carbonyl
Iron, Electrolytic
Iron, Reduced
DL-Isoleucine
L-Isoleucine
Kelp
DL-Leucine
L-Leucine
L-Lysine Monohydrochloride
Magnesium Phosphate, Dibasic
Magnesium Phosphate, Tribasic
Magnesium Sulfate
Manganese Chloride
Manganese Gluconate
Manganese Glycerophosphate
Manganese Hypophosphite
Manganese Sulfate
Mannitol
DL-Methionine
L-Methionine
Niacin
Niacinamide
Niacinamide Ascorbate
DL-Panthenol
DL-Phenylalanine
L-Phenylalanine
Potassium Chloride
Potassium Gluconate
Potassium Glycerophosphate
Potassium Iodide
L-Proline
Pyridoxine Hydrochloride
Riboflavin
Riboflavin 5'-Phosphate Sodium
DL-Serine
L-Serine
Sodium Ascorbate
Sodium Chloride
Sodium Ferric Pyrophosphate
Sodium Gluconate
Sodium Phosphate, Dibasic
Sodium Phosphate, Monobasic
Sodium Phosphate, Tribasic
Sodium Pyrophosphate
Thiamin Hydrochloride
Thiamin Mononitrate
L-Threonine
dl-α-Tocopherol
d-α-Tocopherol Concentrate
Tocopherols Concentrate, Mixed
d-α-Tocopheryl Acetate
dl-α-Tocopheryl Acetate
d-α-Tocopheryl Acetate Concentrate
d-α-Tocopheryl Acid Succinate
DL-Tryptophan
L-Tryptophan
L-Tyrosine
L-Valine
Vitamin A
Vitamin B_{12}

Vitamin D_2
Vitamin D_3
Zinc Gluconate
Zinc Oxide
Zinc Sulfate

Nutritive Sweeteners
Dextrose
Fructose
Xylitol

Preservatives
Ascorbic Acid
Benzoic Acid
Calcium Disodium EDTA
Calcium Propionate
Dehydroacetic Acid
Disodium EDTA
Erythorbic Acid
Gum Guaiac
Heptylparaben
Hydrogen Peroxide
Methylparaben
Potassium Metabisulfite
Potassium Nitrate
Potassium Sorbate
Potassium Sulfite
Propionic Acid
Propylparaben
Sodium Benzoate
Sodium Bisulfite
Sodium Dehydroacetate
Sodium Diacetate
Sodium Erythorbate
Sodium Hypophosphite
Sodium Metabisulfite
Sodium Nitrate
Sodium Nitrite
Sodium Propionate
Sodium Sulfite
Sorbic Acid
Sulfur Dioxide

Salt Substitutes
L-Glutamic Acid
L-Glutamic Acid Hydrochloride
Monoammonium L-Glutamate
Monopotassium L-Glutamate
Potassium Chloride

Sequestrants
Calcium Acetate
Calcium Chloride
Calcium Chloride, Anhydrous
Calcium Chloride Solution
Calcium Citrate
Calcium Disodium EDTA
Calcium Gluconate
Calcium Phosphate, Monobasic
Calcium Sulfate
Citric Acid
Disodium EDTA
Glucono Delta-Lactone
Oxystearin
Phosphoric Acid
Potassium Citrate
Potassium Gluconate
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic
Sodium Acid Pyrophosphate
Sodium Citrate
Sodium Diacetate
Sodium Gluconate
Sodium Metaphosphate, Insoluble
Sodium Polyphosphates, Glassy
Sodium Potassium Tartrate
Sodium Tartrate
Sodium Thiosulfate
Sorbitol
Sorbitol Solution
Tartaric Acid
Triethyl Citrate

Solvents, Vehicles, Solubilizers
Acetone
Acetylated Monoglycerides
1,3-Butylene Glycol
Ethyl Alcohol
Ethylene Dichloride
Glycerin
Isopropyl Alcohol
Methyl Alcohol
Methylene Chloride
Poloxamer 331
Poloxamer 407
Propylene Glycol
Triacetin
Trichloroethylene

Stabilizers, Suspending Agents
Acacia
Agar
Alginic Acid
Ammonium Alginate
Brominated Vegetable Oil
Calcium Alginate
Calcium Stearoyl Lactylate
Carrageenan
Dextrin
Disodium EDTA
Food Starch, Modified
Glycerol Ester of Wood Rosin
Guar Gum
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose
Karaya Gum
Lactated Mono-Diglycerides
Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol
Locust (Carob) Bean Gum
Methylcellulose
Methyl Ethyl Cellulose
Mono- and Diglycerides
Pectin
Poloxamer 331
Poloxamer 407
Polysorbate 20
Polysorbate 60
Polysorbate 65
Polysorbate 80
Potassium Alginate
Propylene Glycol Alginate
Propylene Glycol Mono- and Diesters
PVP
Sodium Alginate
Sodium Carboxymethylcellulose
Sodium Stearoyl Lactylate
Sorbitan Monostearate
Stearyl Monoglyceridyl Citrate
Tragacanth
Xanthan Gum

Starch-Modifying Agents
Hydrogen Peroxide
Sodium Trimetaphosphate

Surface-Active Agents, Wetting Agents
Dioctyl Sodium Sulfosuccinate
Lactylated Fatty Acid Esters of Glycerol and Propylene Glycol
Lactylic Esters of Fatty Acids
Propylene Glycol
Sodium Lauryl Sulfate

Surface-Finishing Agents, Glazes, Polishes, Coating Agents, Film Formers
Acetylated Monoglycerides
Beeswax, White
Beeswax, Yellow
Candelilla Wax
Carnauba Wax
Castor Oil
Dextrin
Ethyl Cellulose
Hydroxypropyl Cellulose
Methylcellulose
Mineral Oil, White
Petrolatum
Petroleum Wax
Petroleum Wax, Synthetic
Polyethylene Glycols
Rice Bran Wax
Shellac, Bleached
Shellac, Bleached, Wax Free
Talc

Texturizers, Texture-Modifying Agents
Acetylated Monoglycerides
Cellulose, Powdered
Dextrose
Mannitol
Potassium Pyrophosphate
Potassium Tripolyphosphate
Sodium Metaphosphate, Insoluble
Sodium Phosphate, Dibasic
Sodium Polyphosphates, Glassy
Sodium Tripolyphosphate
Sorbitol
Sorbitol Solution
Talc

Thickeners, Gelling Agents
Acacia
Agar
Alginic Acid

Ammonium Alginate
Calcium Alginate
Carrageenan
Cellulose, Powdered
Dextrin
Food Starch, Modified
Guar Gum
Hydroxypropyl Cellulose
Hydroxypropyl Methylcellulose
Karaya Gum
Locust (Carob) Bean Gum
Methylcellulose
Pectin
Potassium Alginate
Potassium Chloride
Propylene Glycol Alginate
Sodium Alginate
Sodium Carboxymethylcellulose
Tragacanth
Xanthan Gum

Yeast Foods
Ammonium Chloride
Ammonium Phosphate, Dibasic
Ammonium Phosphate, Monobasic
Ammonium Sulfate
Calcium Carbonate
Calcium Lactate
Calcium Oxide
Calcium Phosphate, Dibasic
Calcium Phosphate, Monobasic
Calcium Sulfate
Potassium Chloride
Potassium Phosphate, Dibasic
Potassium Phosphate, Monobasic

Other Functional Uses, Miscellaneous
Caffeine (STIMULANT)
Carbon, Activated (DECOLORIZING AGENT, TASTE- AND ODOR-REDUCING AGENT, PURIFICATION AGENT)
Hydroxylated Lecithin (CLOUDING AGENT)
Hydroxypropyl Cellulose (PROTECTIVE COLLOID)
Methyl Formate (FUMIGANT)
Mineral Oil, White (FERMENTATION AID)
Monoglyceride Citrate (ANTIOXIDANT ADJUNCT)
Oxystearin (CRYSTALLIZATION INHIBITOR)
Potassium Sulfate (WATER CORRECTIVE)
Silicon Dioxide (CONDITIONING AGENT, CHILLPROOFING AGENT)
Sodium Chloride (CURING AGENT)
Sodium Citrate (NUTRIENT FOR CULTURED BUTTERMILK)
Sodium Methylate (CATALYST)
Sodium Sulfate (CARAMEL PRODUCTION)
Stannous Chloride (REDUCING AGENT)

9 / *Infrared Spectra*

INTRODUCTION

The infrared absorption spectra contained in this section are provided in conjunction with the requirement for *Identification* as specified for a number of substances in *Sections 2* and *3* of this edition.

ORGANIZATION OF SPECTRA

This section contains reproductions of infrared spectra for three major groups of substances.

Series A (pages 584–612), for the essential oils
Series B (pages 613–712), for the flavor aromatic chemicals and isolates
Series C (pages 713–721), for miscellaneous other substances

Most of the spectra were prepared especially for use in the *Food Chemicals Codex* by the Scientific Committee of the Essential Oil Association and the Technical Committee of the Flavor and Extract Manufacturers Association. It was not feasible for them to be recorded in a single uniform format, however. Consequently, spectra of several shapes and sizes, contributed by different laboratories using various types of infrared spectrophotometers, will be found. Within each series, however, spectra of the same format have been grouped together in uniform subseries. An alphabetical listing, with page references, is provided at the beginning of each series to aid the reader in locating the desired spectrum.

SAMPLE PREPARATION

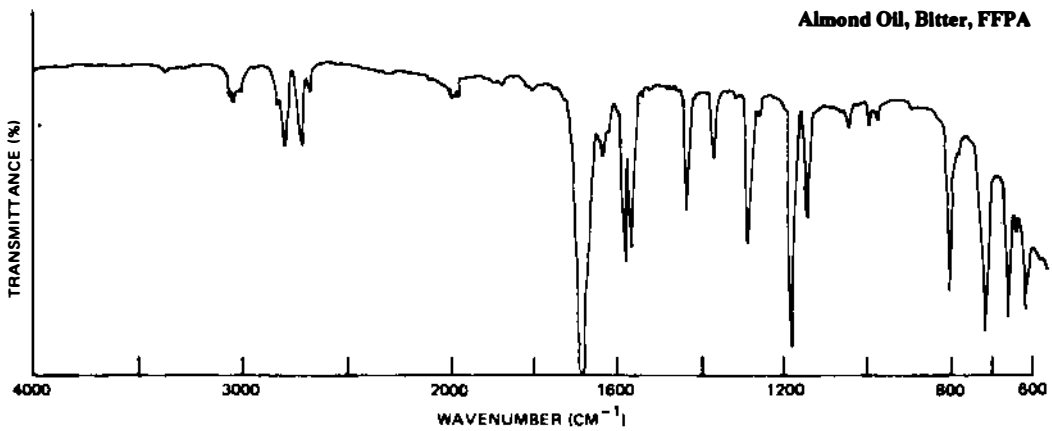
Most of the substances for which spectra are provided are liquids at or near room temperature. Unless otherwise noted in the caption for an individual spectrum, the spectra for substances in *Series A* and *B* were obtained on the neat liquids contained in fixed-volume sodium chloride cells or between salt plates. For substances in *Series A* and *B* that are not liquids, the sample was prepared as a potassium bromide pellet or a mineral oil (Nujol or equivalent) dispersion, as indicated in the individual spectrum caption. For substances in *Series C*, the samples were prepared as directed under *Identification* in the individual monographs.

Series A: Essential Oils

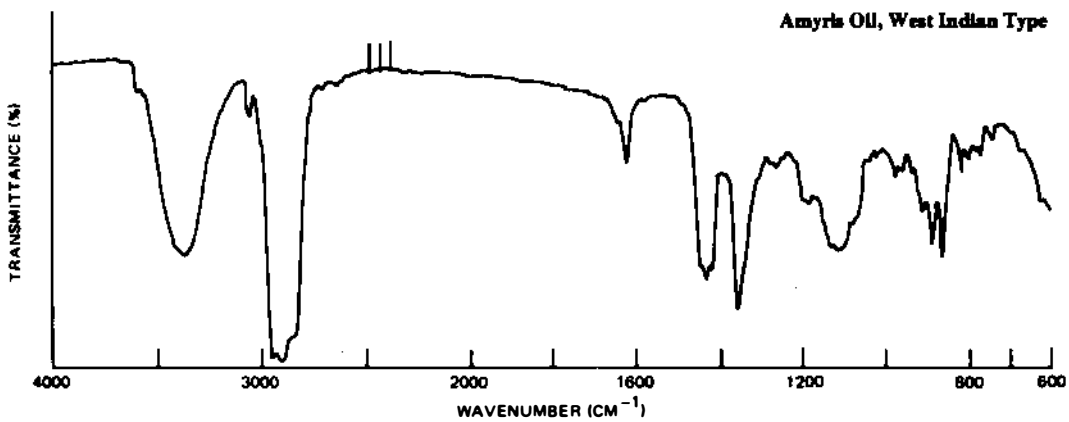
This series is divided into two subseries, depending upon the format of the spectra involved. The substances are listed below alphabetically.

SERIES A-1

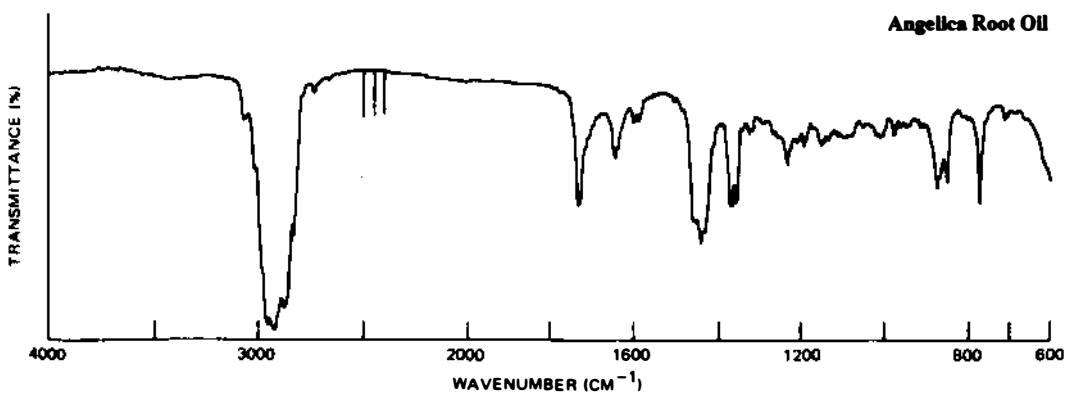
Almond Oil, Bitter, FFPA



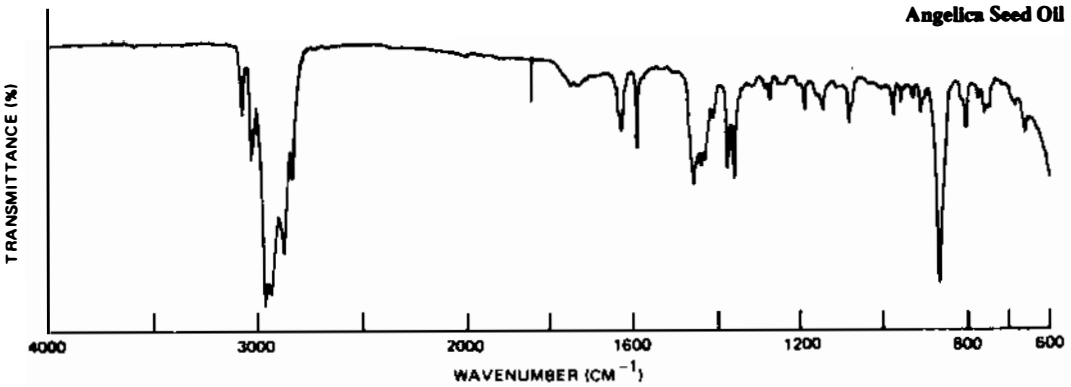
Amyris Oil, West Indian Type



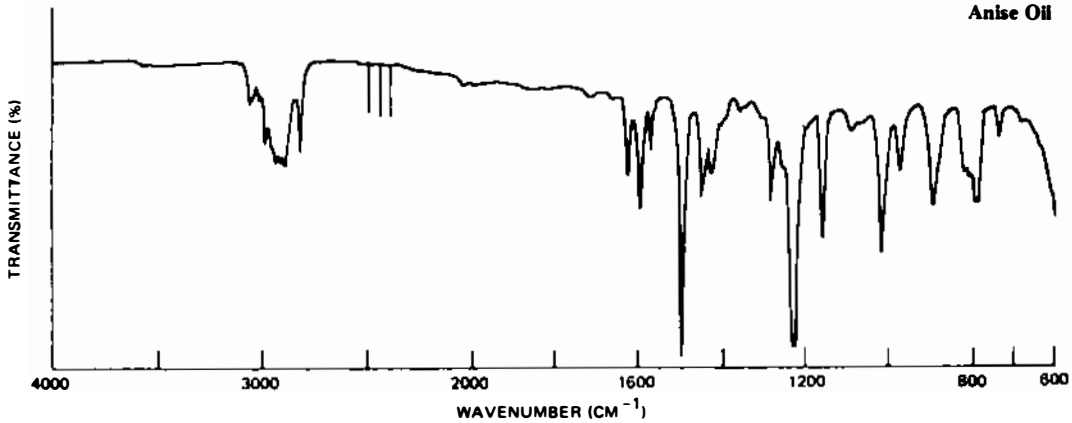
Angelica Root Oil



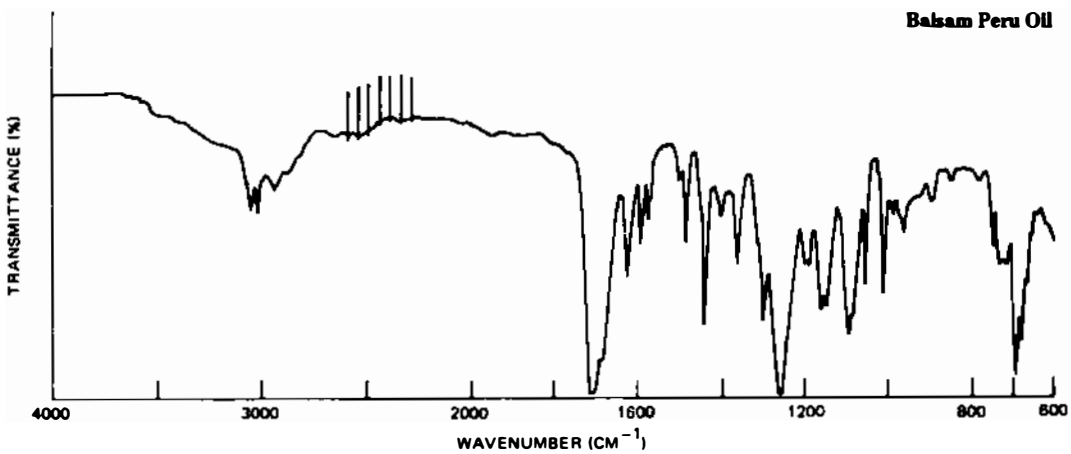
Angelica Seed Oil



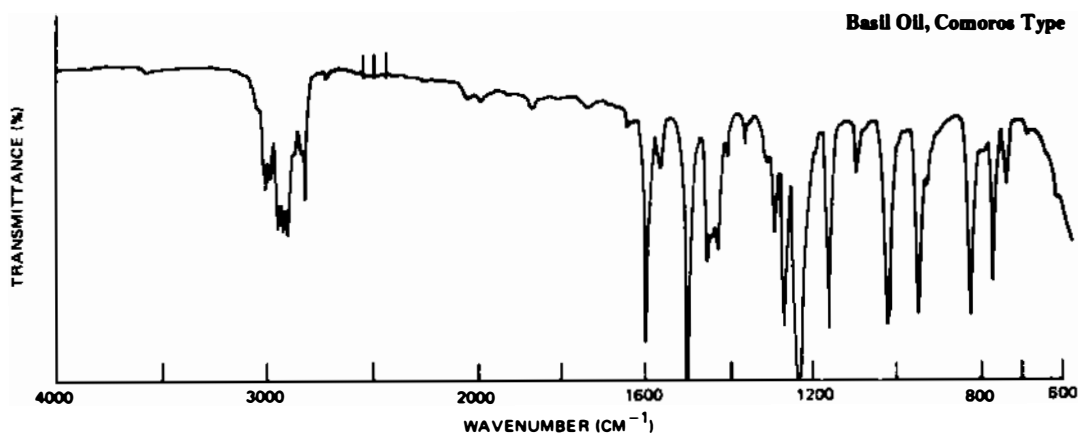
Anise Oil



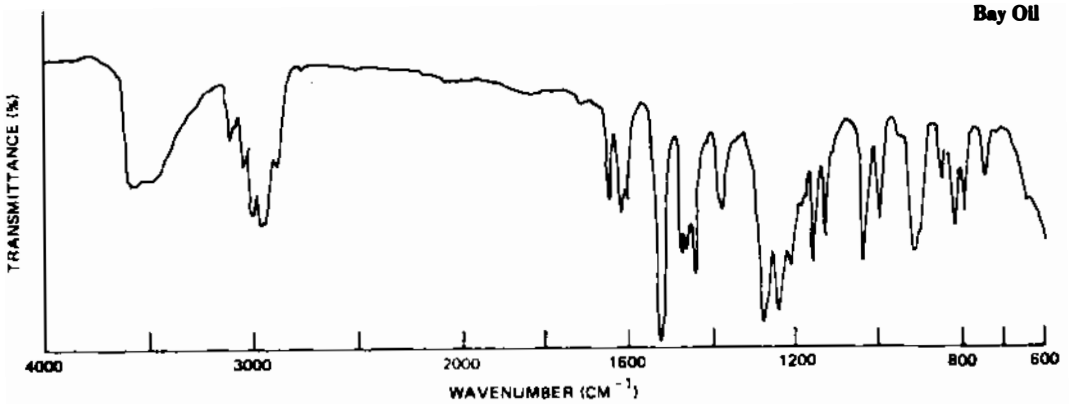
Balsam Peru Oil



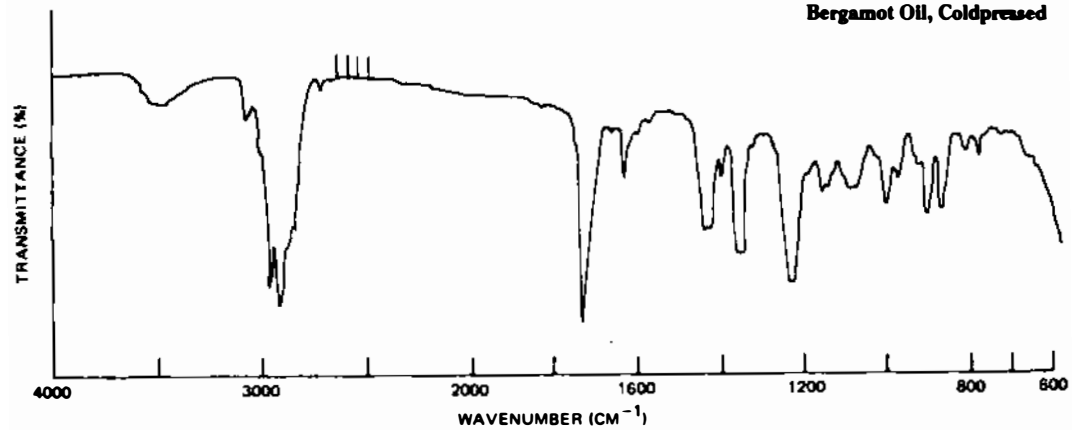
Basil Oil, Comoros Type



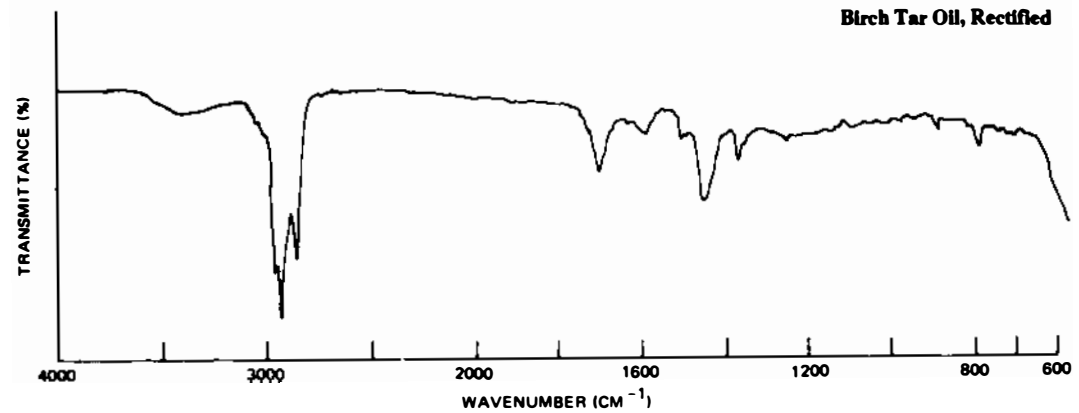
Bay Oil



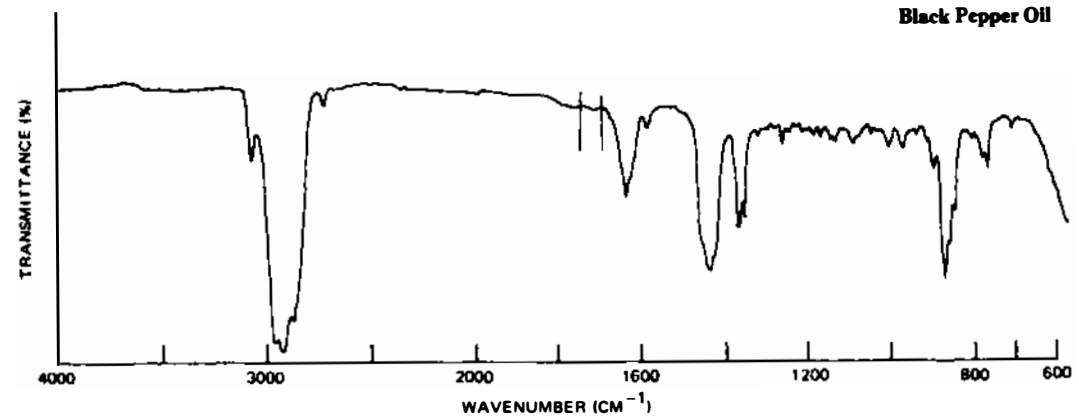
Bergamot Oil, Coldpressed



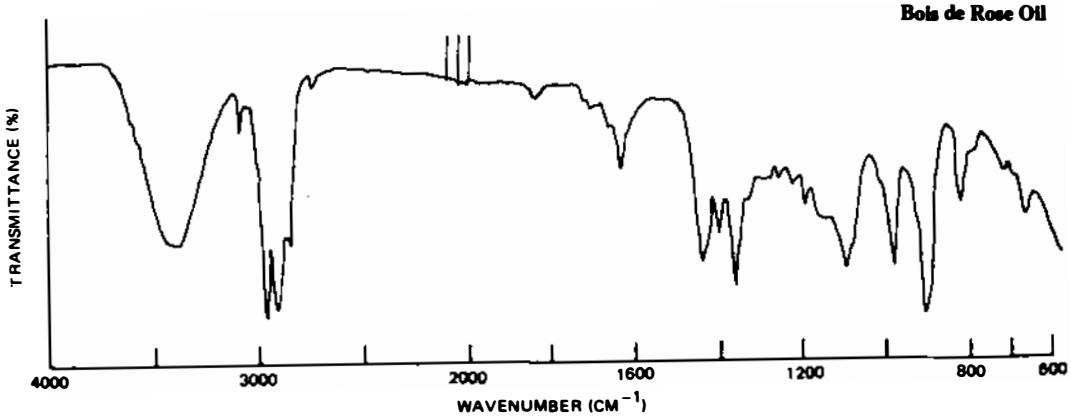
Birch Tar Oil, Rectified



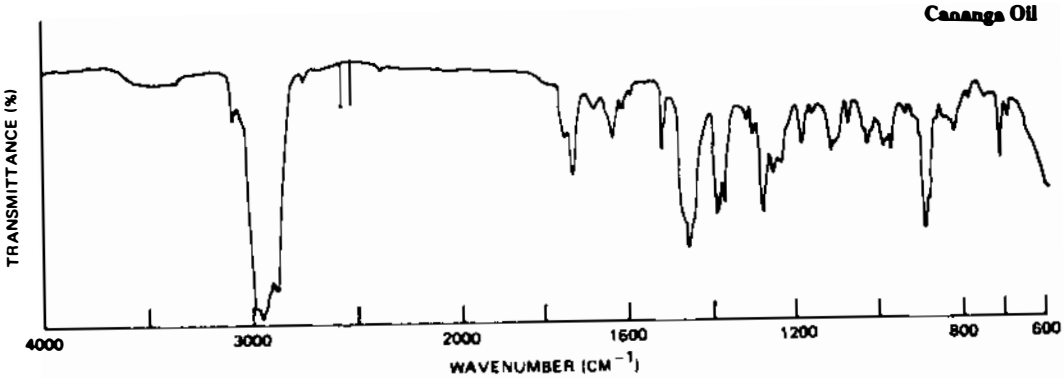
Black Pepper Oil



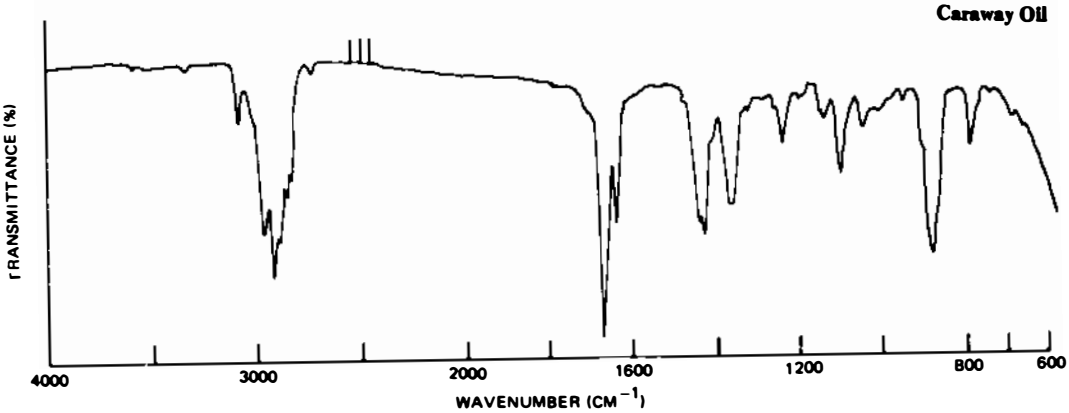
Bois de Rose Oil



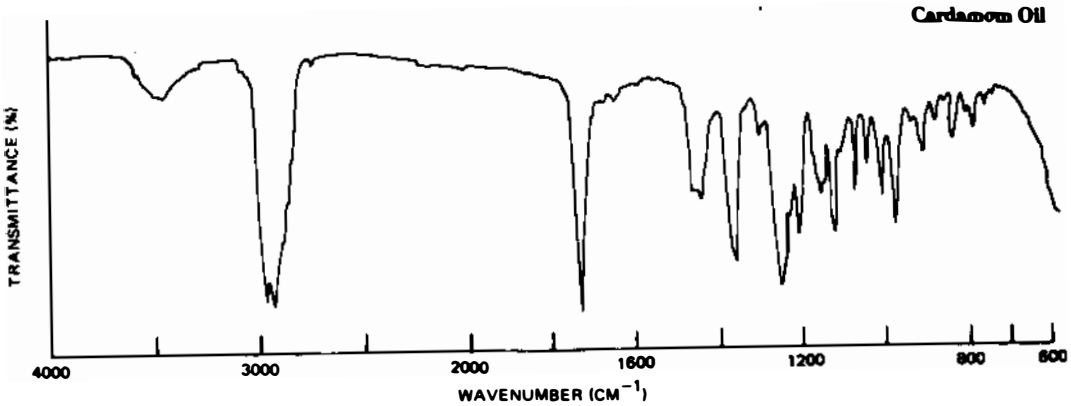
Cananga Oil



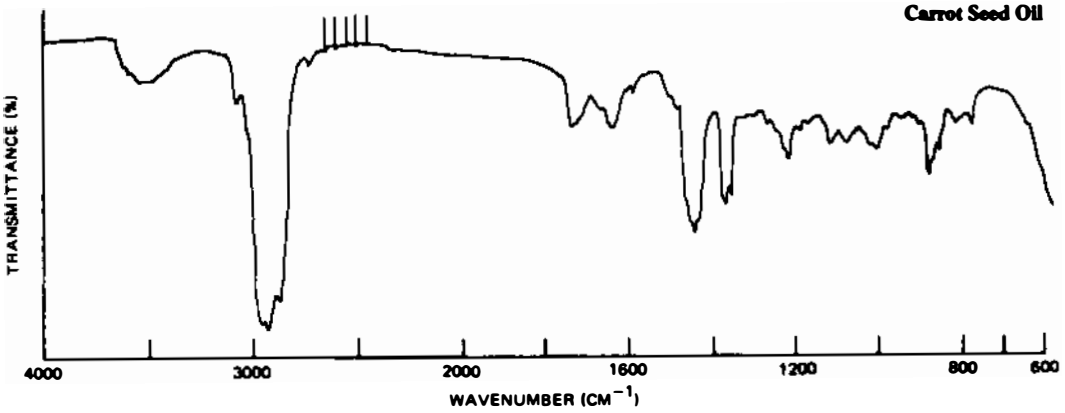
Caraway Oil



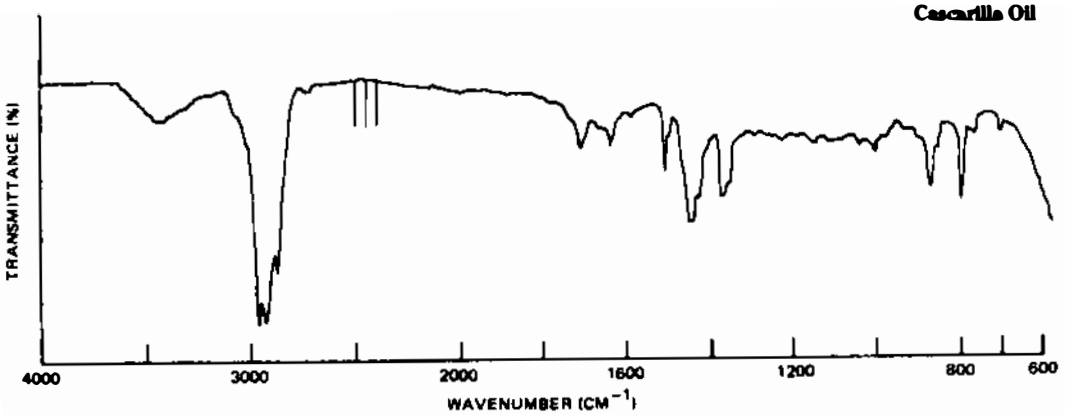
Cardamom Oil



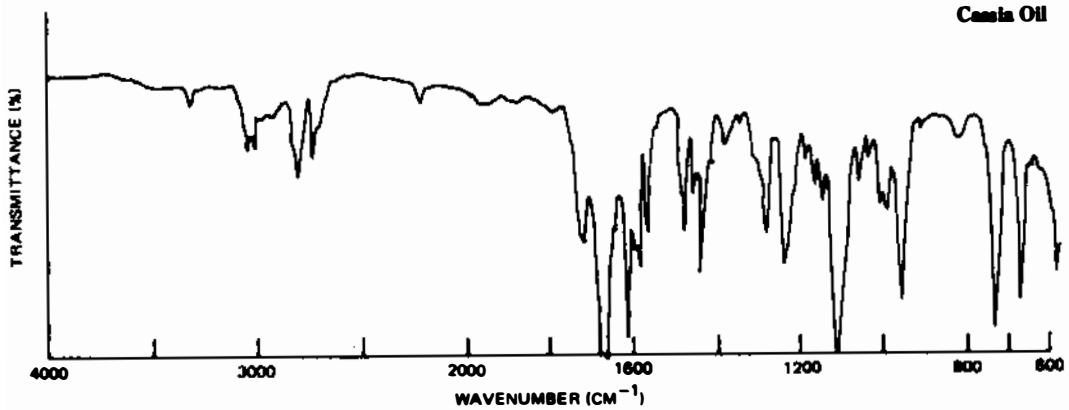
Carrot Seed Oil



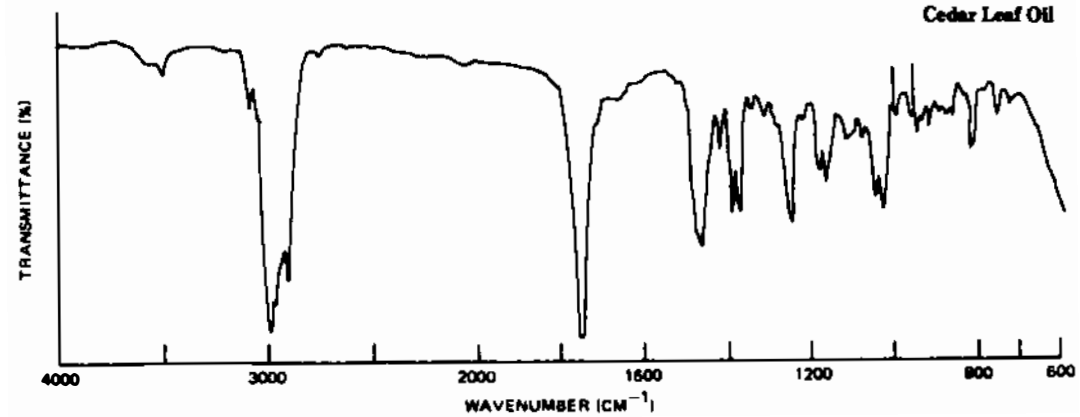
Cascarilla Oil



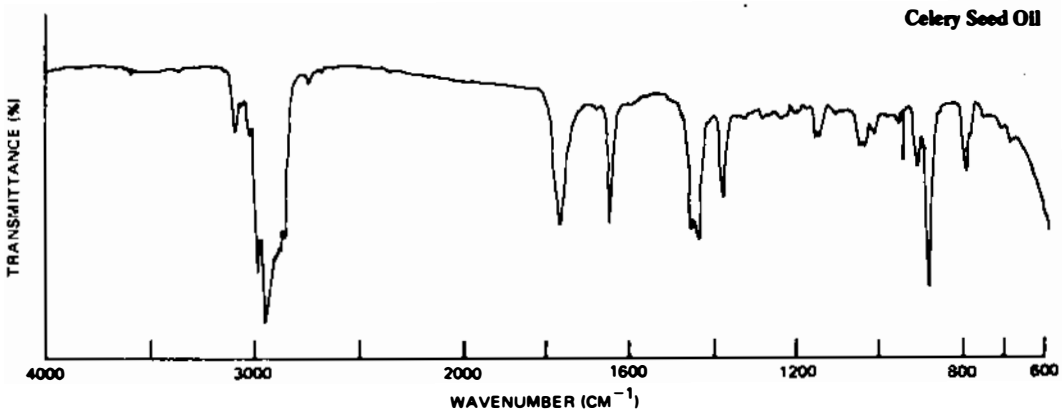
Cassia Oil



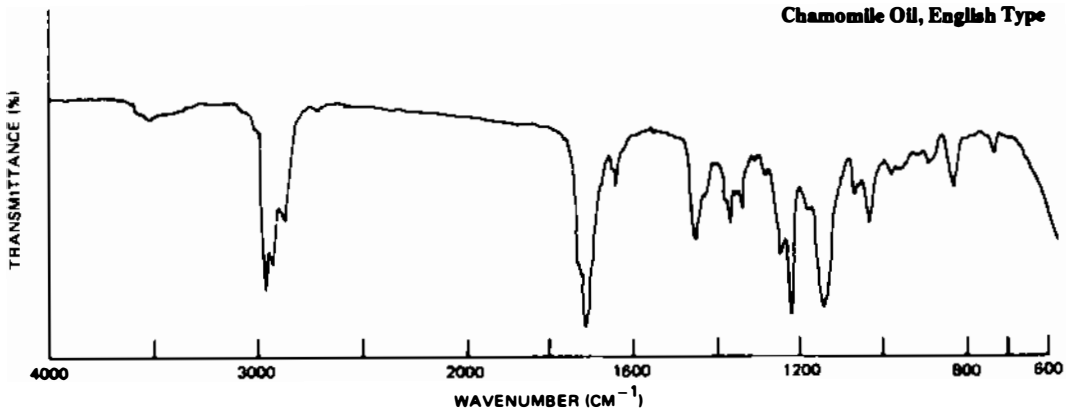
Cedar Leaf Oil



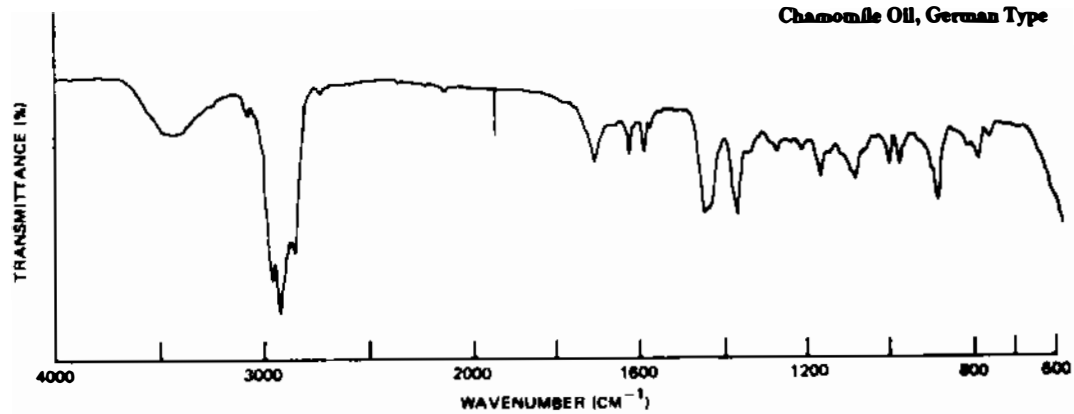
Celery Seed Oil



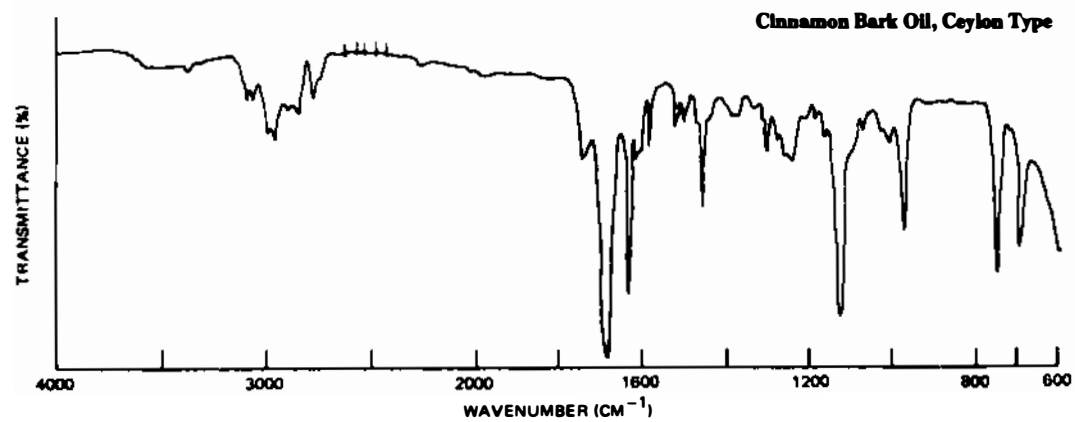
Chamomile Oil, English Type



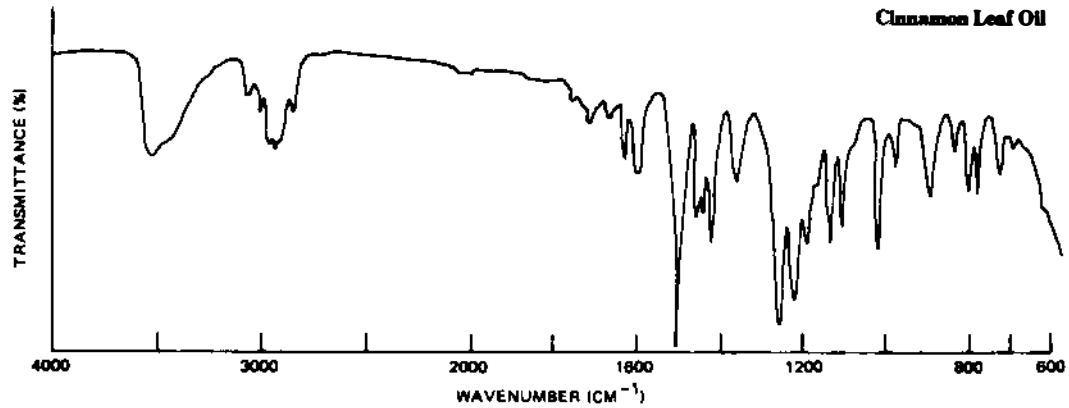
Chamomile Oil, German Type



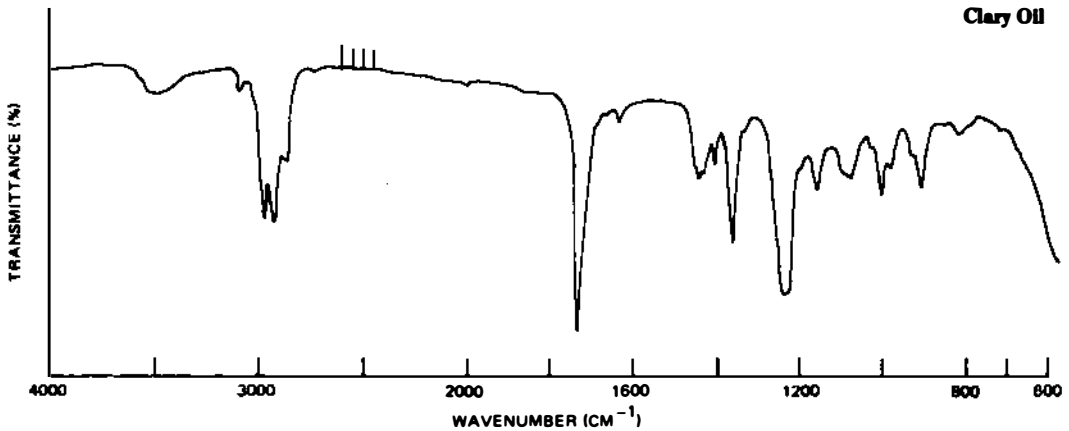
Cinnamon Bark Oil, Ceylon Type



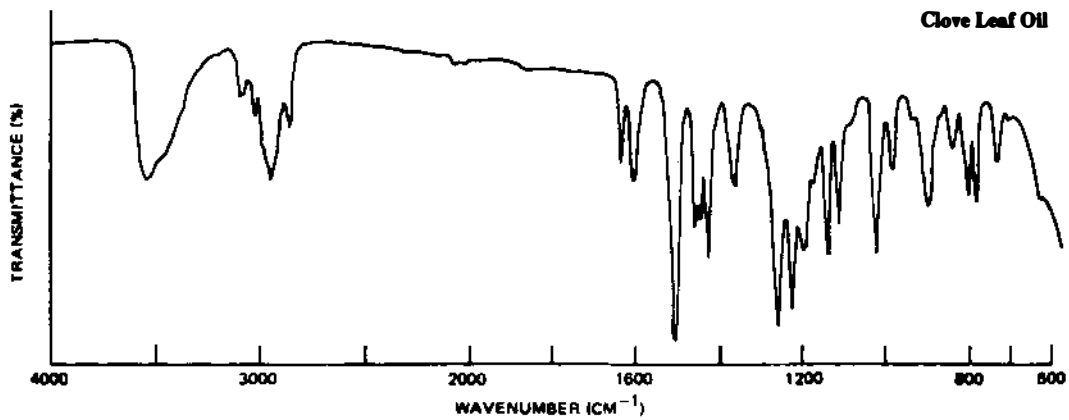
Cinnamon Leaf Oil



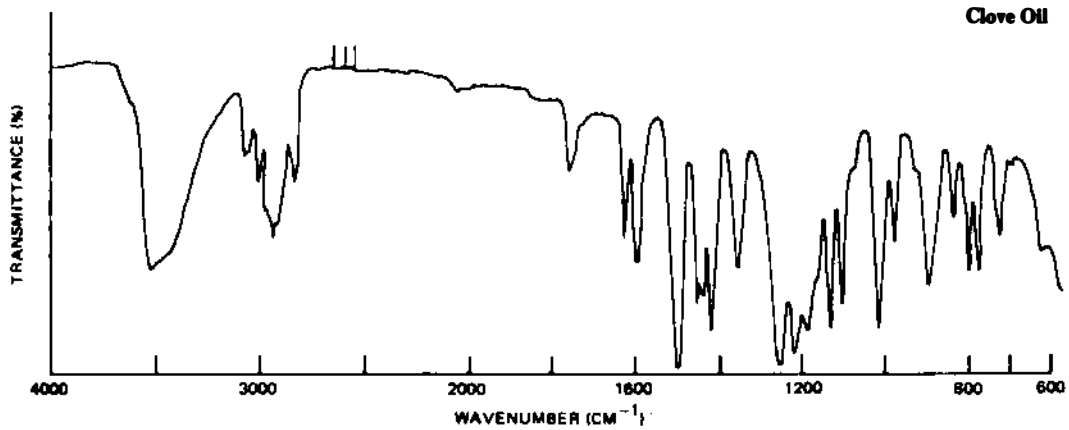
Clary Oil



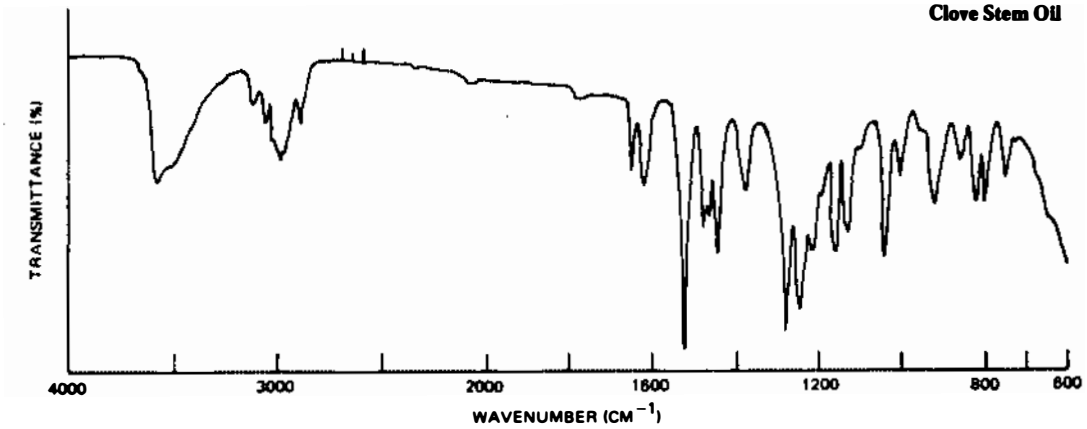
Clove Leaf Oil



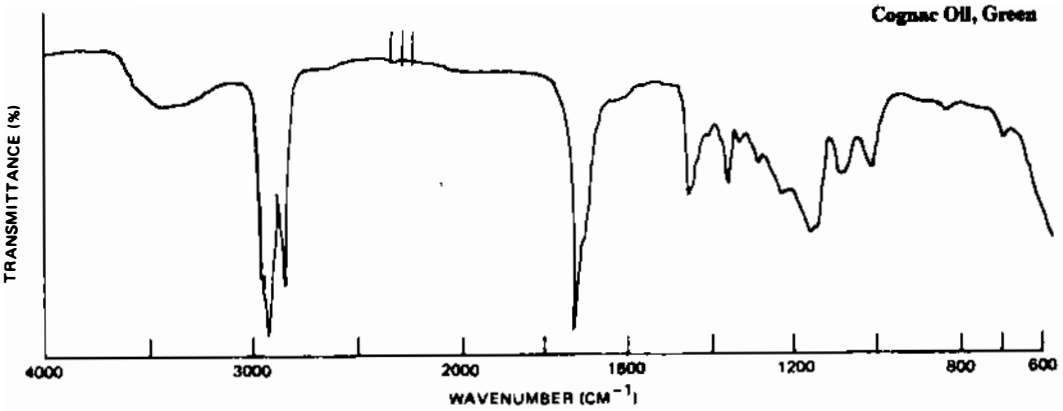
Clove Oil



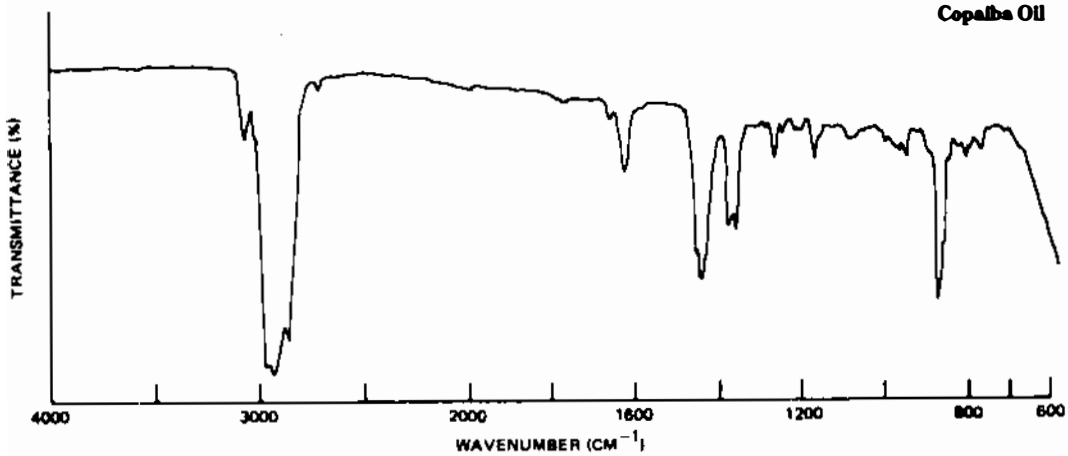
Clove Stem Oil



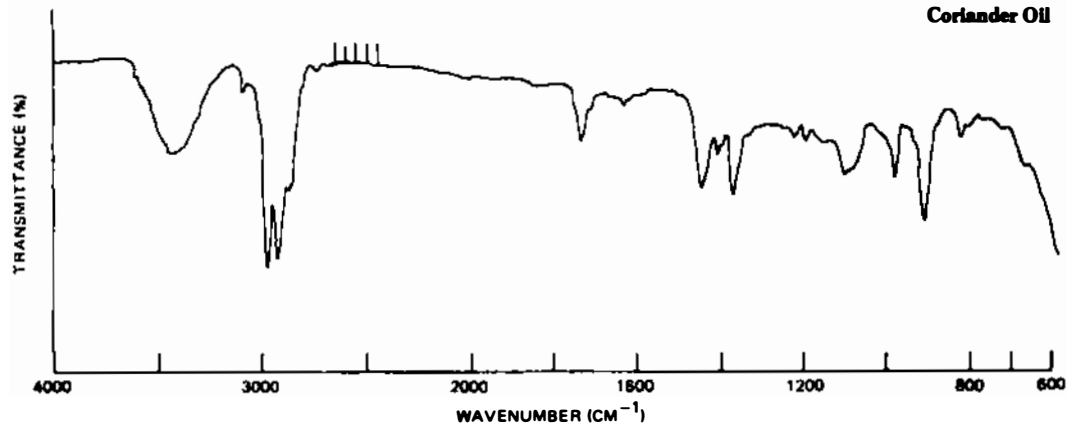
Cognac Oil, Green



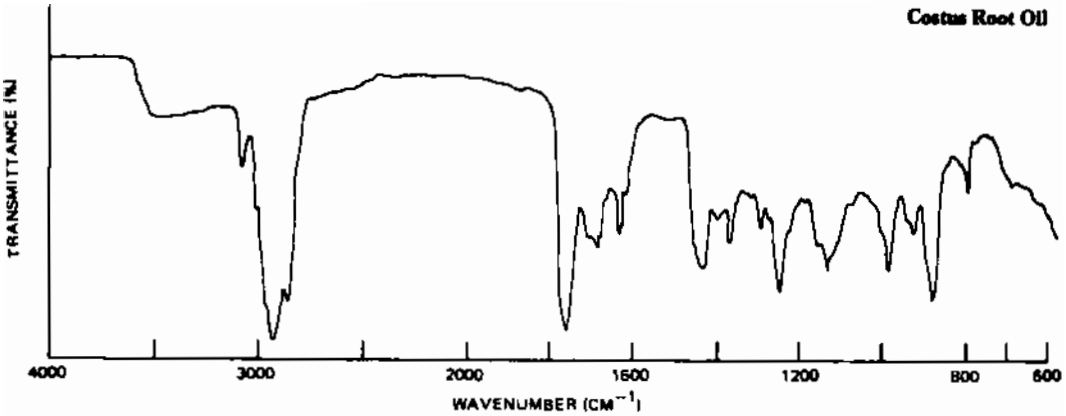
Copaiba Oil



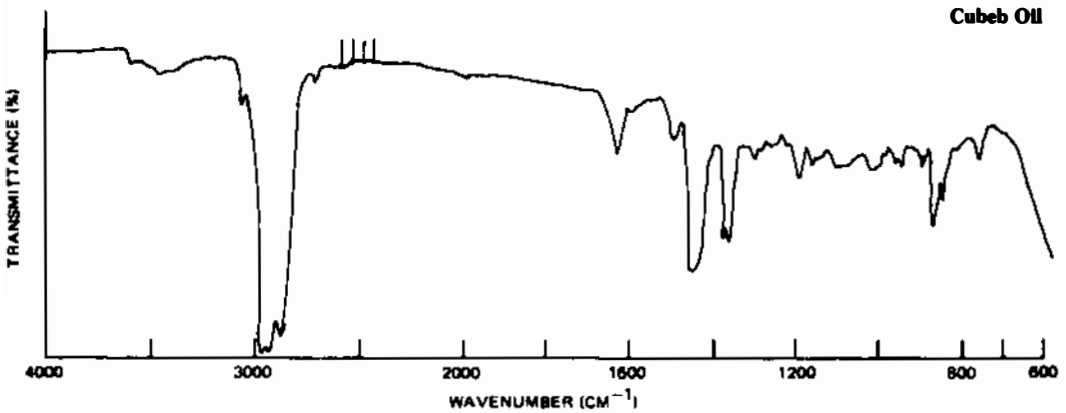
Coriander Oil



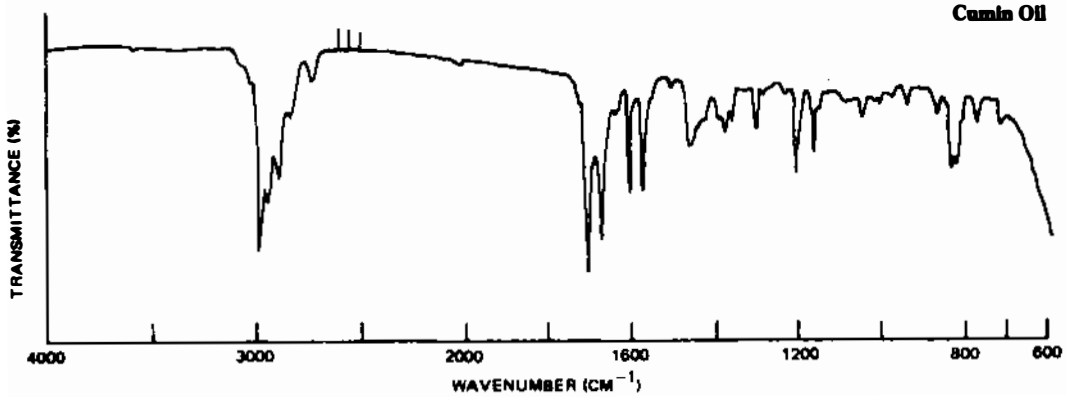
Costus Root Oil



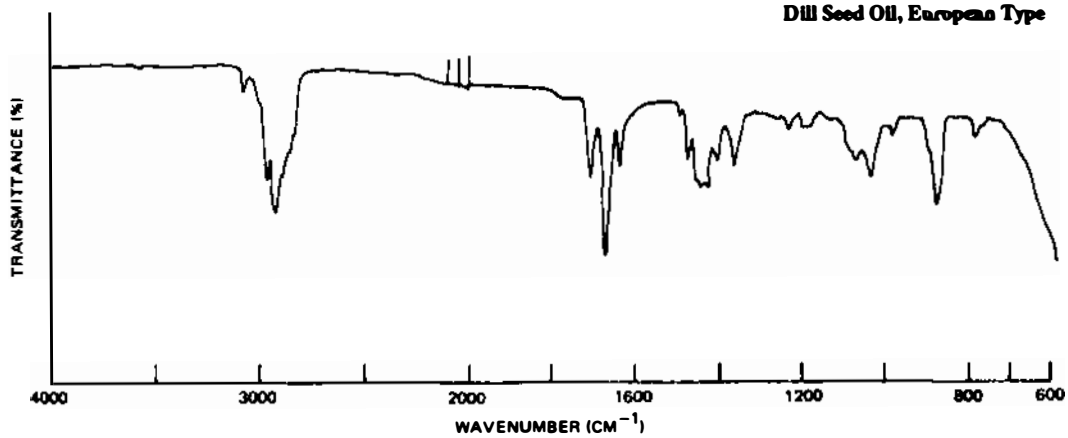
Cubeb Oil



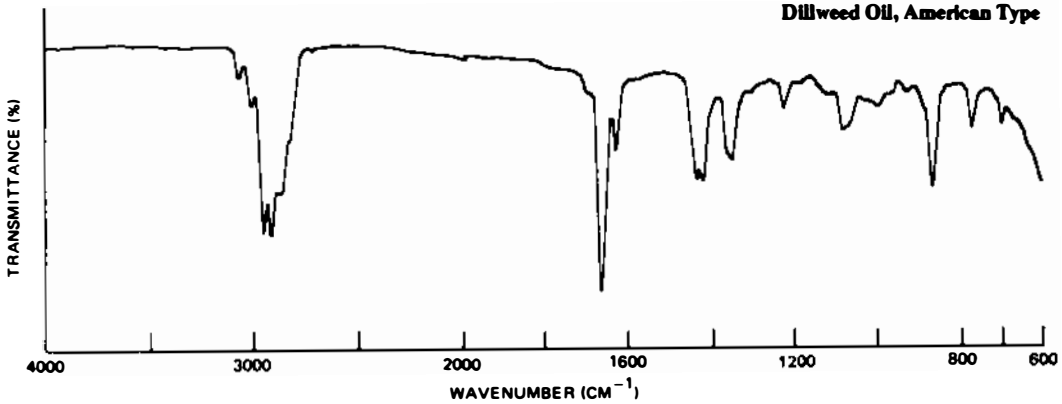
Cumin Oil



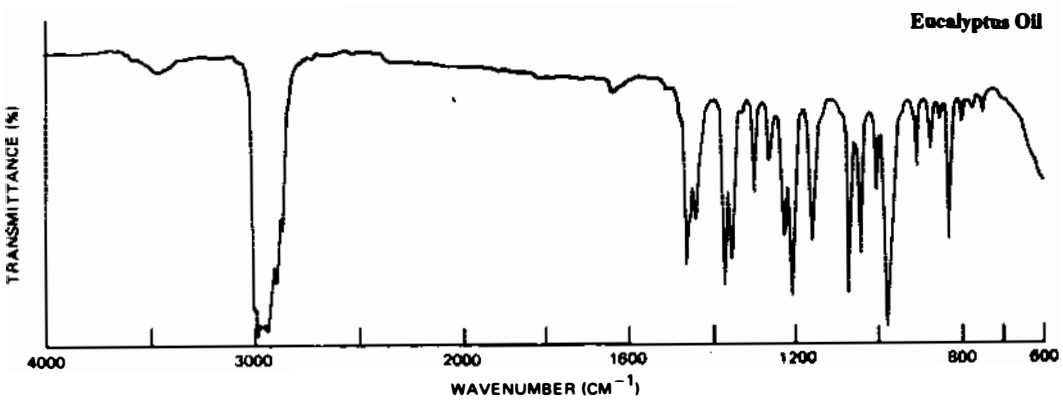
Dill Seed Oil, European Type



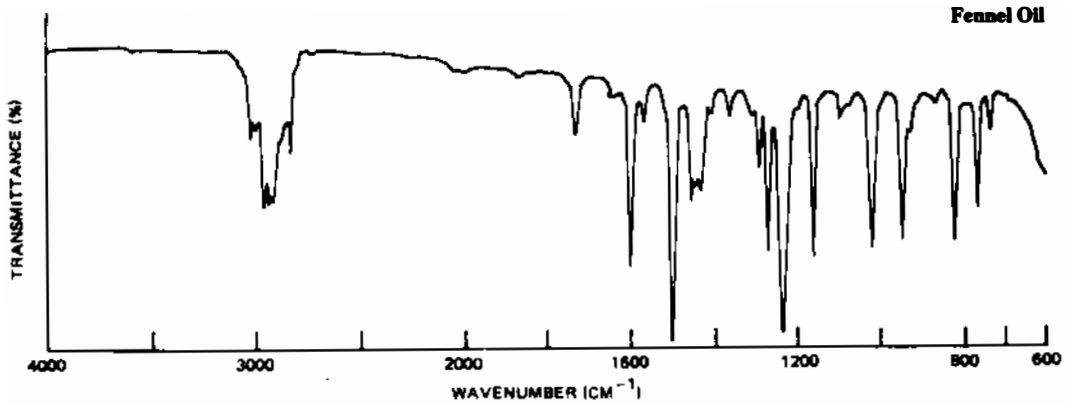
Dillweed Oil, American Type



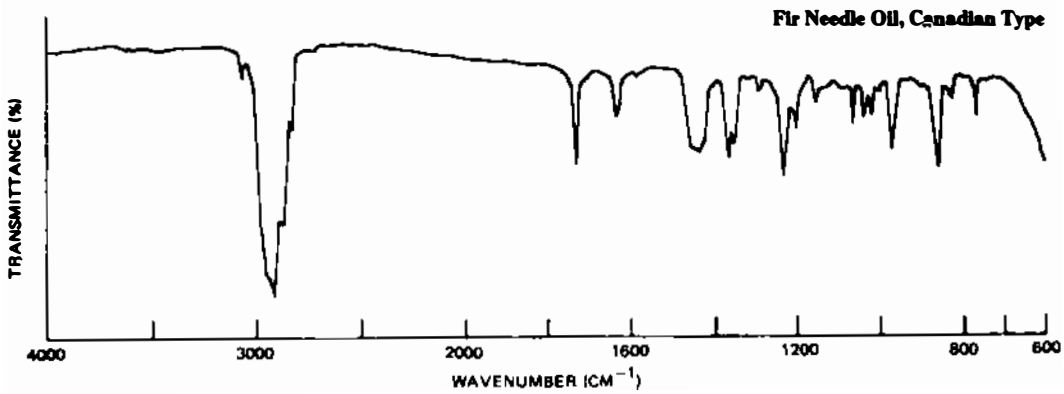
Eucalyptus Oil



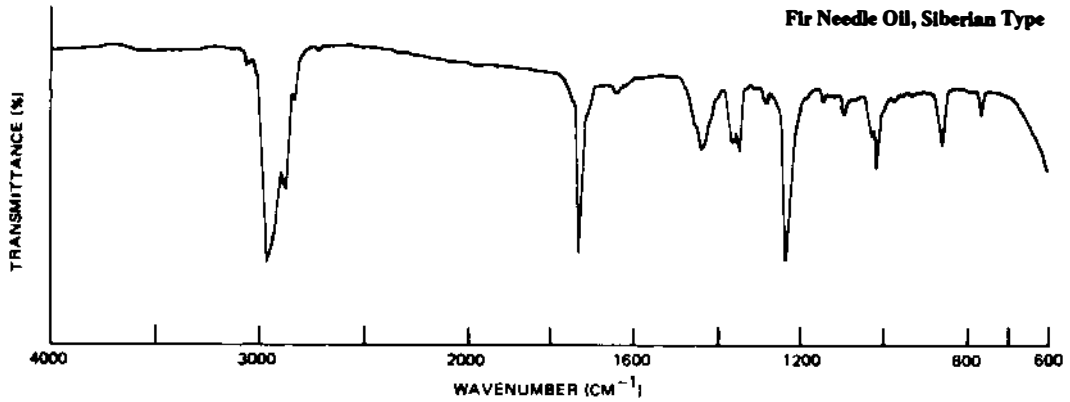
Fennel Oil



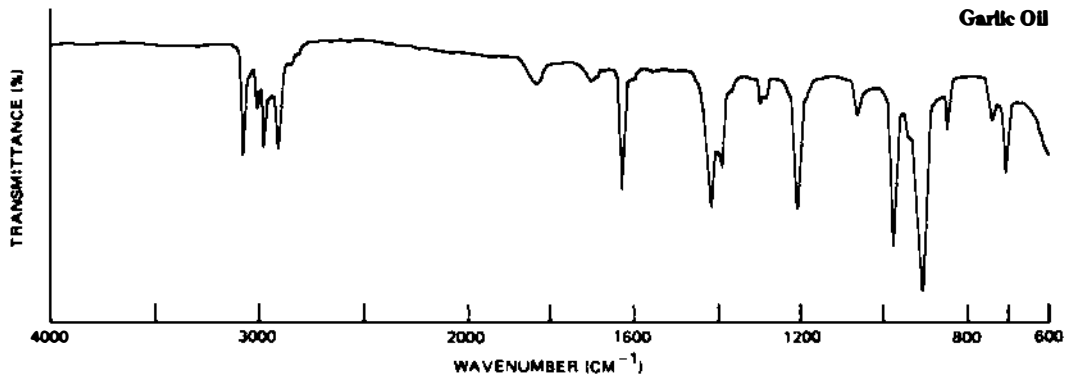
Fir Needle Oil, Canadian Type



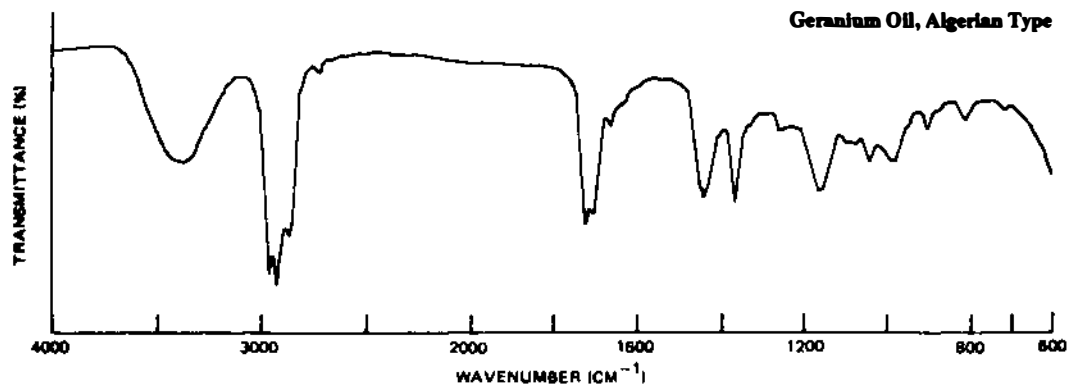
Fir Needle Oil, Siberian Type



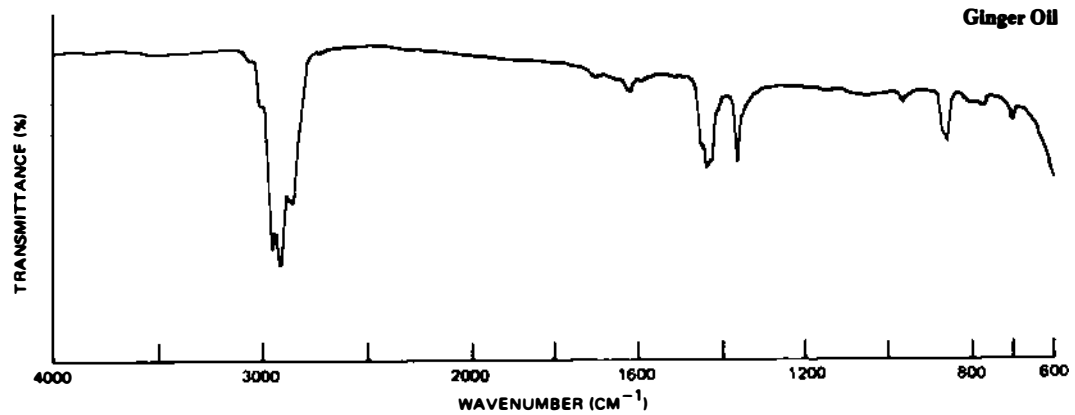
Garlic Oil



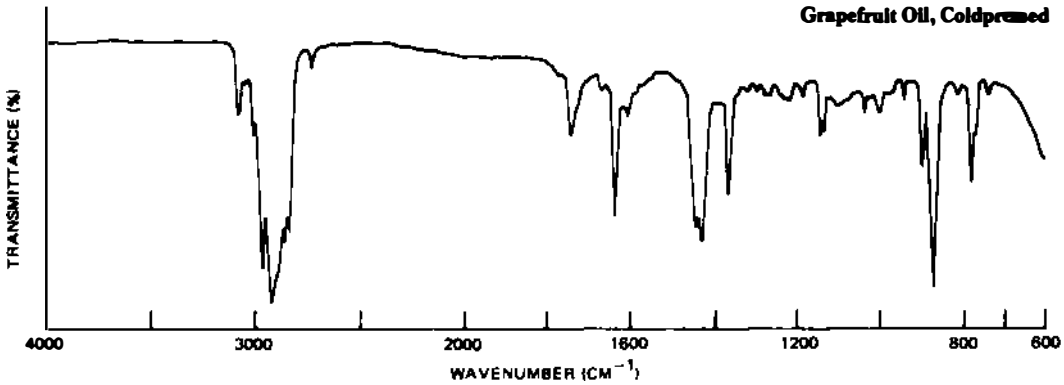
Geranium Oil, Algerian Type



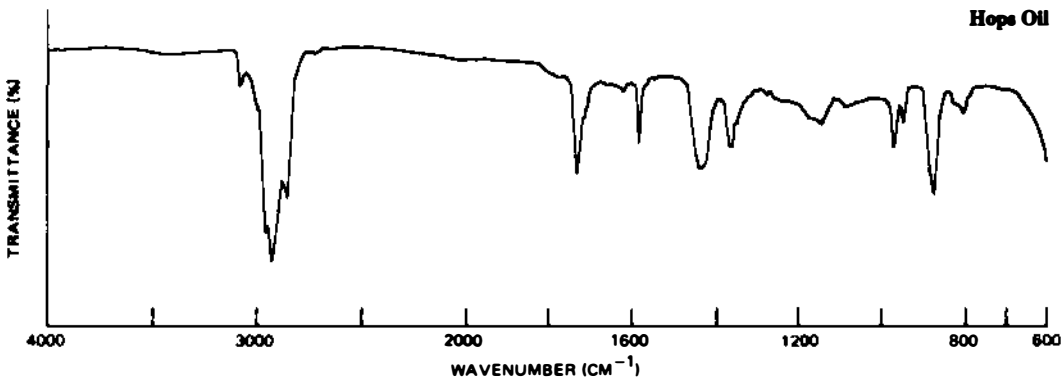
Ginger Oil



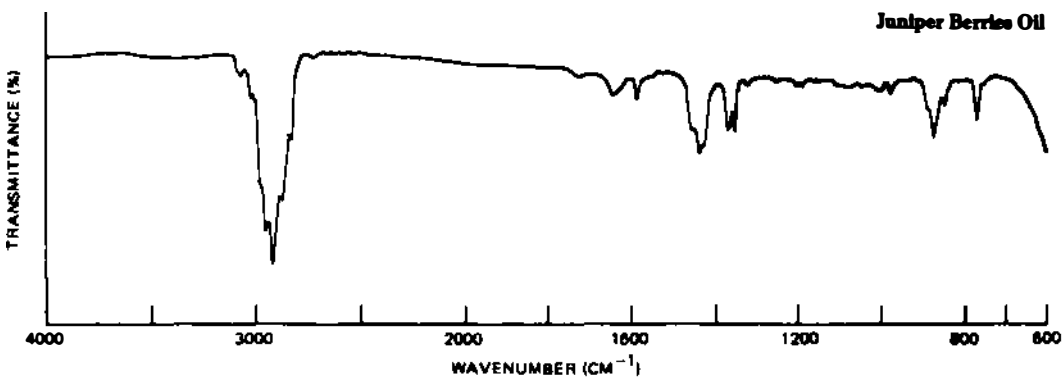
Grapefruit Oil, Coldpressed



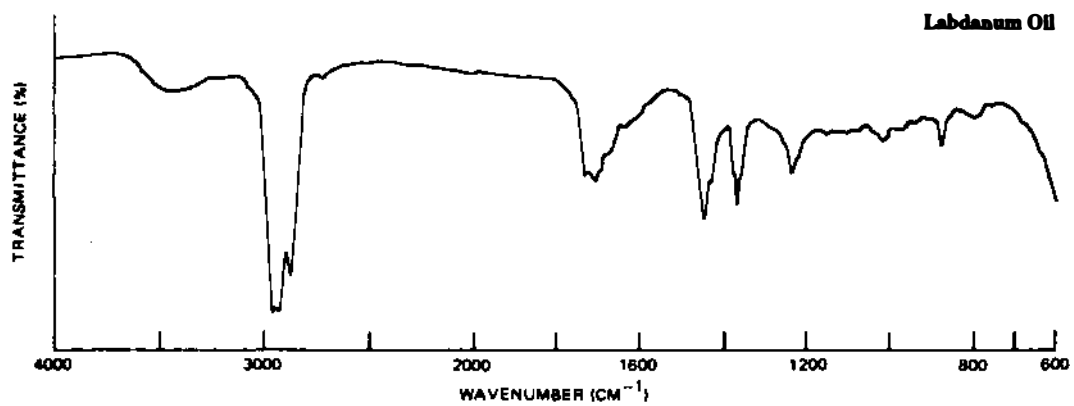
Hops Oil



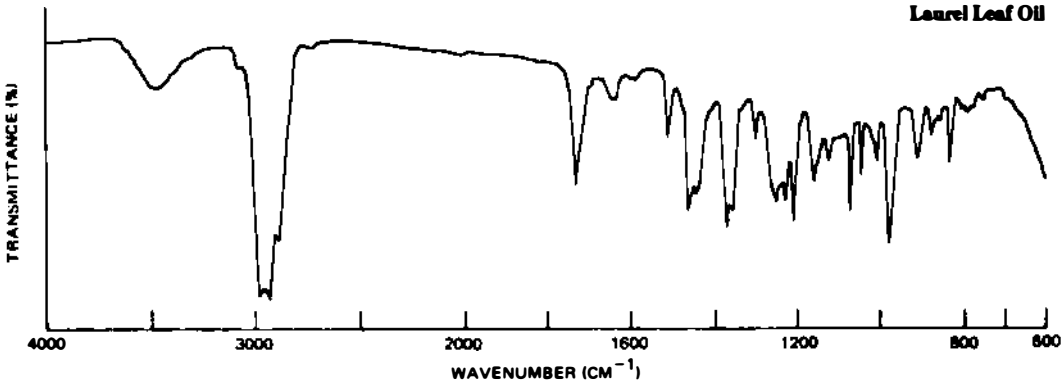
Juniper Berries Oil



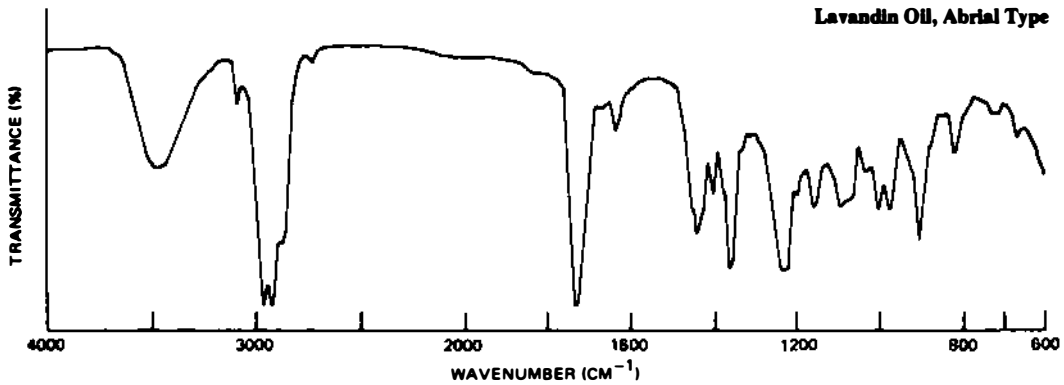
Labdanum Oil



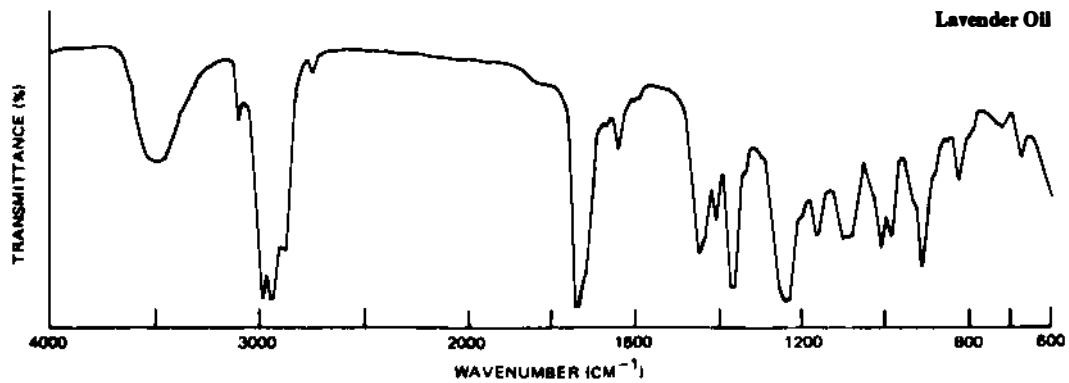
Laurel Leaf Oil



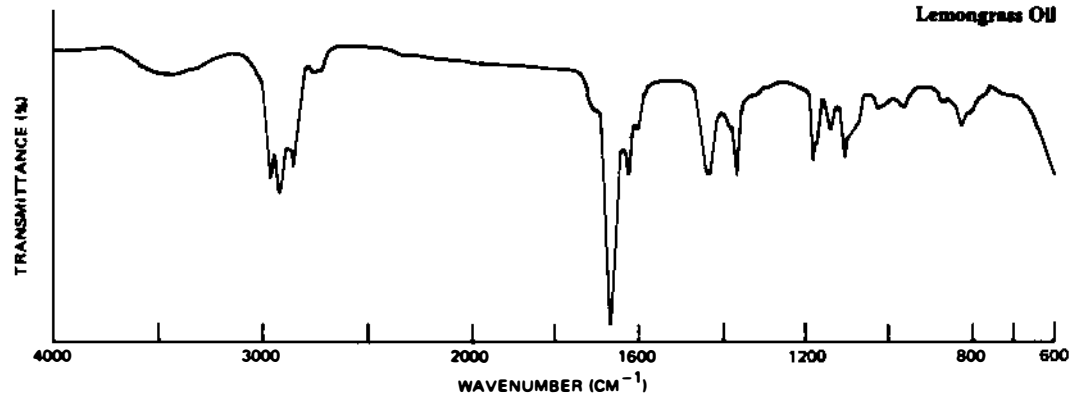
Lavandin Oil, Abrial Type



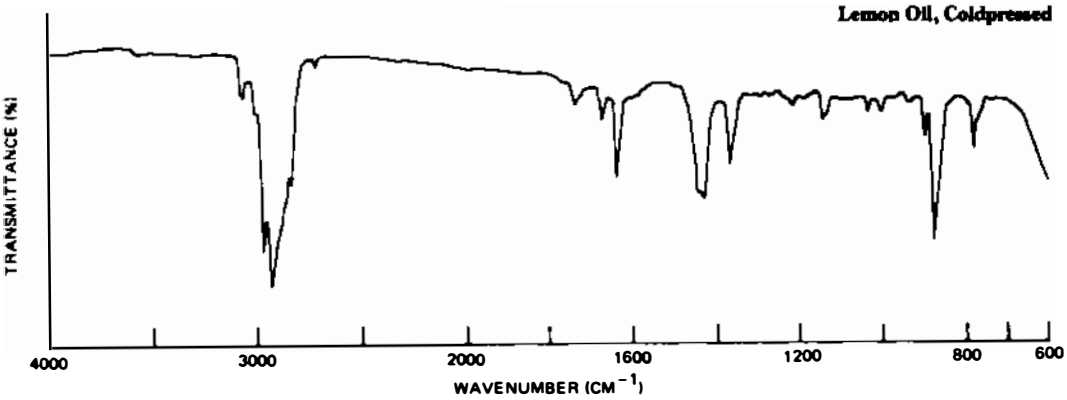
Lavender Oil



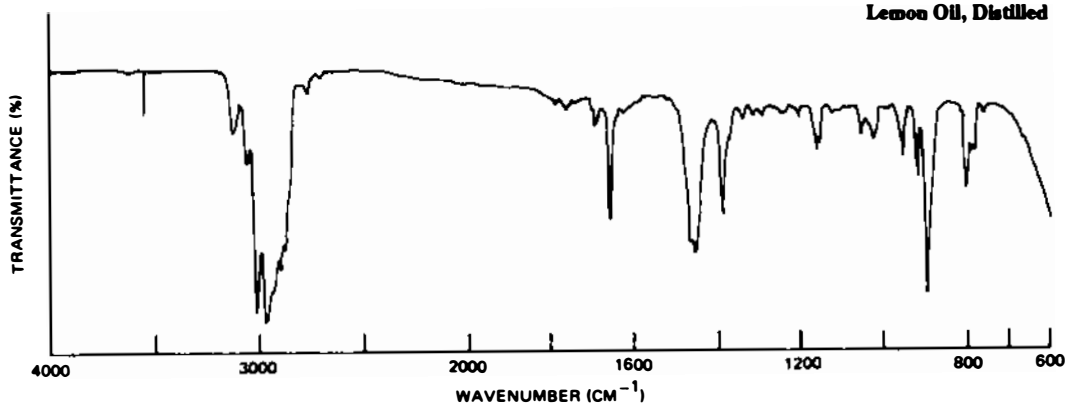
Lemongrass Oil



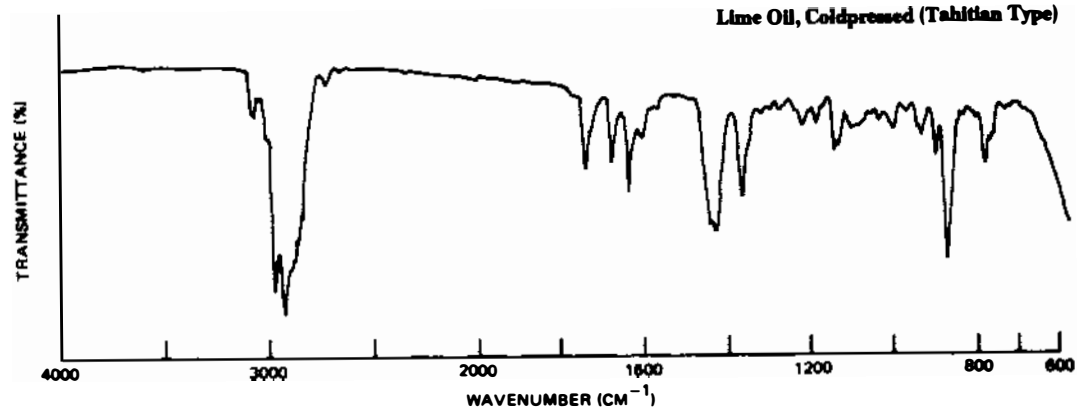
Lemon Oil, Coldpressed



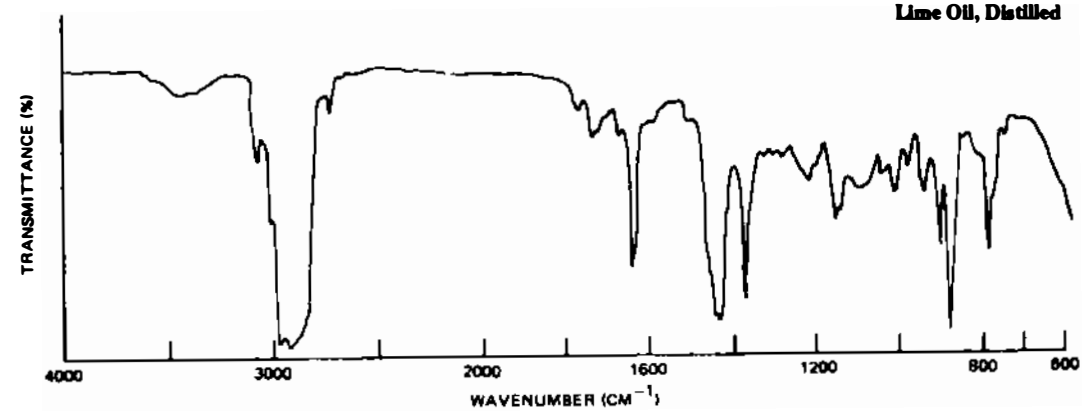
Lemon Oil, Distilled



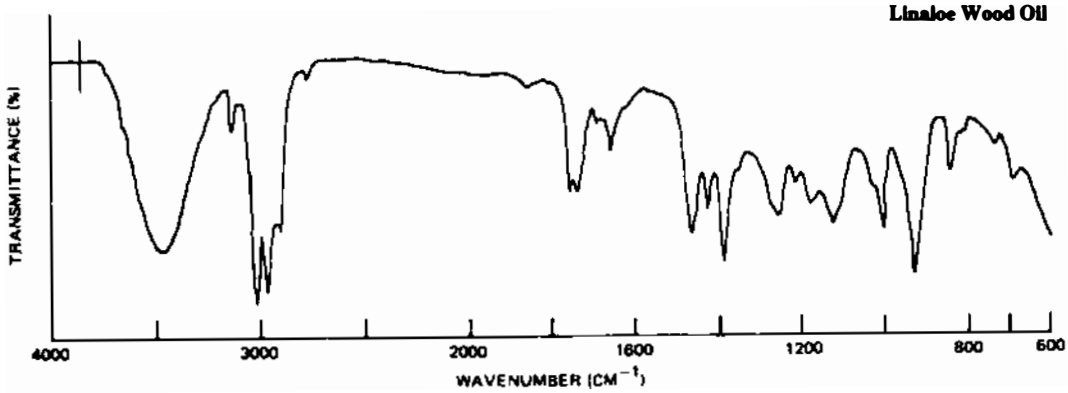
Lime Oil, Coldpressed (Tahitian Type)



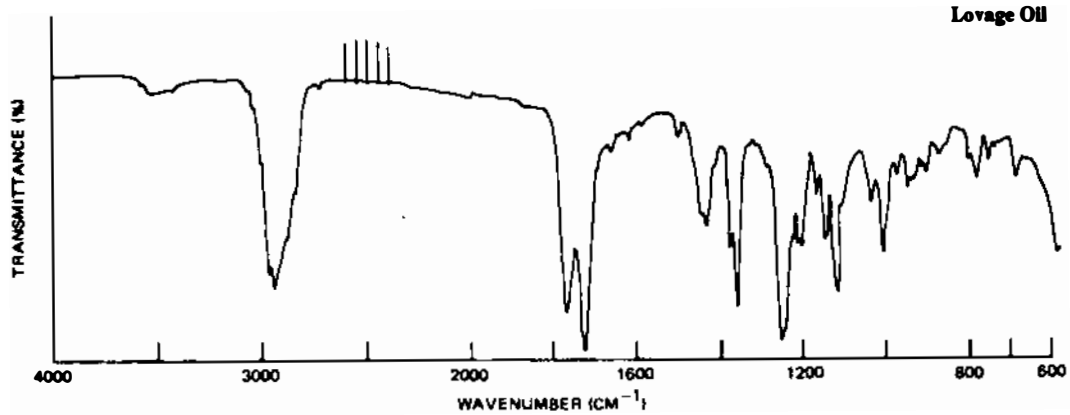
Lime Oil, Distilled



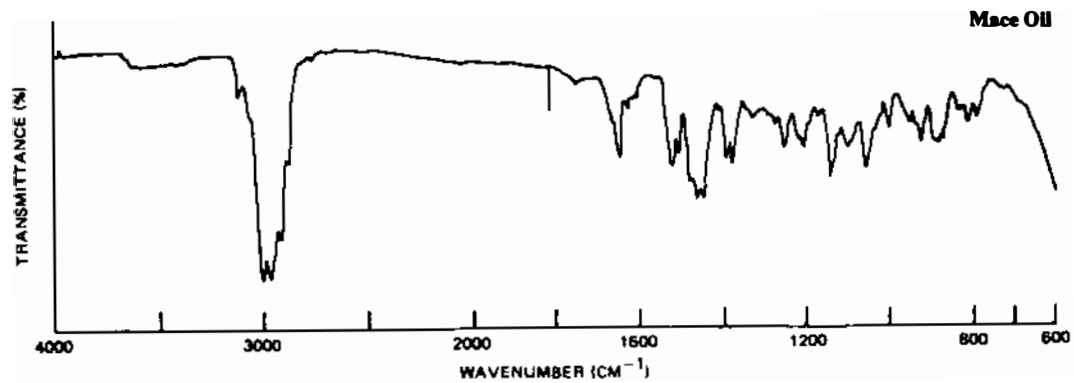
Linaloe Wood Oil



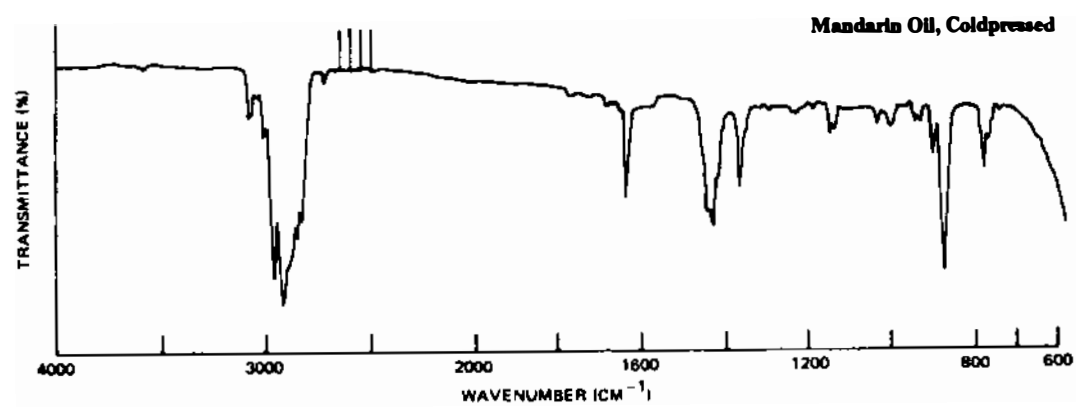
Lovage Oil



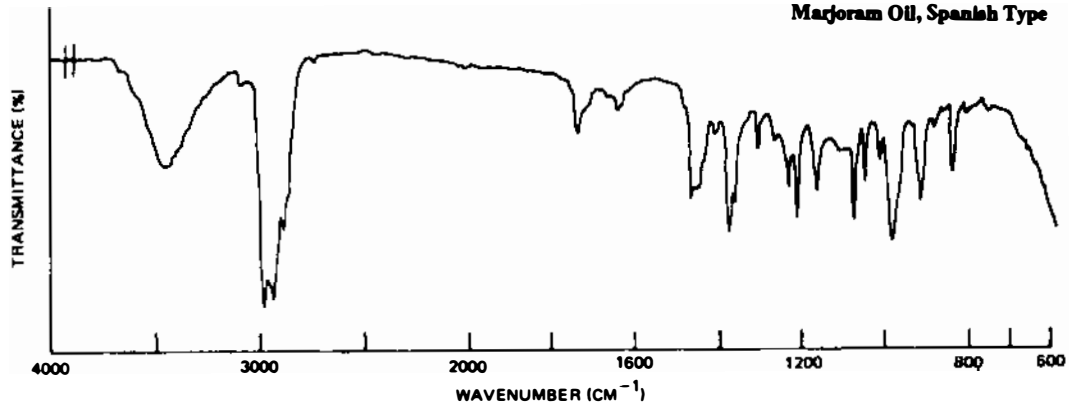
Mace Oil



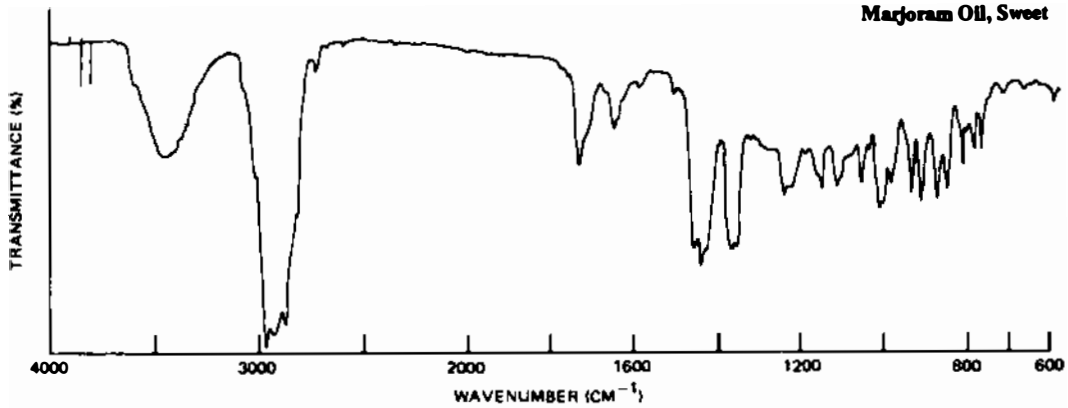
Mandarin Oil, Coldpressed



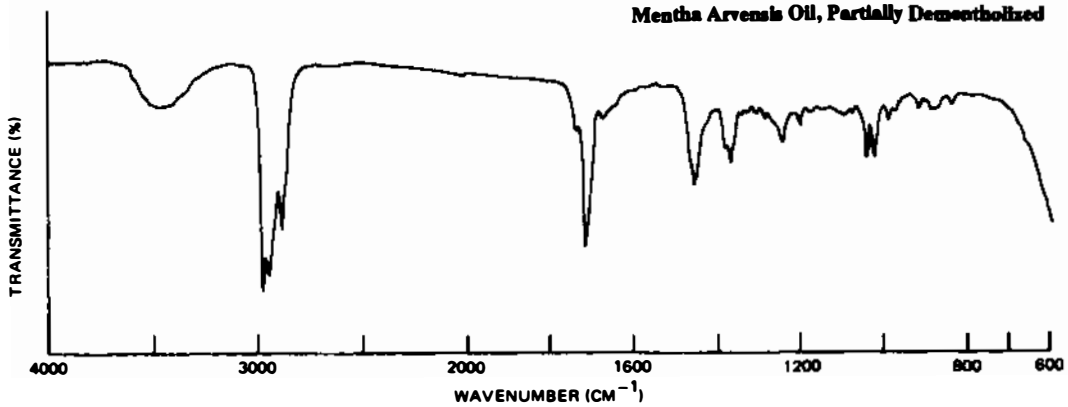
Marjoram Oil, Spanish Type



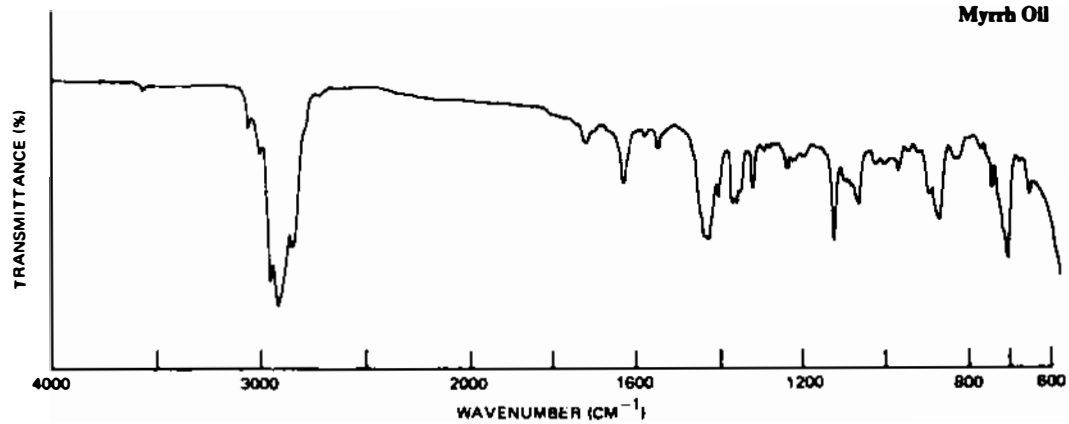
Marjoram Oil, Sweet



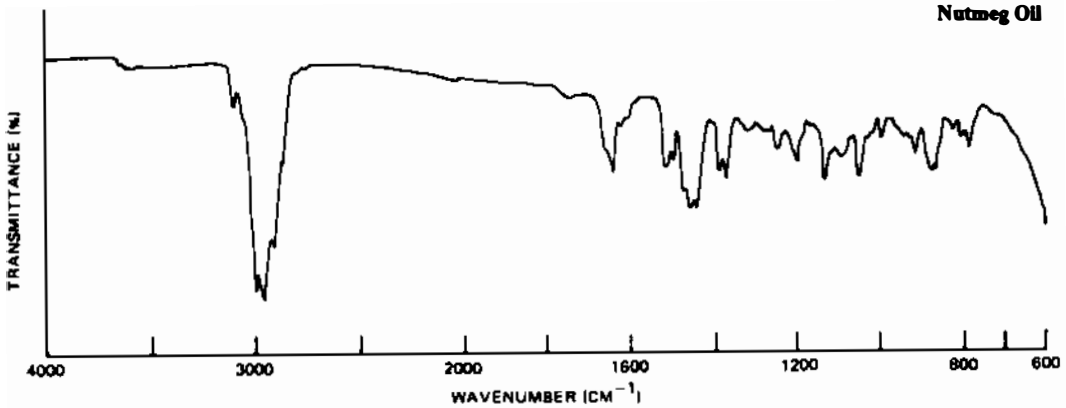
Mentha Arvensis Oil, Partially Dementhollzed



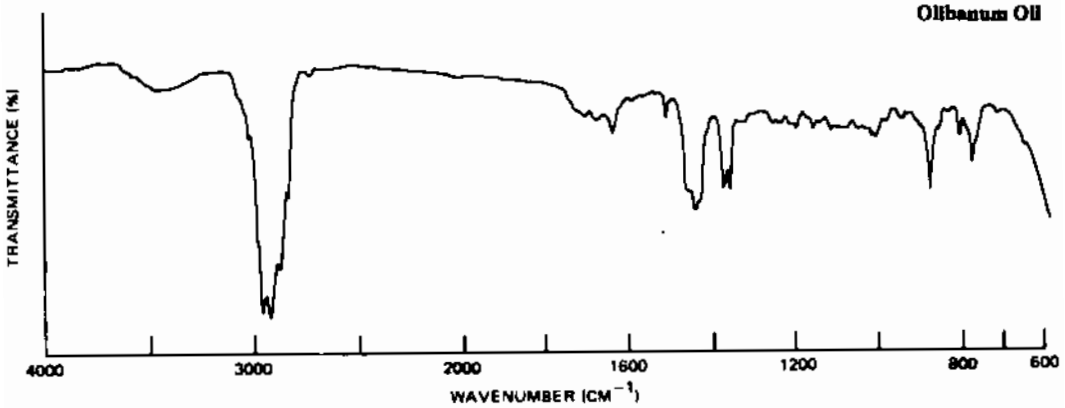
Myrrh Oil



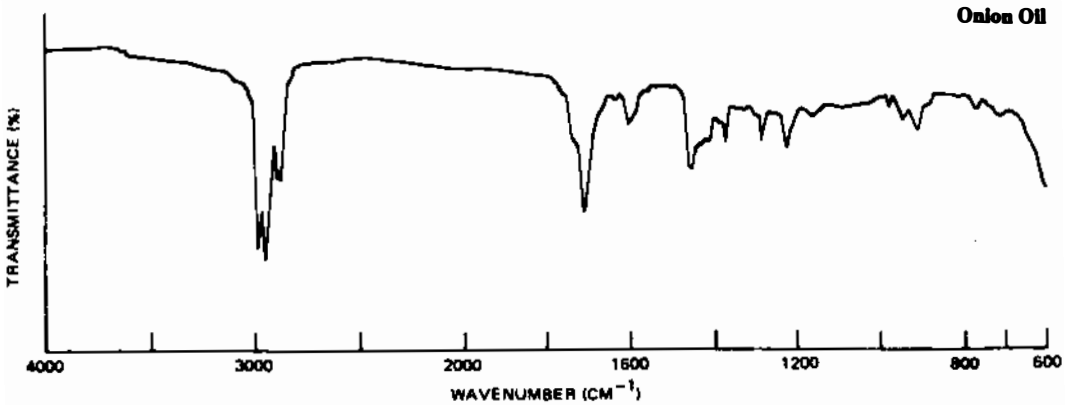
Nutmeg Oil



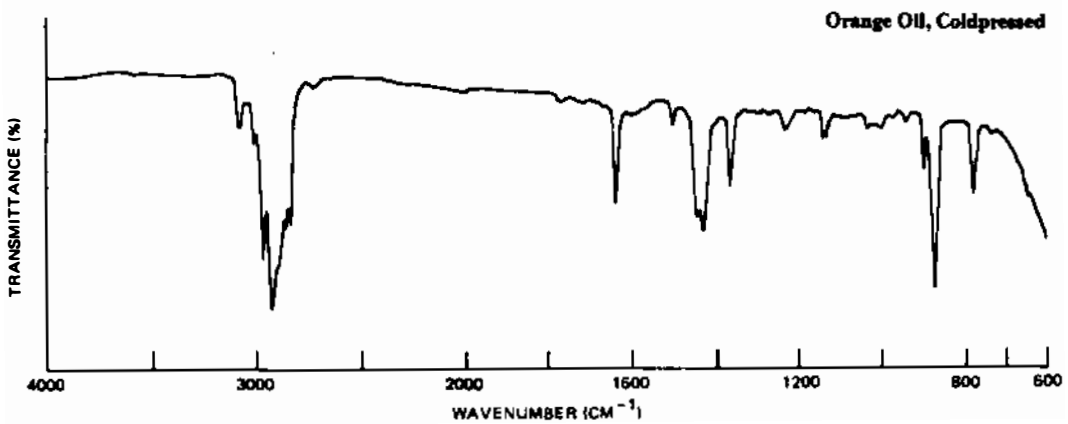
Olibanum Oil



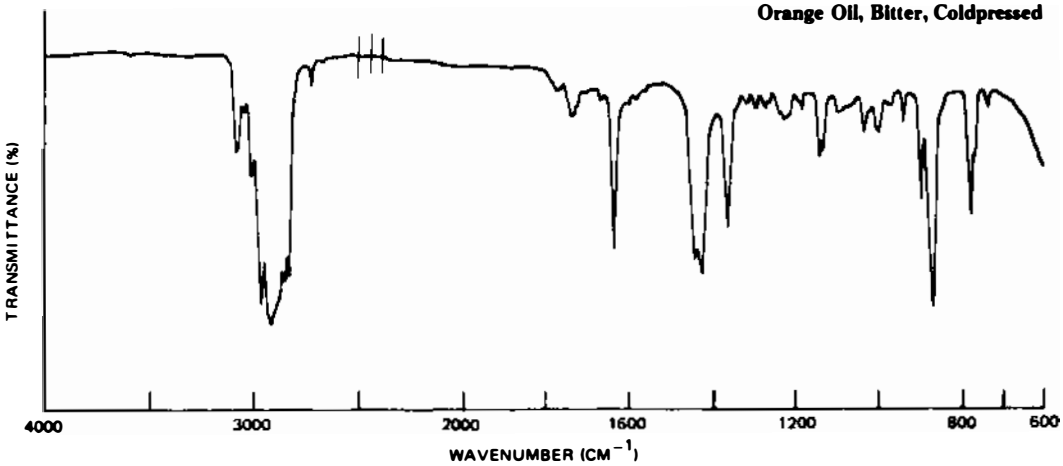
Onion Oil



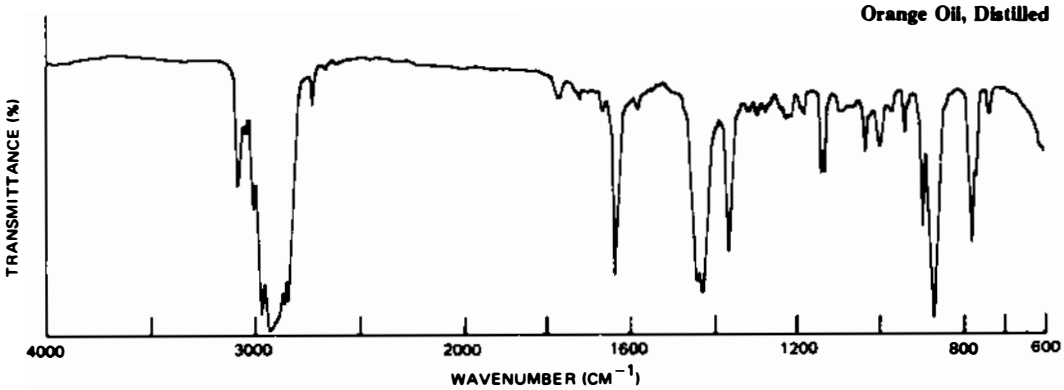
Orange Oil, Coldpressed



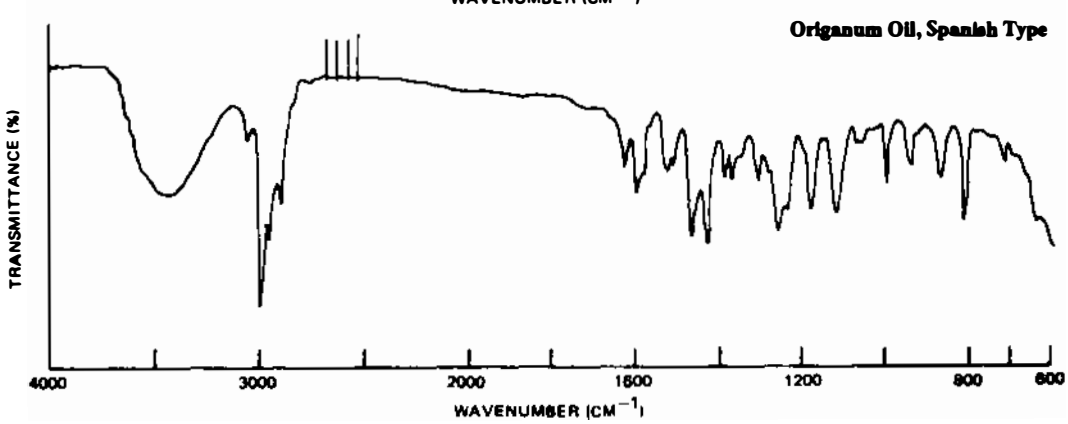
Orange Oil, Bitter, Coldpressed



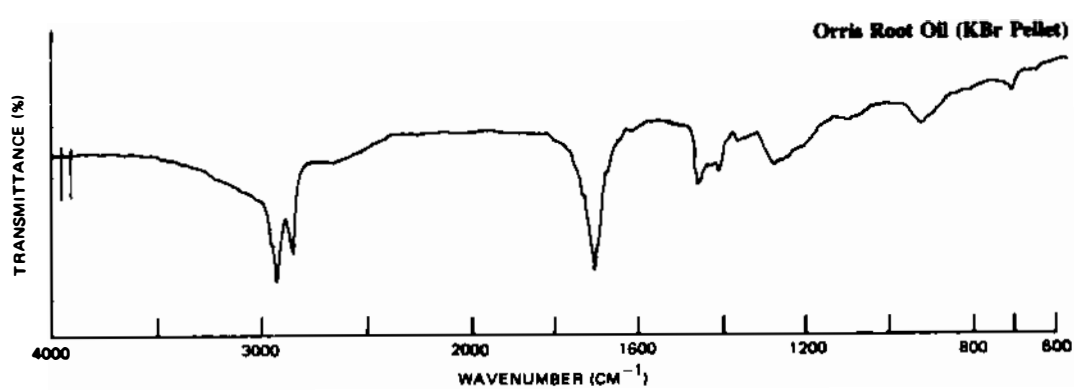
Orange Oil, Distilled



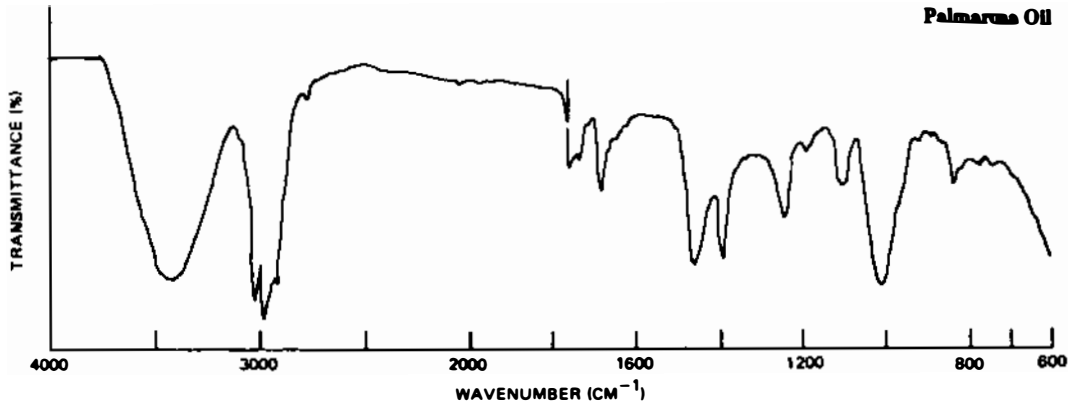
Origanum Oil, Spanish Type



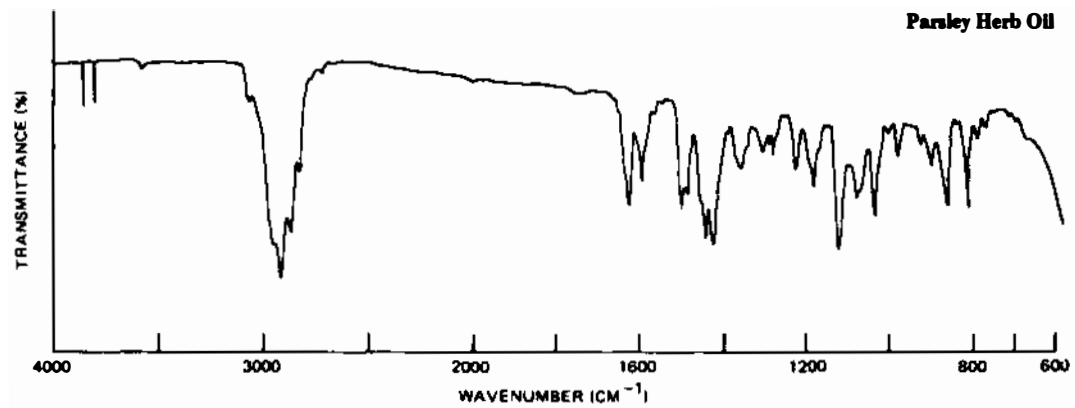
Orris Root Oil (KBr Pellet)



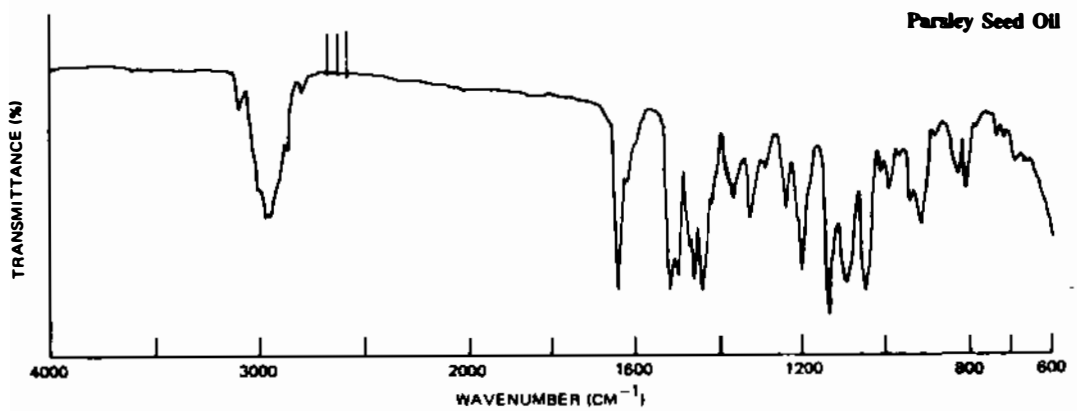
Palmarosa Oil



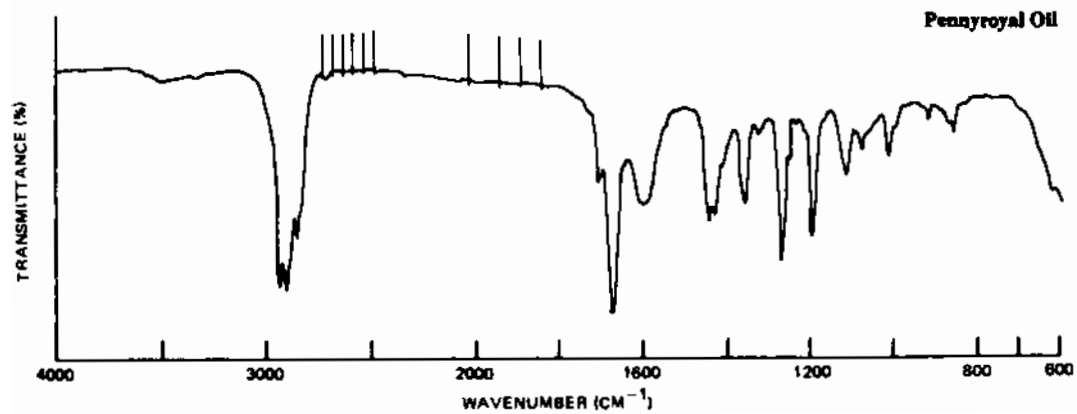
Parsley Herb Oil



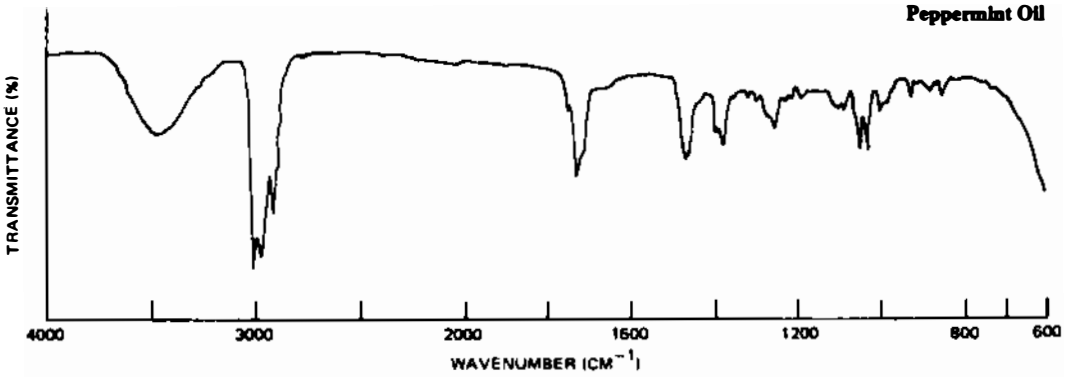
Parsley Seed Oil



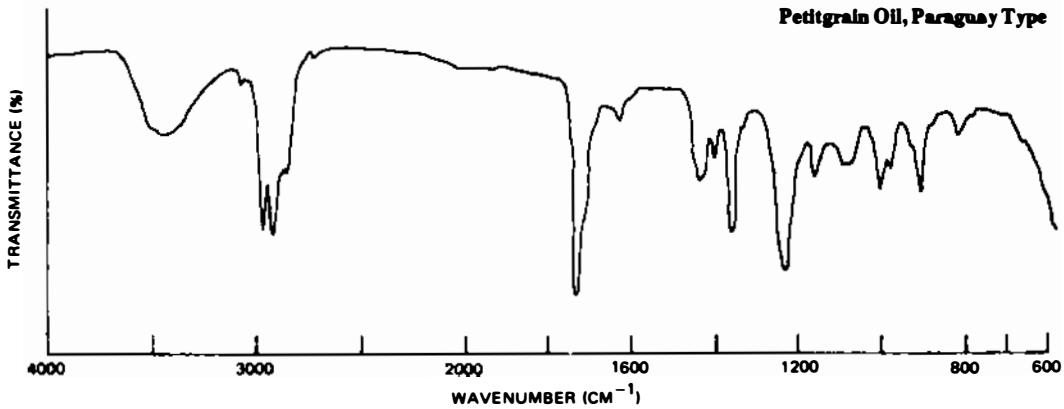
Pennyroyal Oil



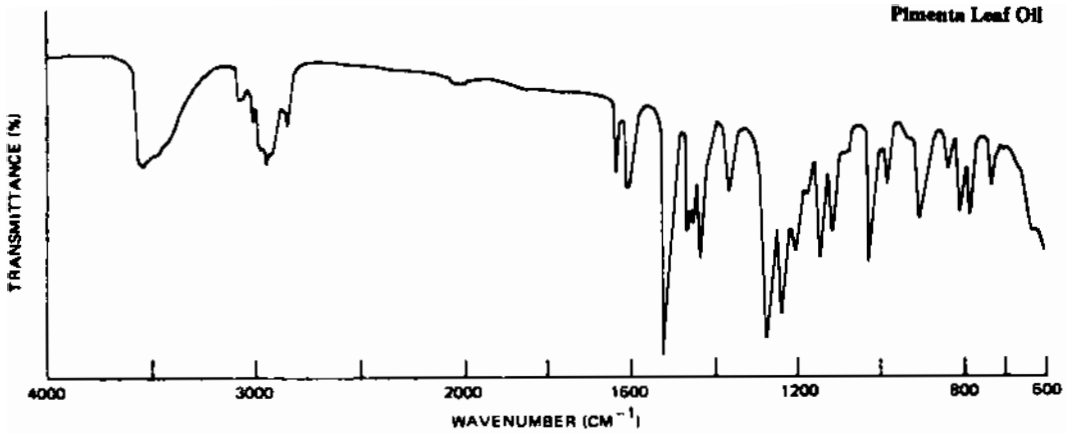
Peppermint Oil



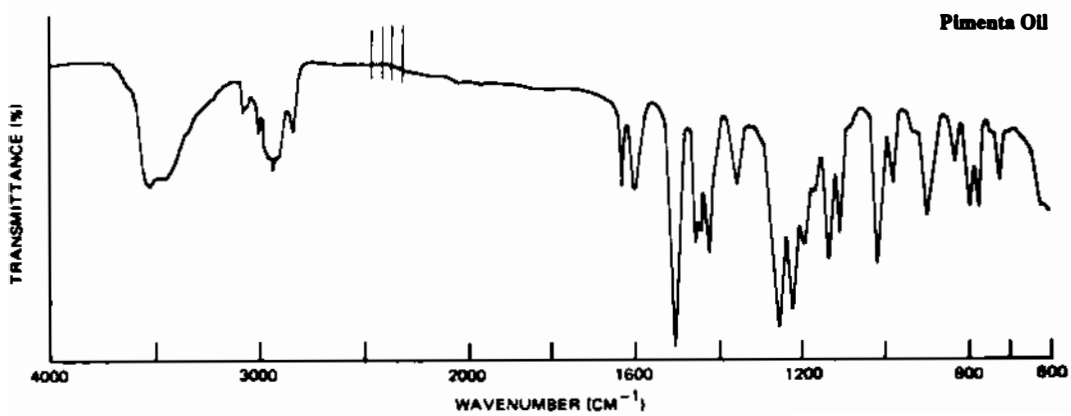
Petitgrain Oil, Paraguay Type



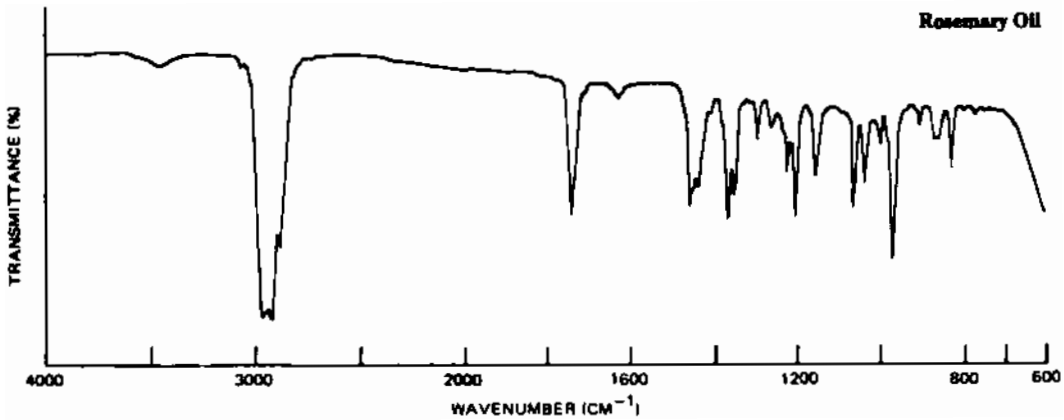
Pimenta Leaf Oil



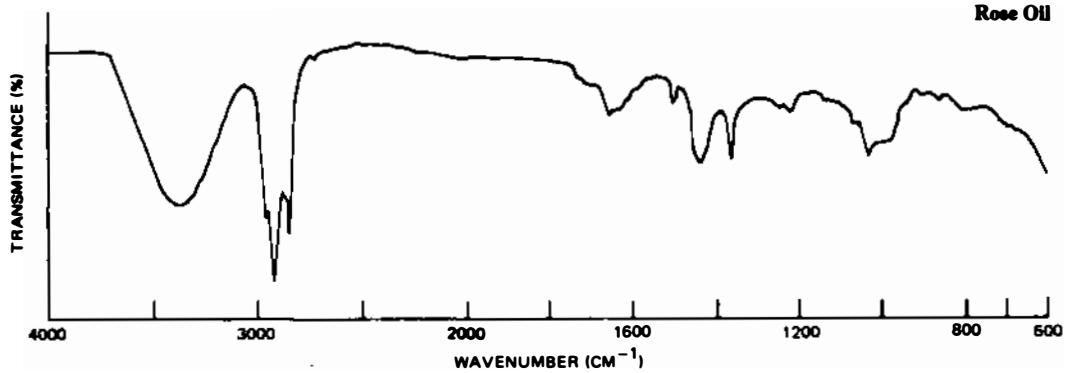
Pimenta Oil



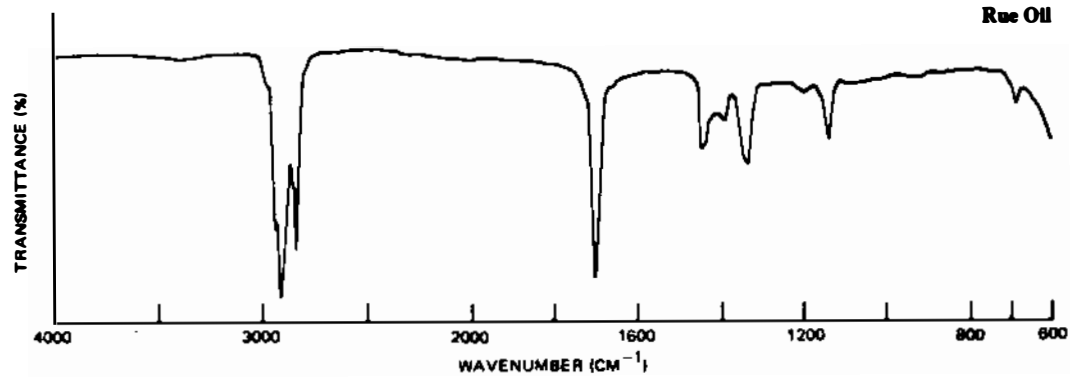
Rosemary Oil



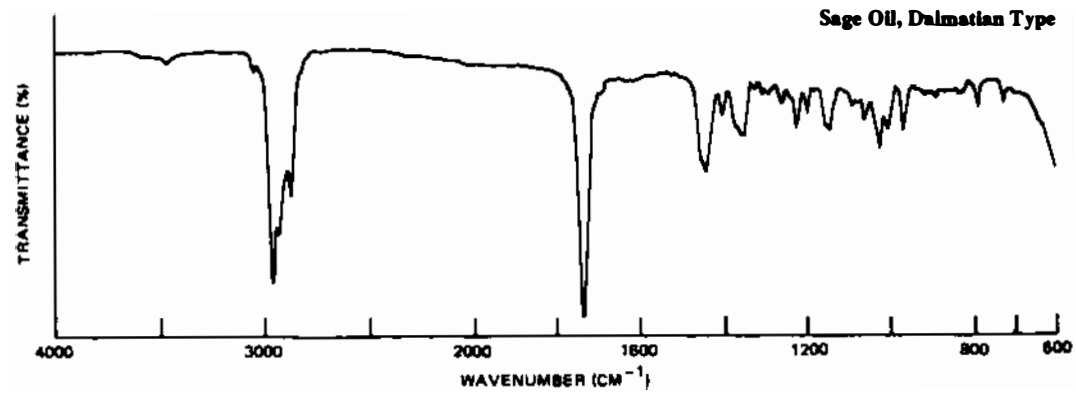
Rose Oil



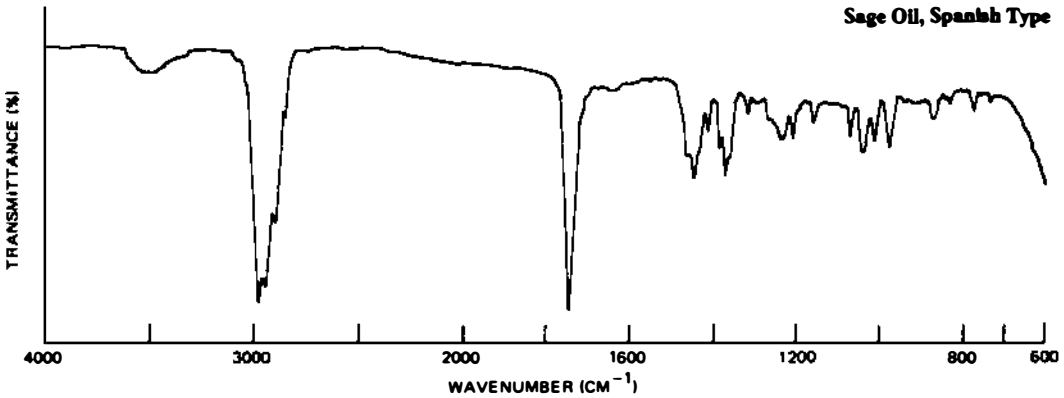
Rue Oil



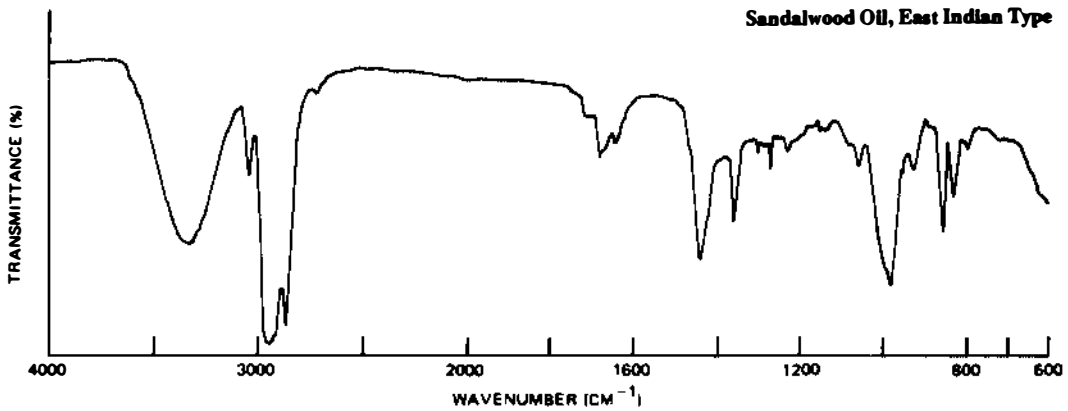
Sage Oil, Dalmatian Type



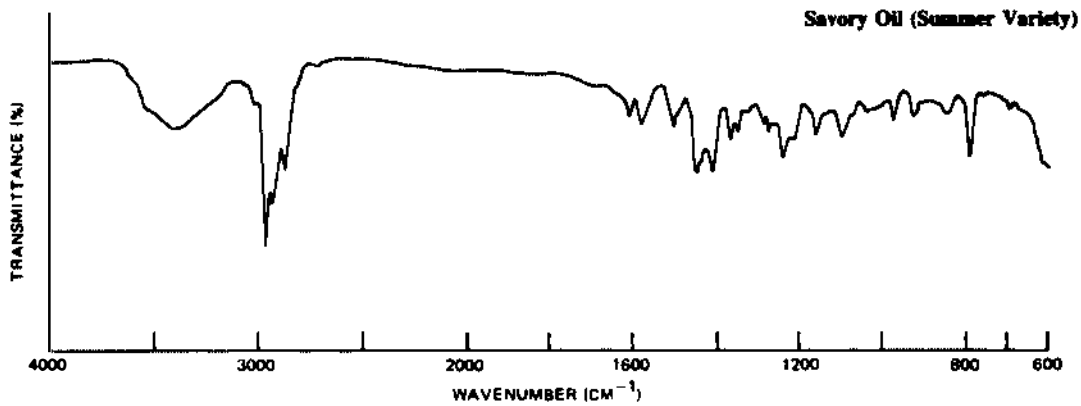
Sage Oil, Spanish Type



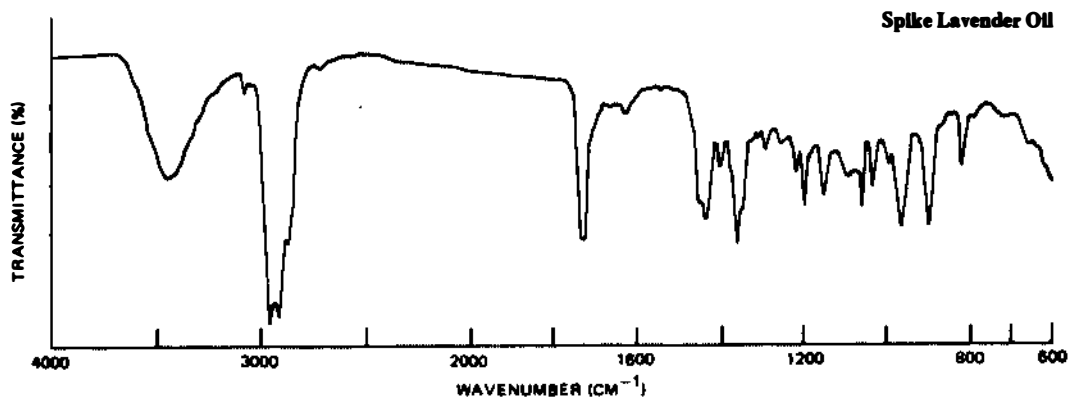
Sandalwood Oil, East Indian Type



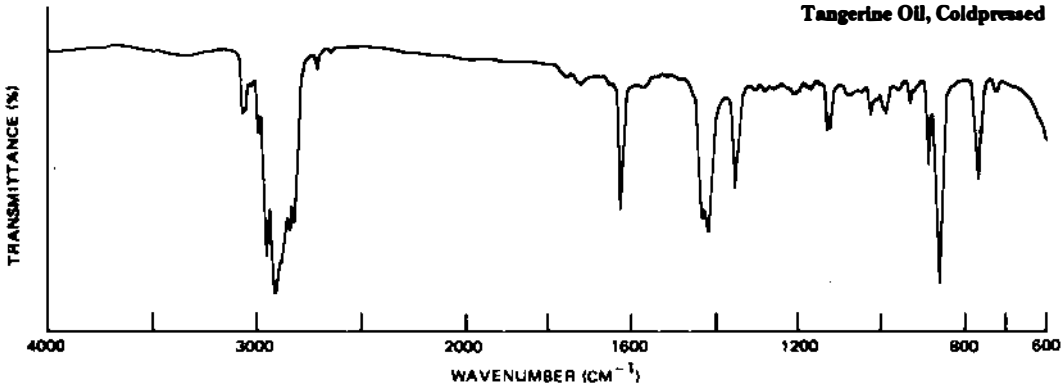
Savory Oil (Summer Variety)



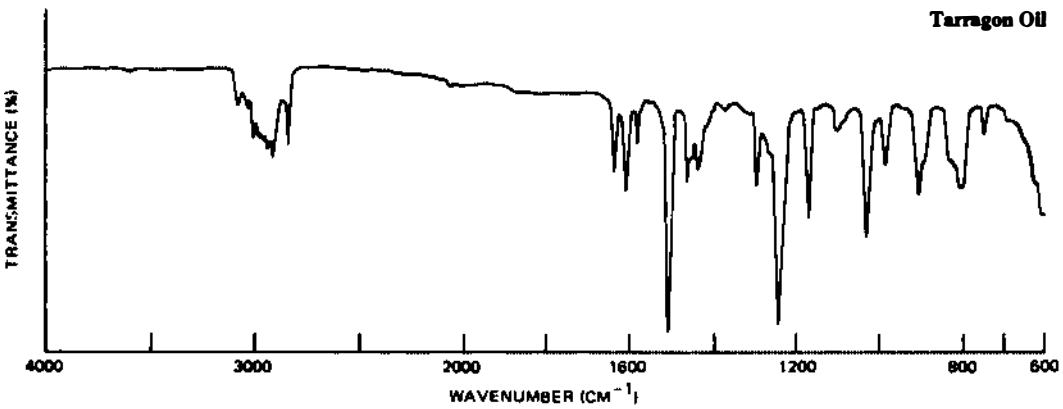
Spike Lavender Oil



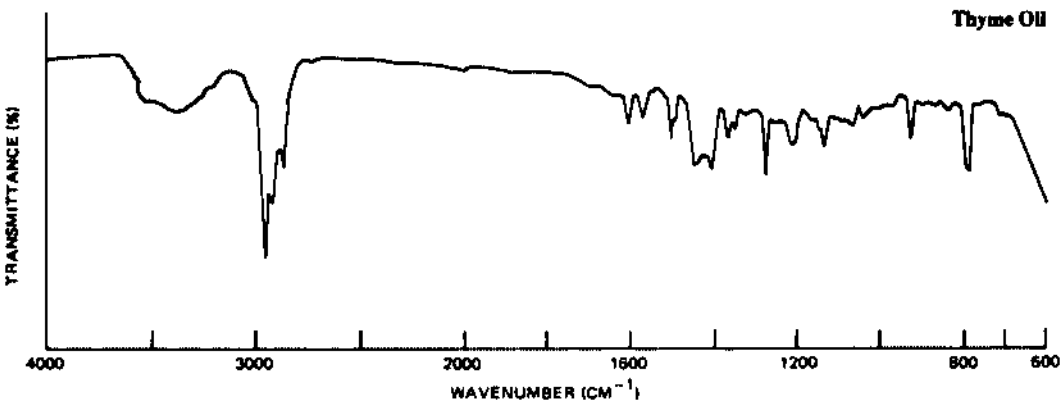
Tangerine Oil, Coldpressed



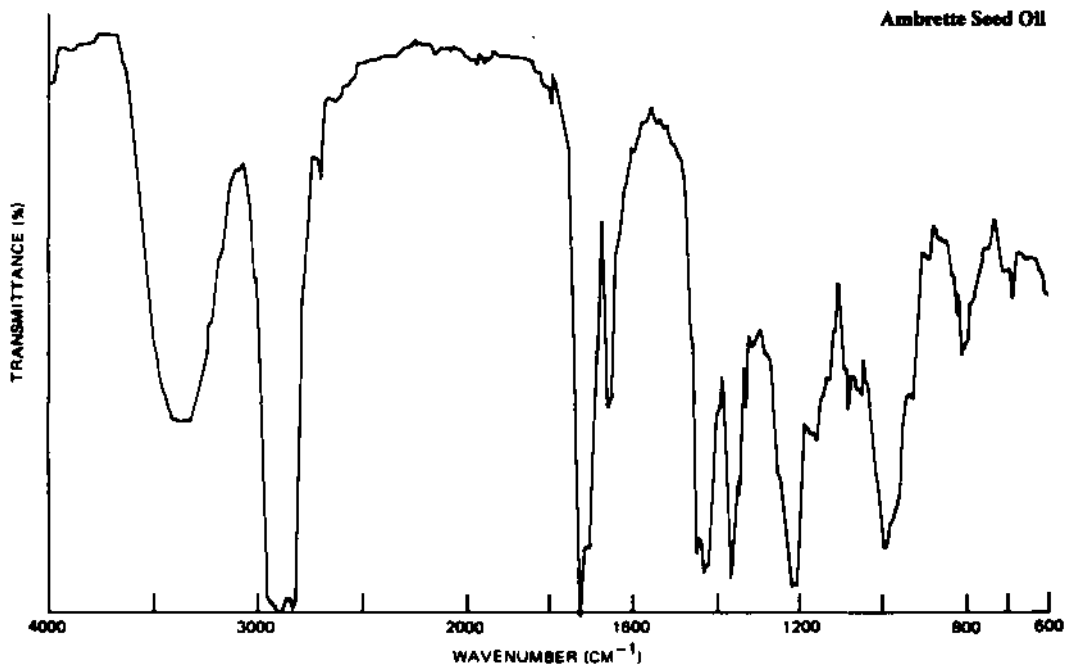
Tarragon Oil



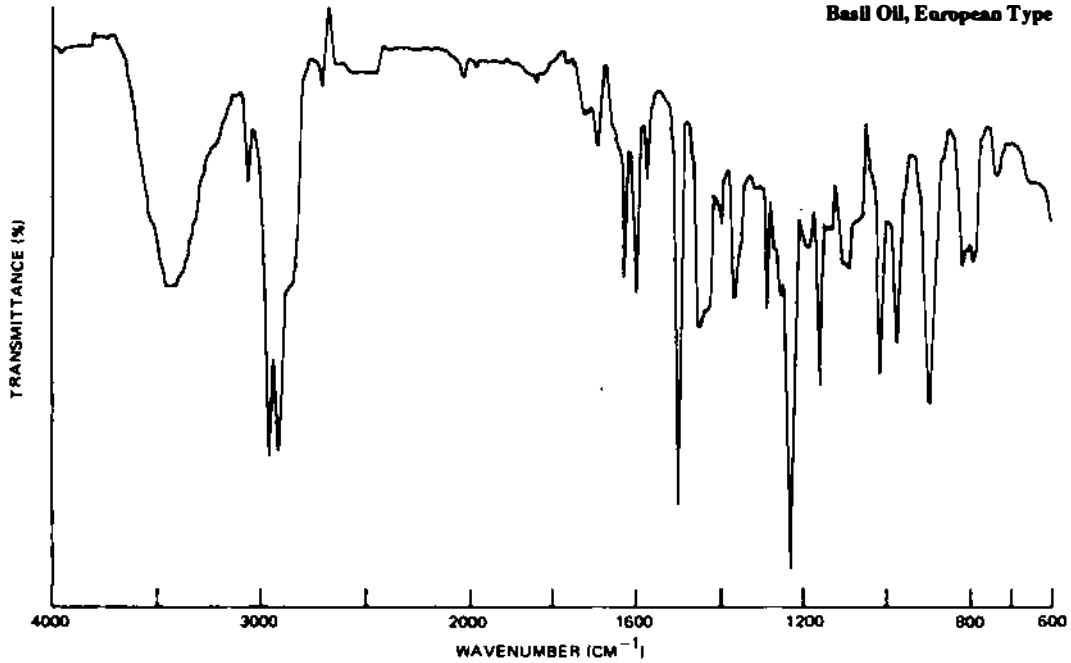
Thyme Oil



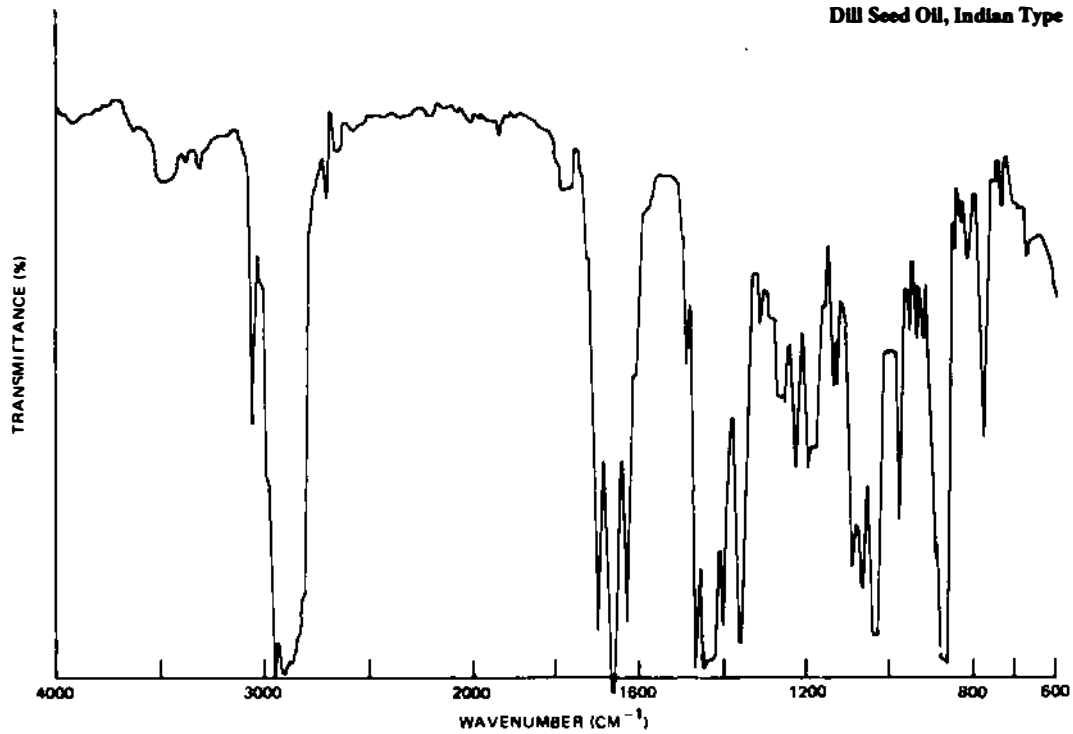
SERIES A-2

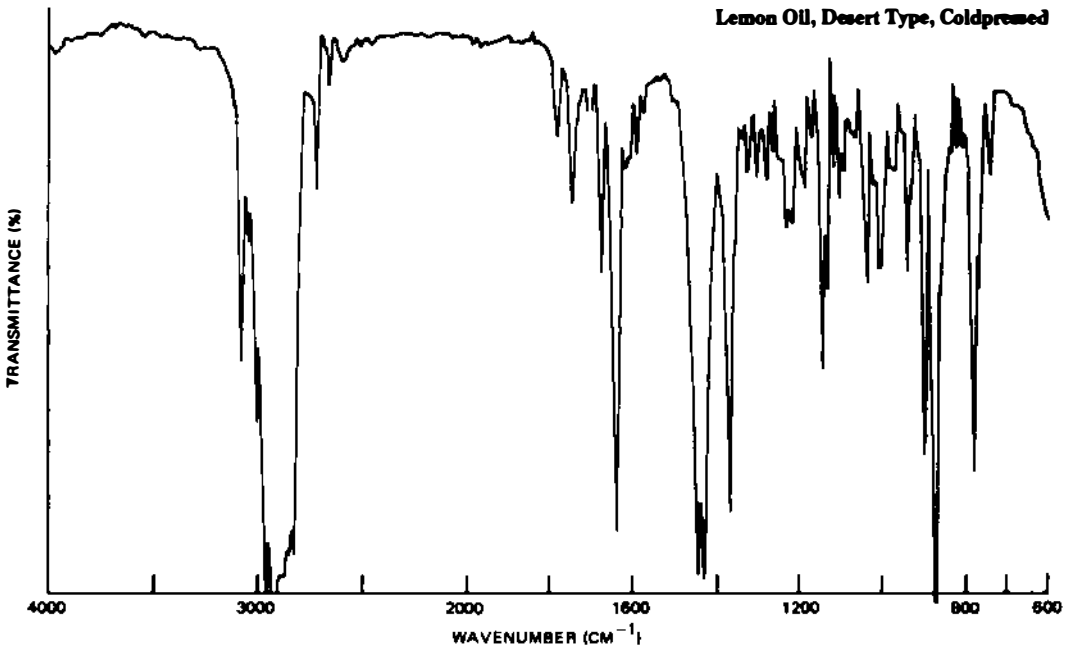


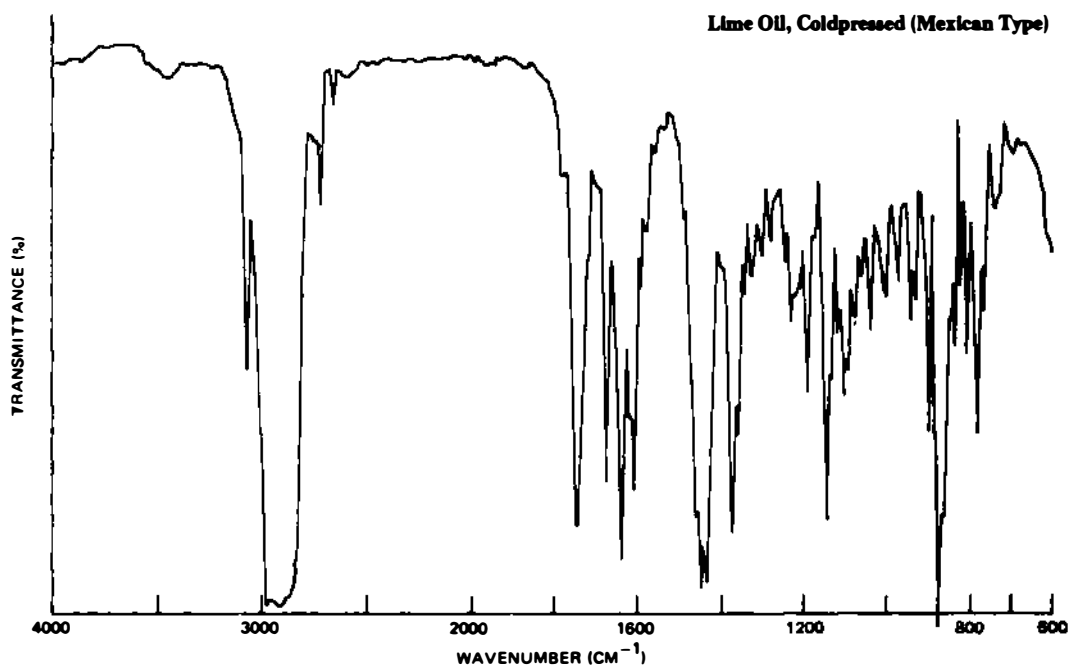
Basil Oil, European Type



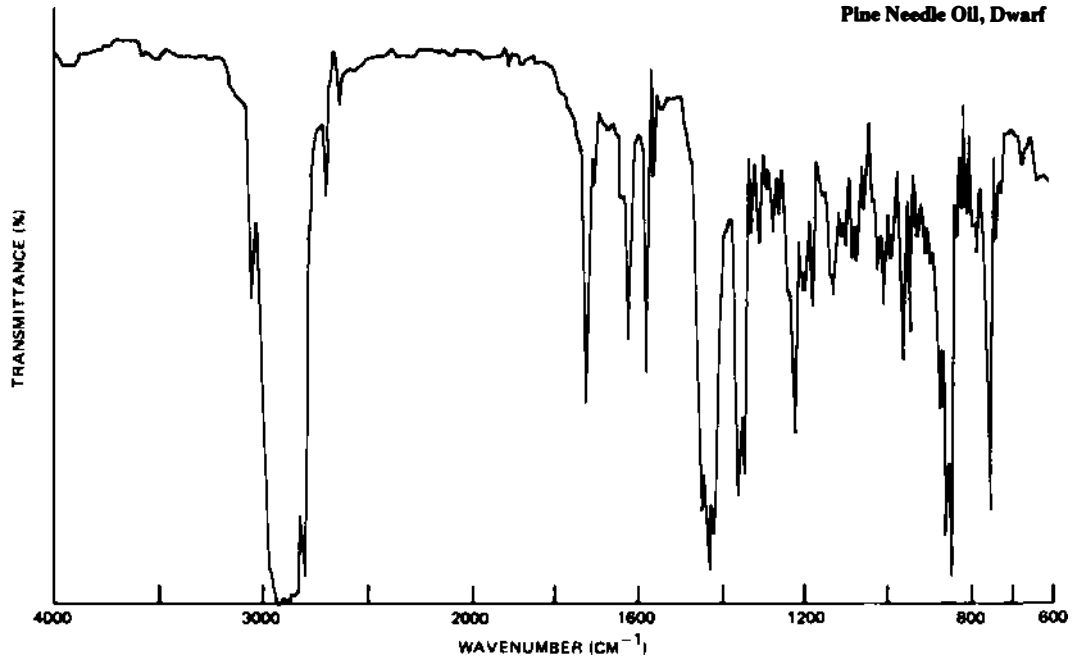
Dill Seed Oil, Indian Type



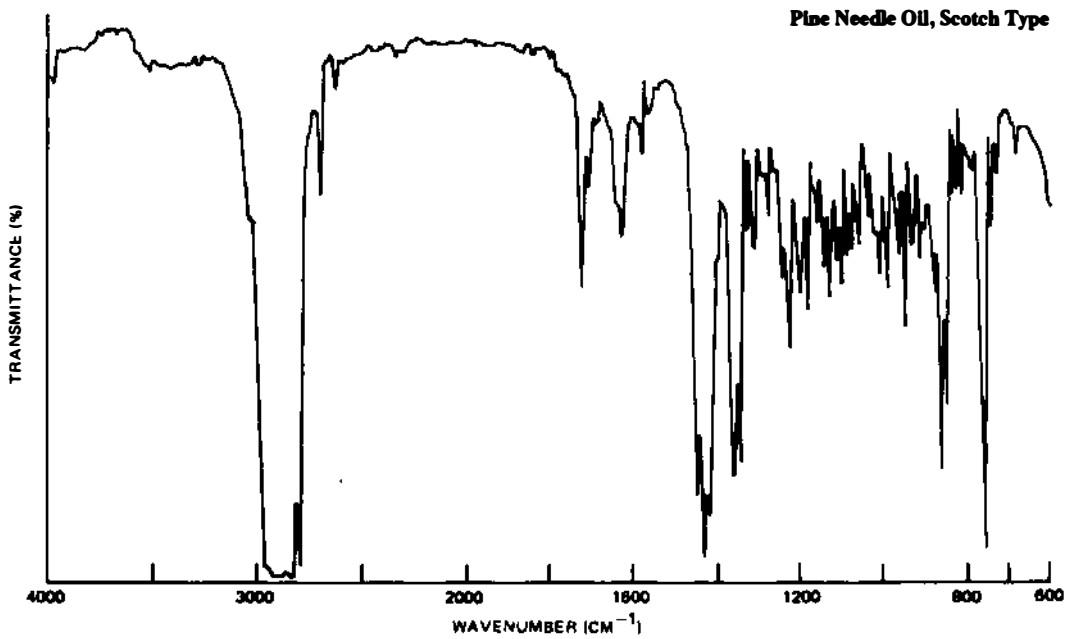




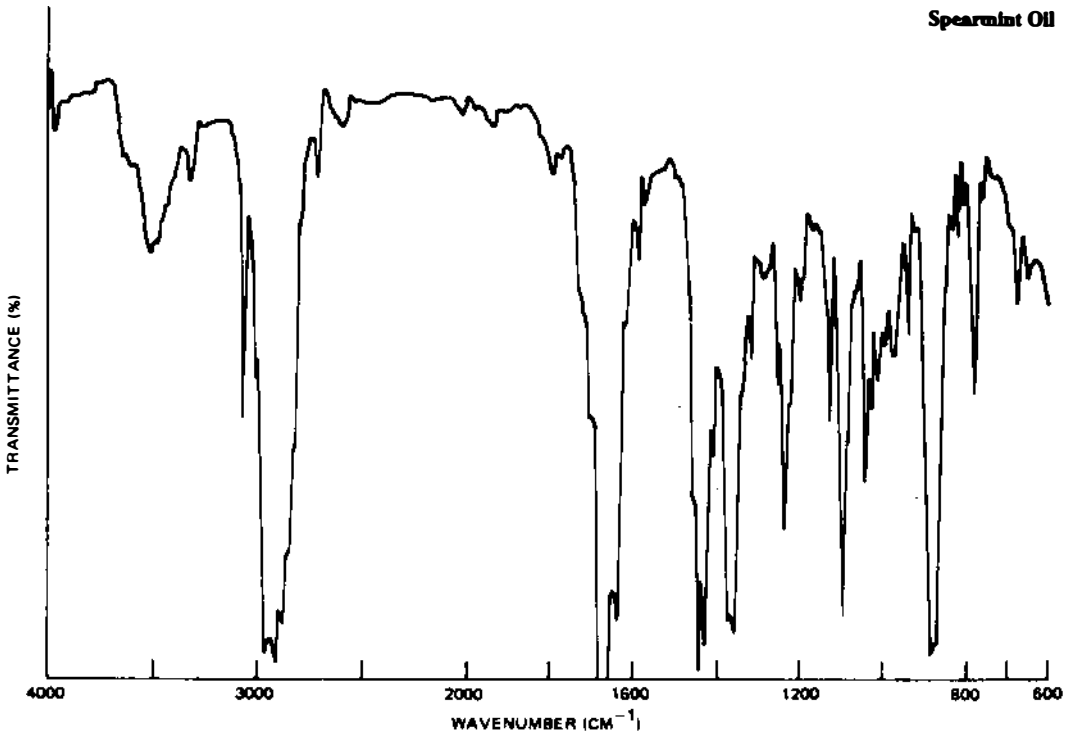
Pine Needle Oil, Dwarf



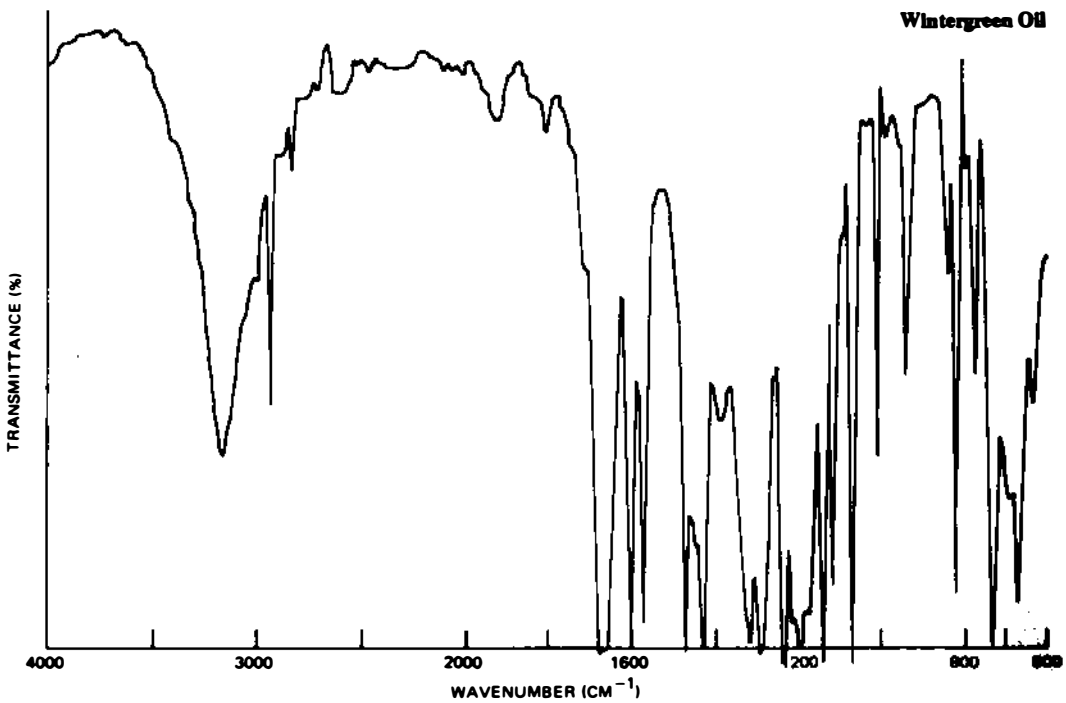
Pine Needle Oil, Scotch Type



Spearmint Oil



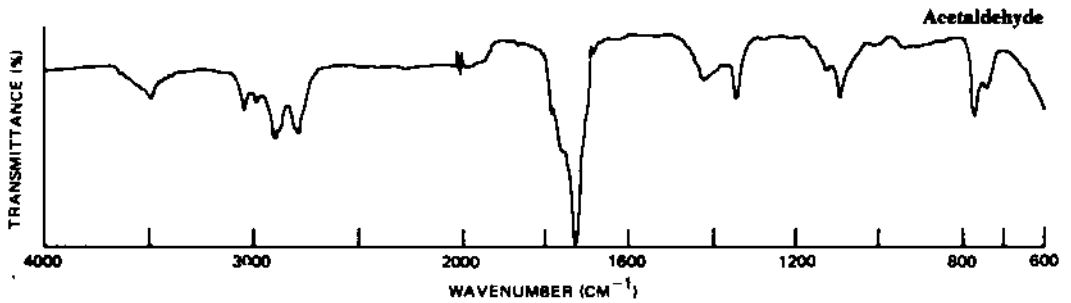
Wintergreen Oil

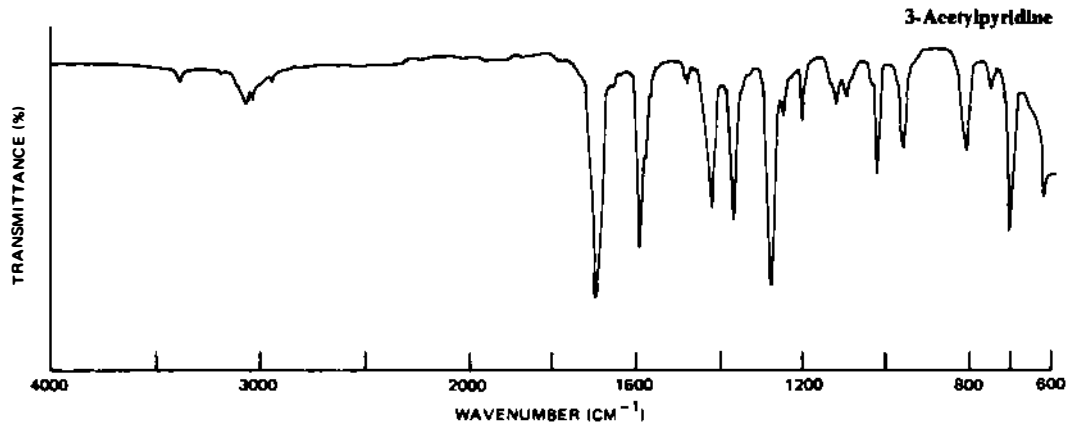


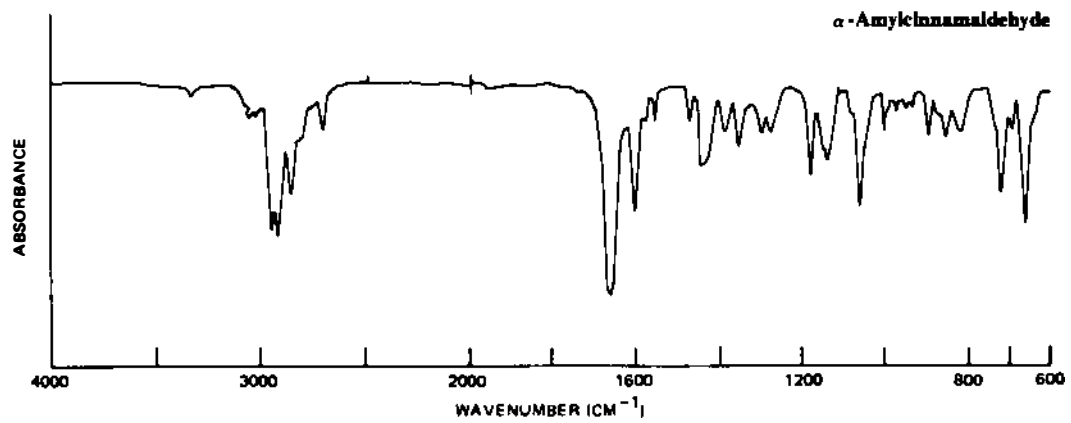
Series B: Flavor Aromatic Chemicals and Isolates

This series is divided into three subseries, depending upon the format of the spectra involved. The substances are listed below alphabetically.

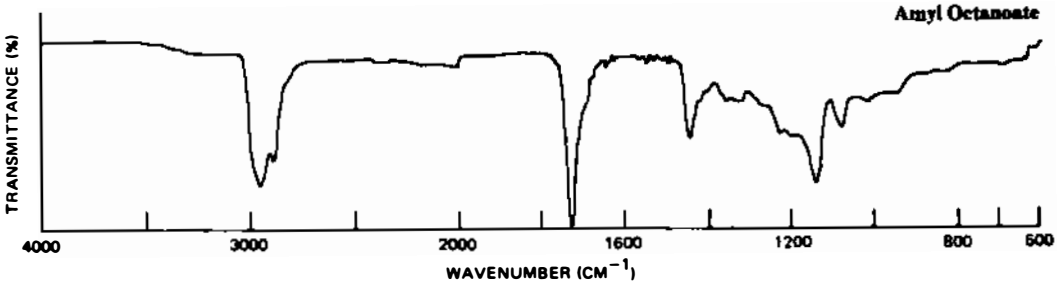
SERIES B-1


Acetaldehyde
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM⁻¹)

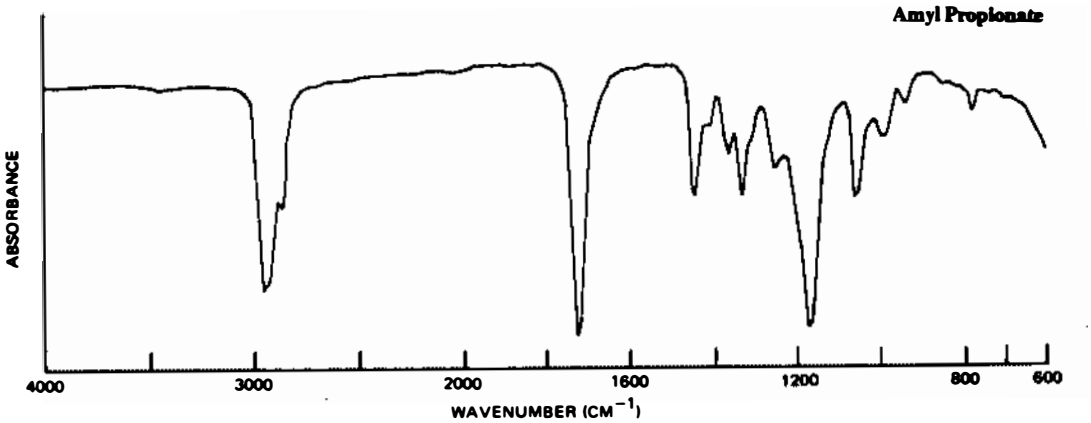

3-Acetylpyridine
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM⁻¹)


α-Amylcinnamaldehyde
ABSORBANCE
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM⁻¹)

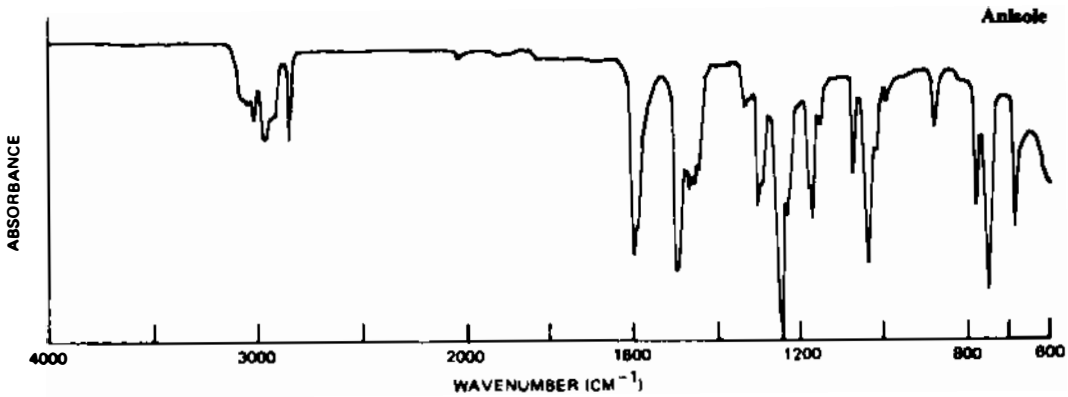
Amyl Octanoate



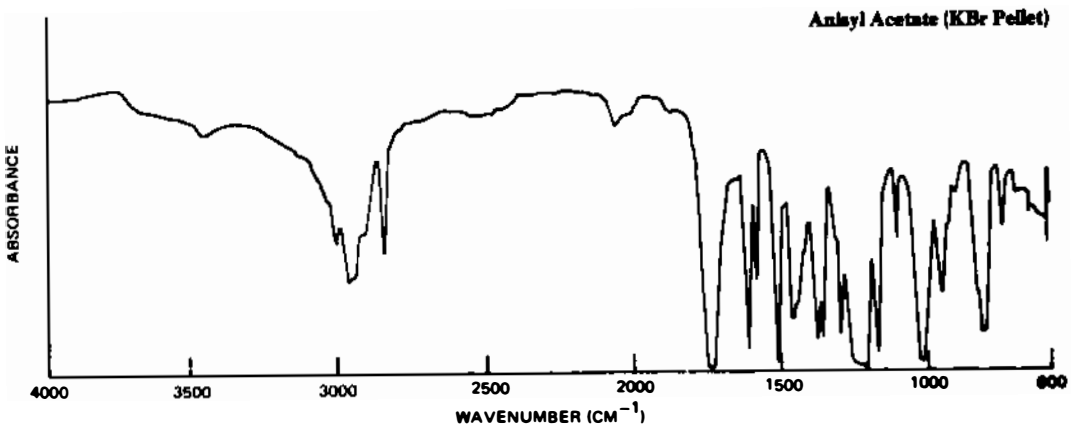
Amyl Propionate



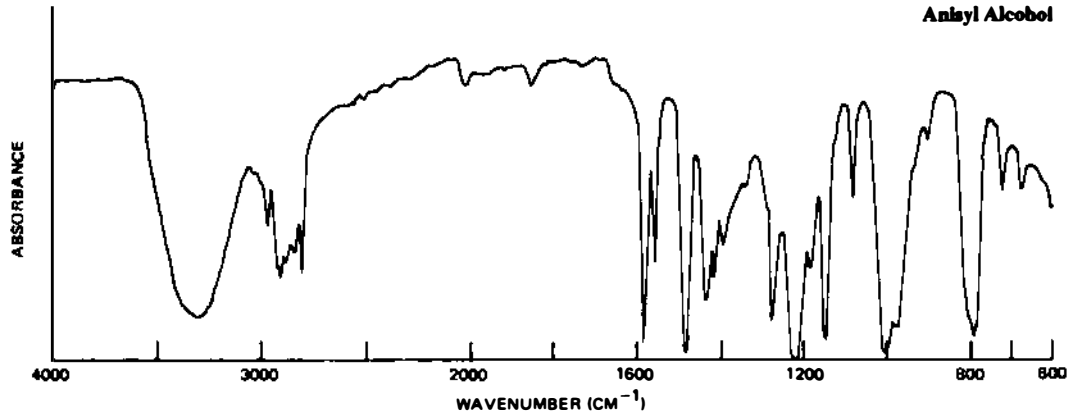
Anisole



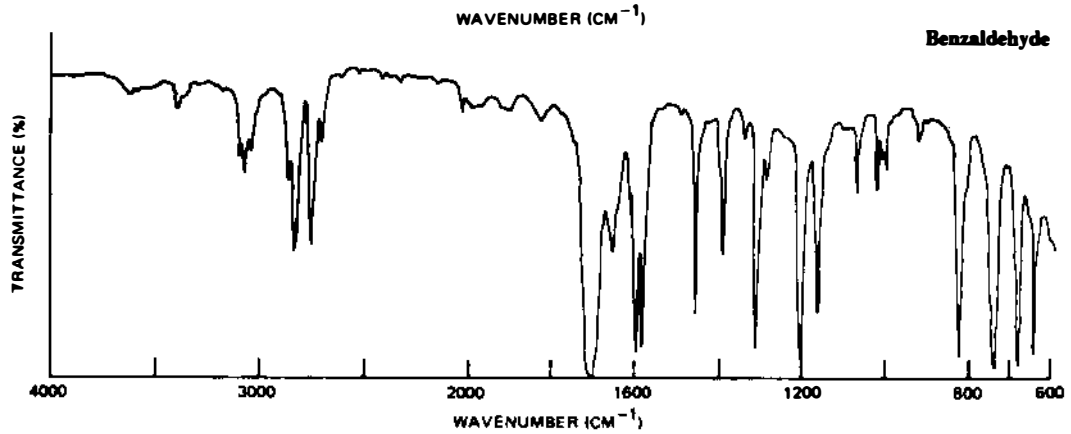
Anisyl Acetate (KBr Pellet)



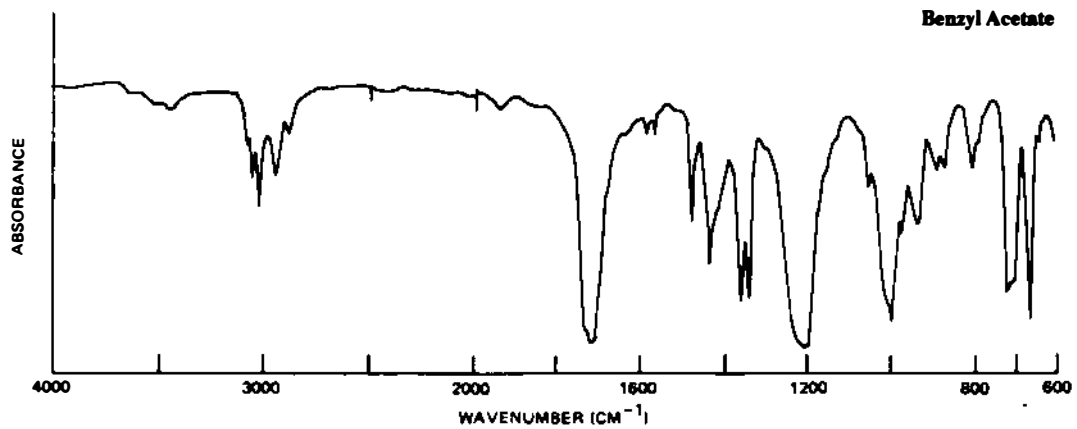
Anisyl Alcohol



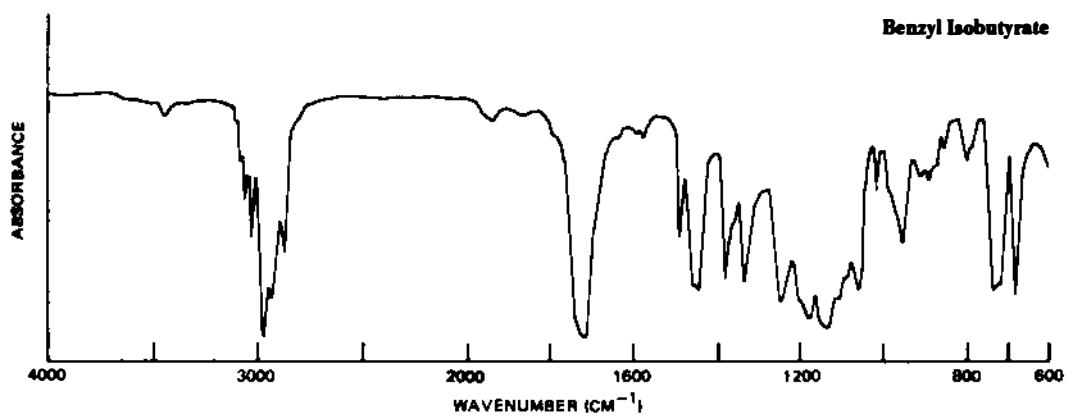
Benzaldehyde



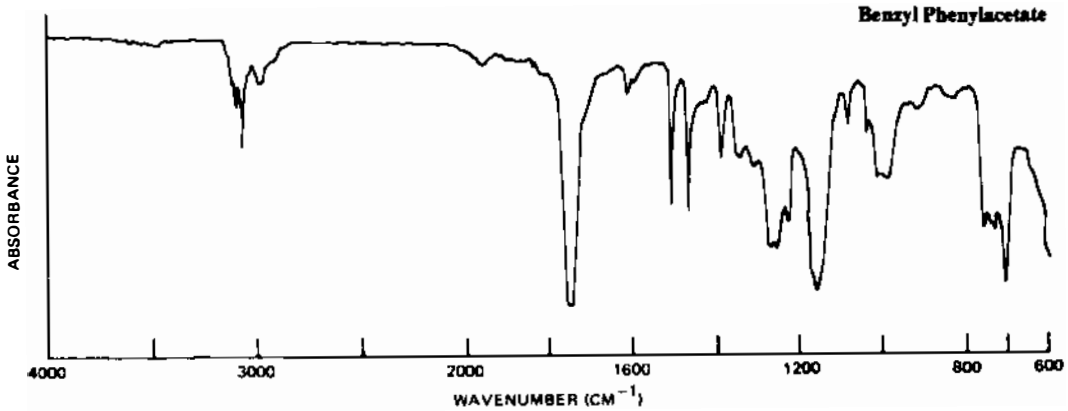
Benzyl Acetate



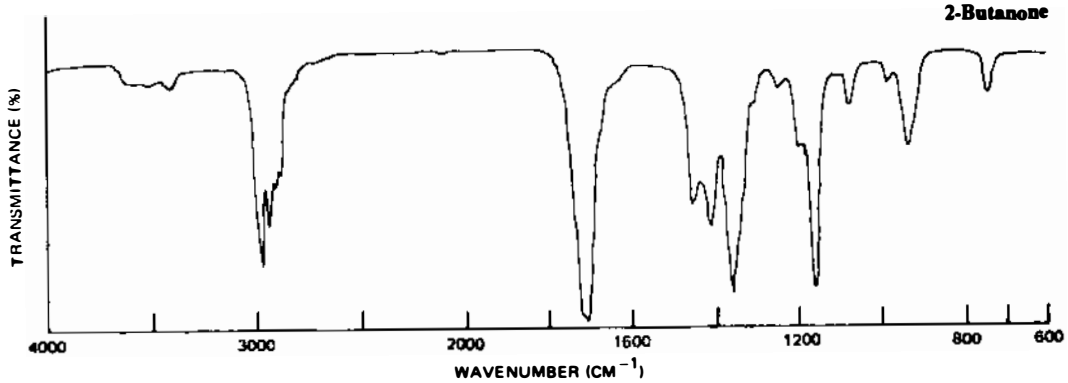
Benzyl Isobutyrate



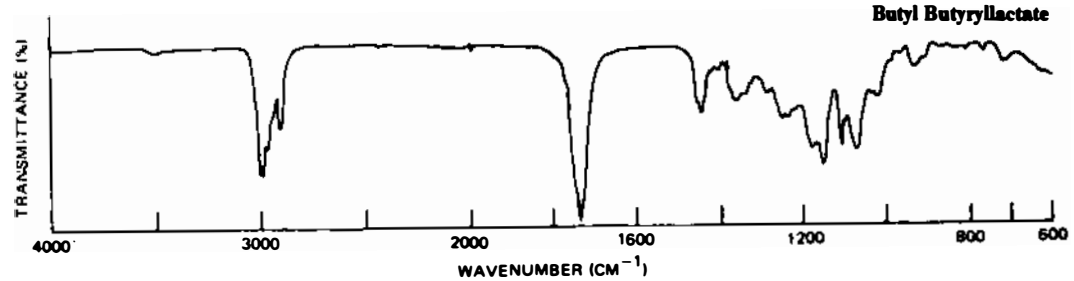
Benzyl Phenylacetate



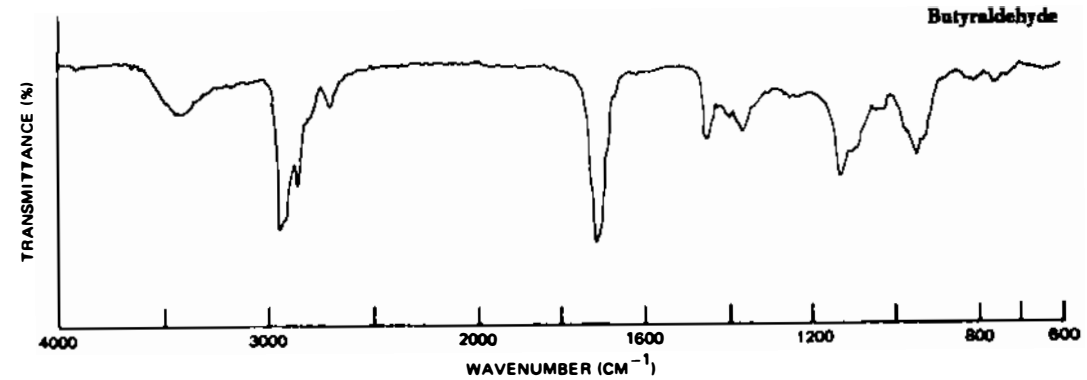
2-Butanone



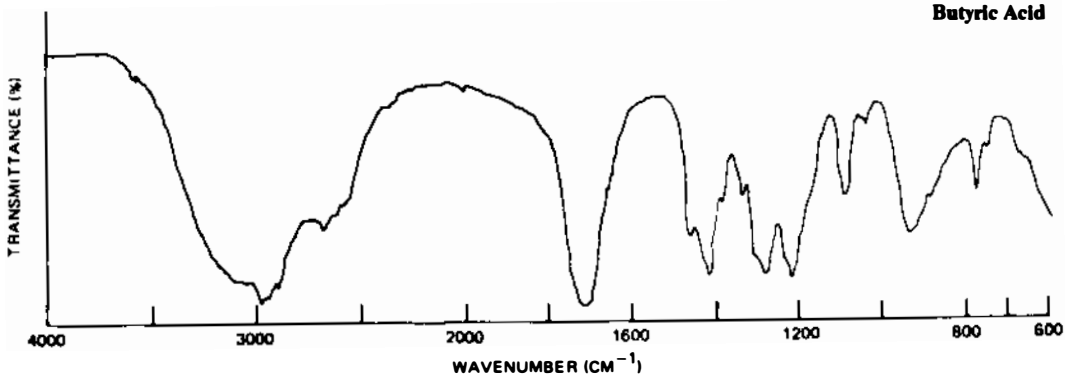
Butyl Butyryllactate



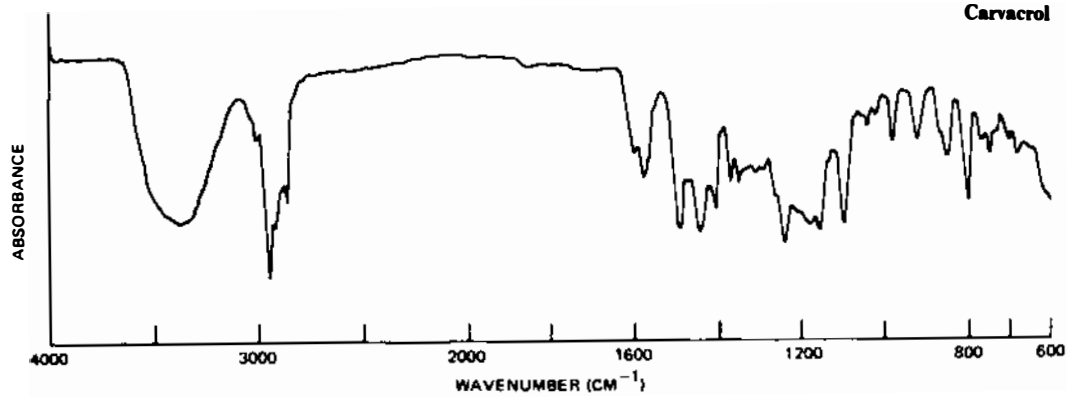
Butyraldehyde



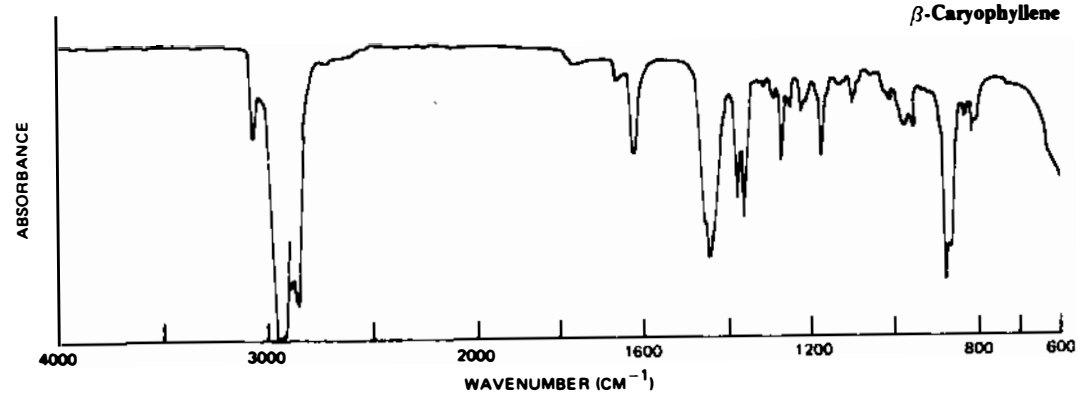
Butyric Acid



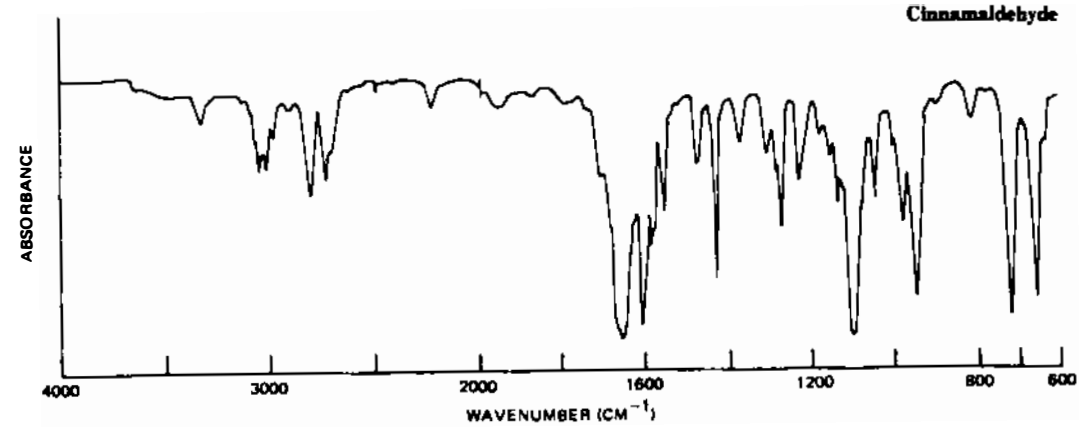
Carvacrol



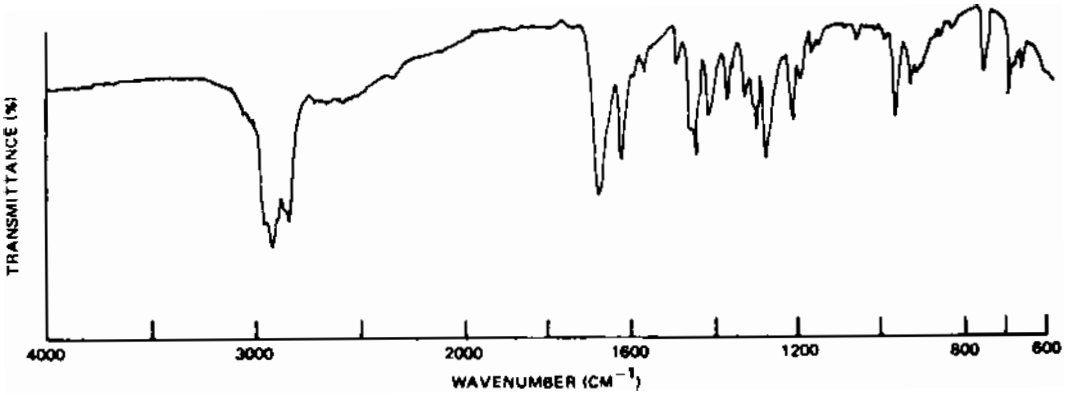
β-Caryophyllene



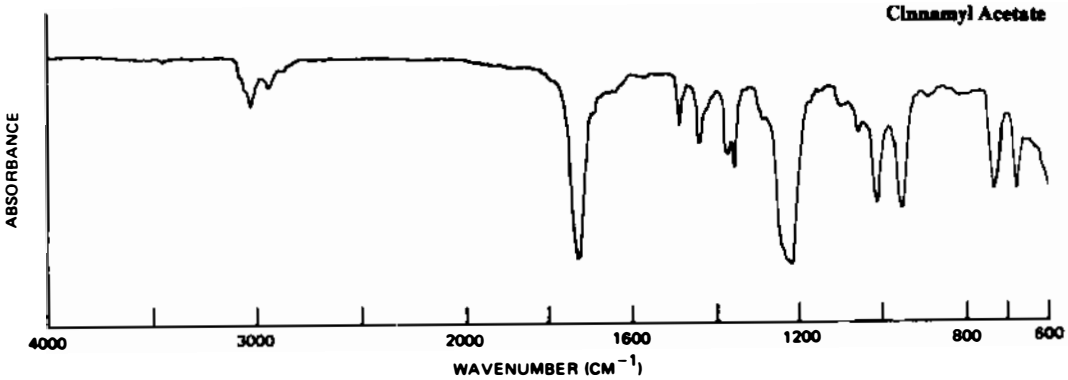
Cinnamaldehyde



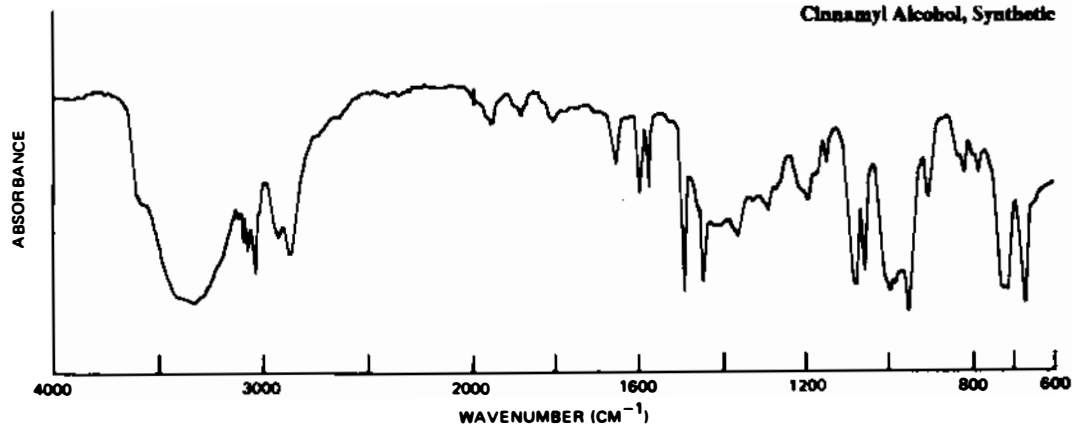
Cinnamic Acid (Nujol Mull)



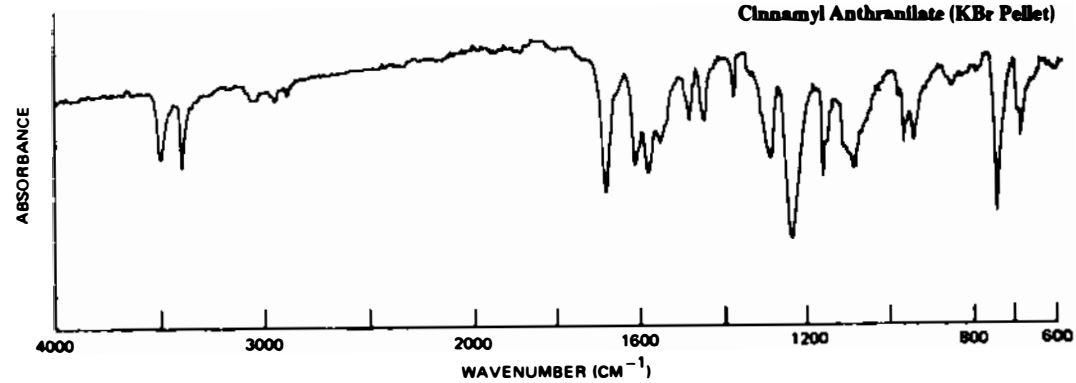
Cinnamyl Acetate



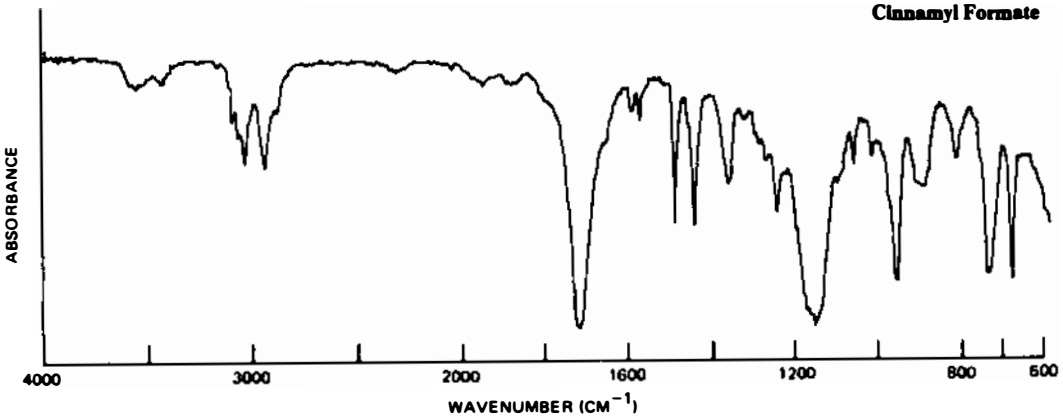
Cinnamyl Alcohol, Synthetic



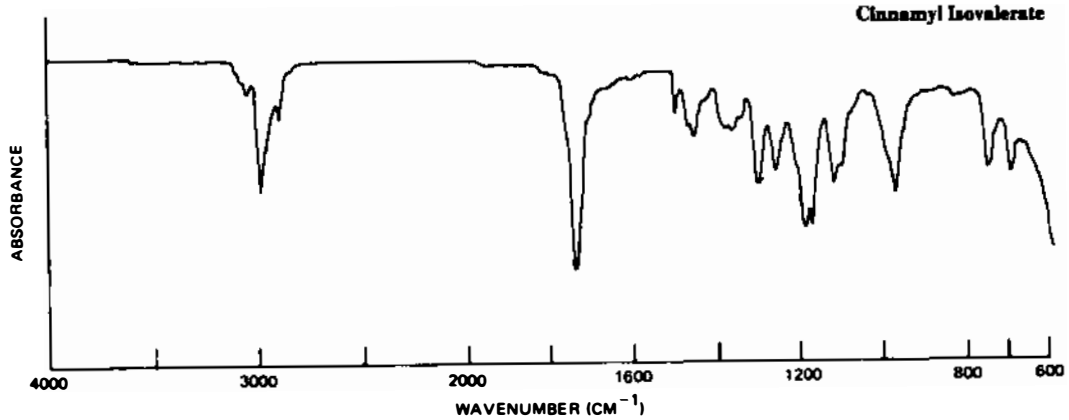
Cinnamyl Anthranilate (KBr Pellet)



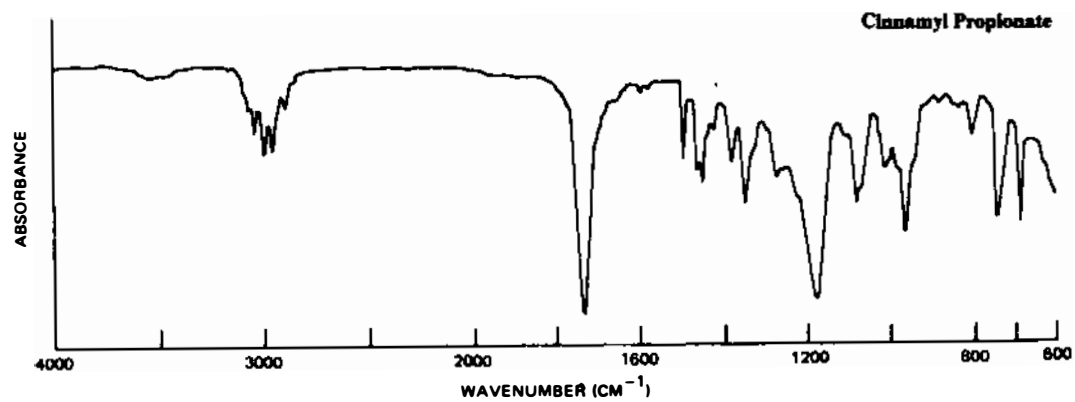
Cinnamyl Formate



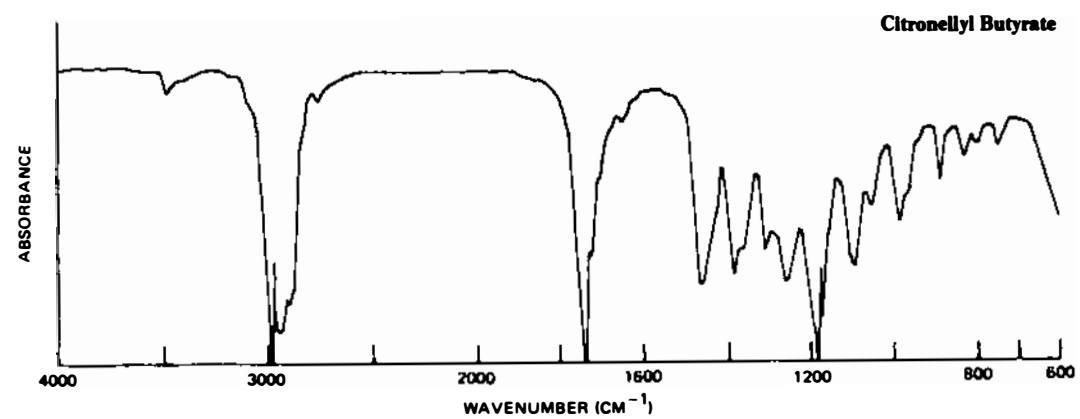
Cinnamyl Isovalerate



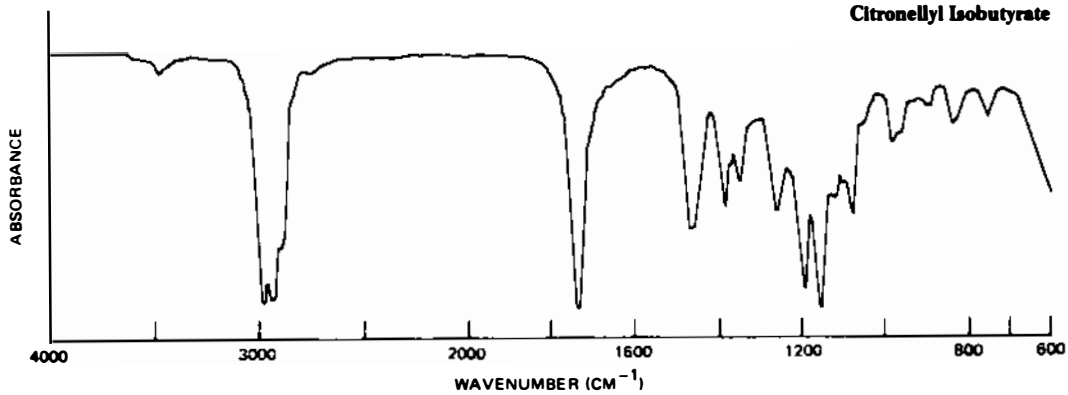
Cinnamyl Propionate



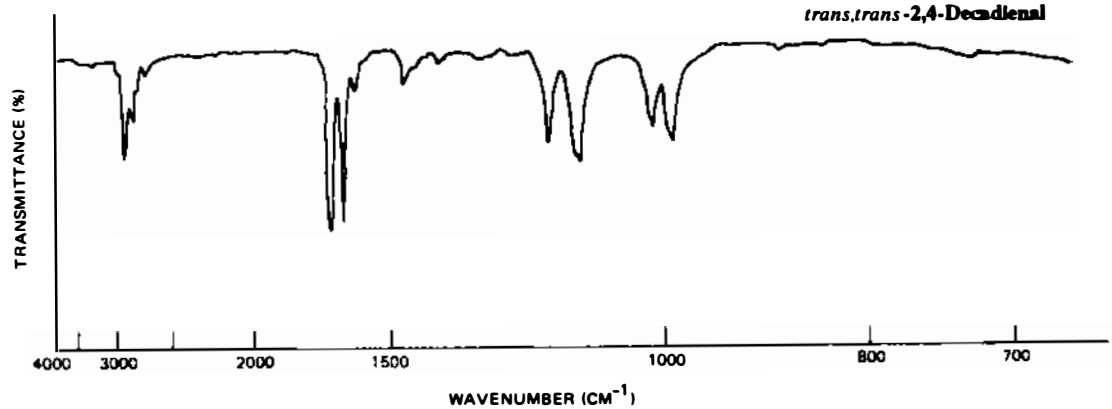
Citronellyl Butyrate



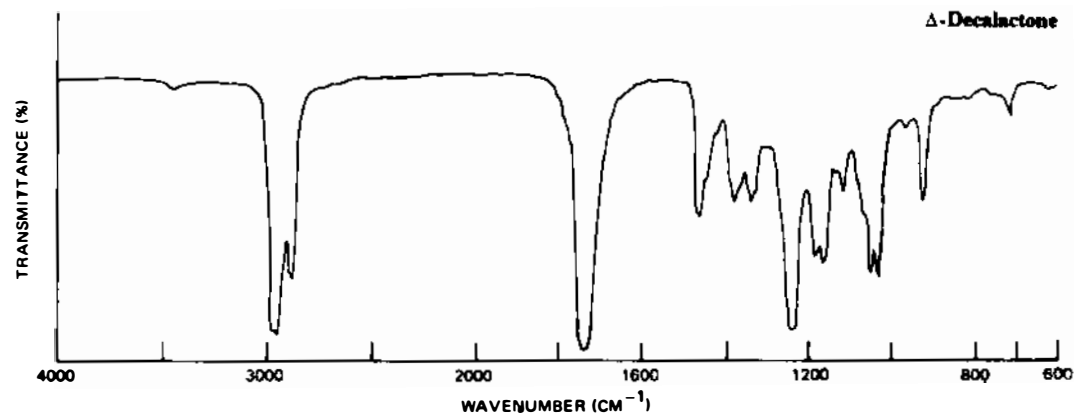
Citronellyl Isobutyrate



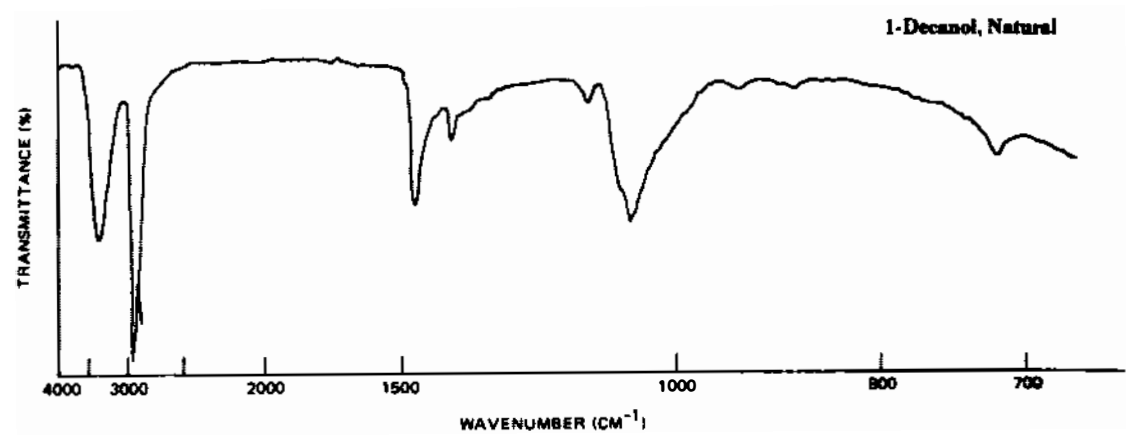
***trans,trans*-2,4-Decadienal**



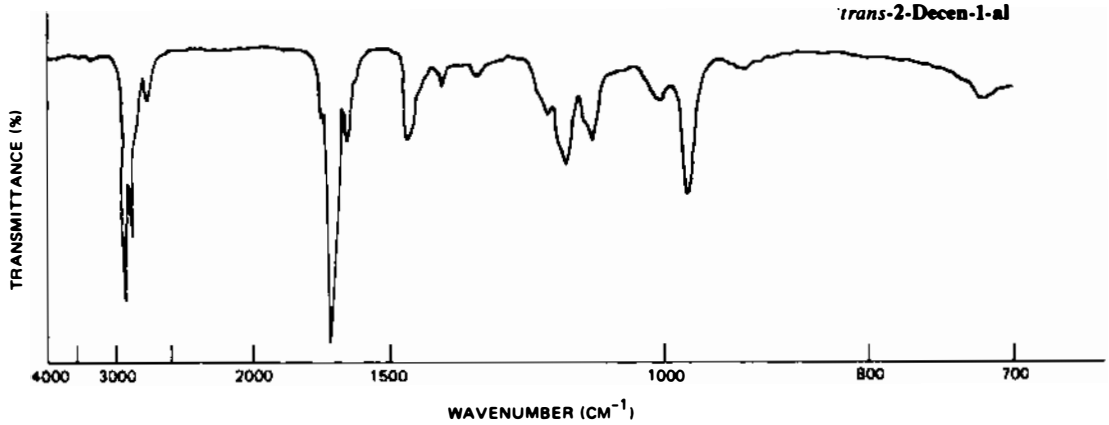
Δ-Decalactone



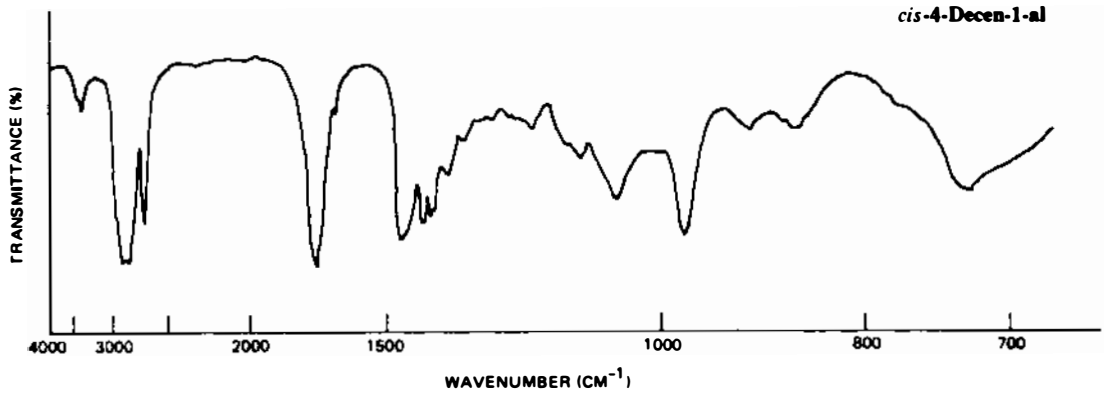
1-Decanol, Natural



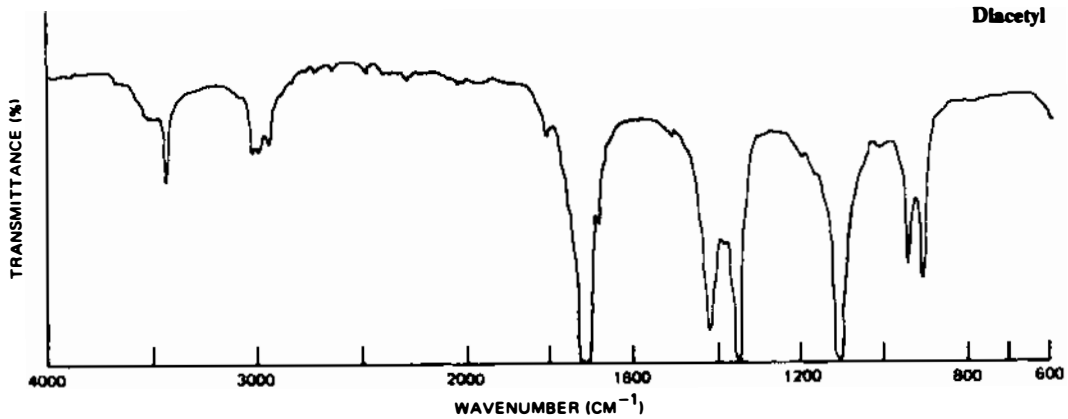
***trans*-2-Decen-1-al**



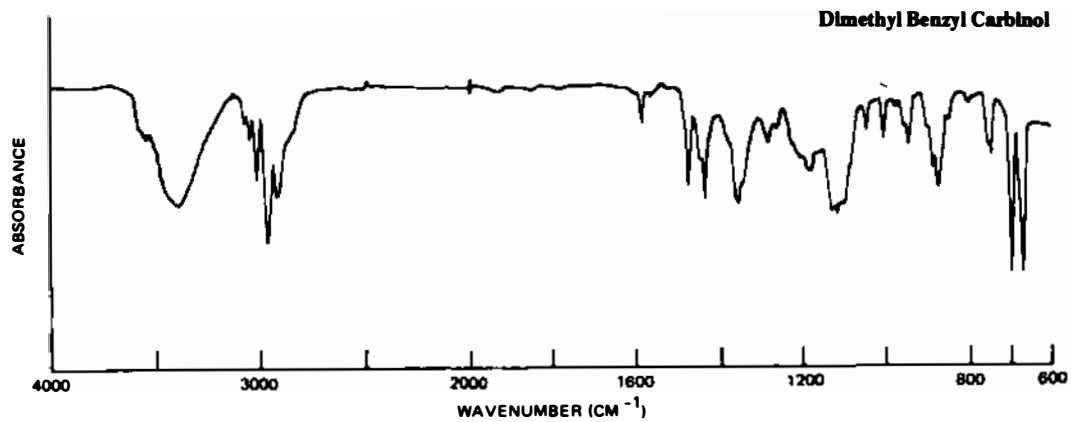
***cis*-4-Decen-1-al**



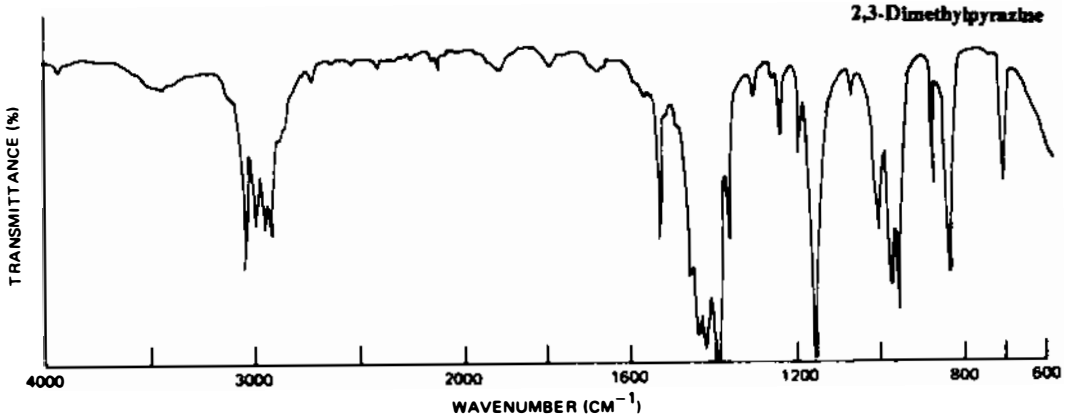
Diacetyl



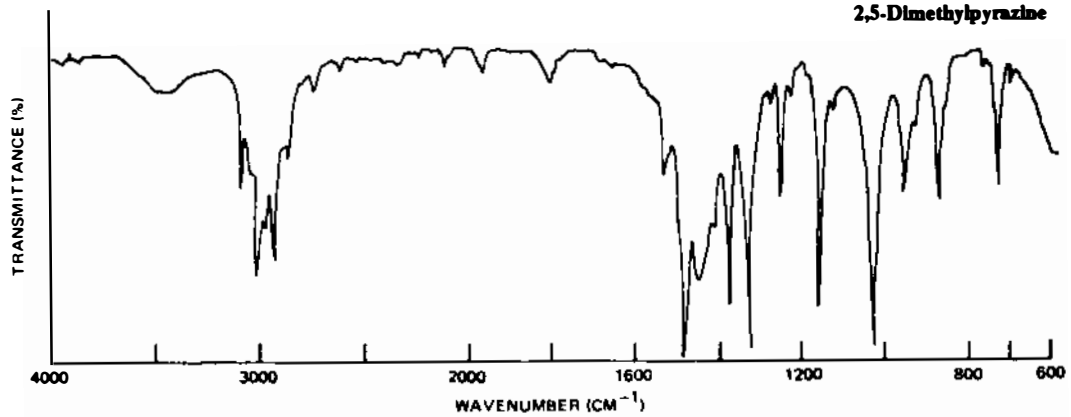
Dimethyl Benzyl Carbinol



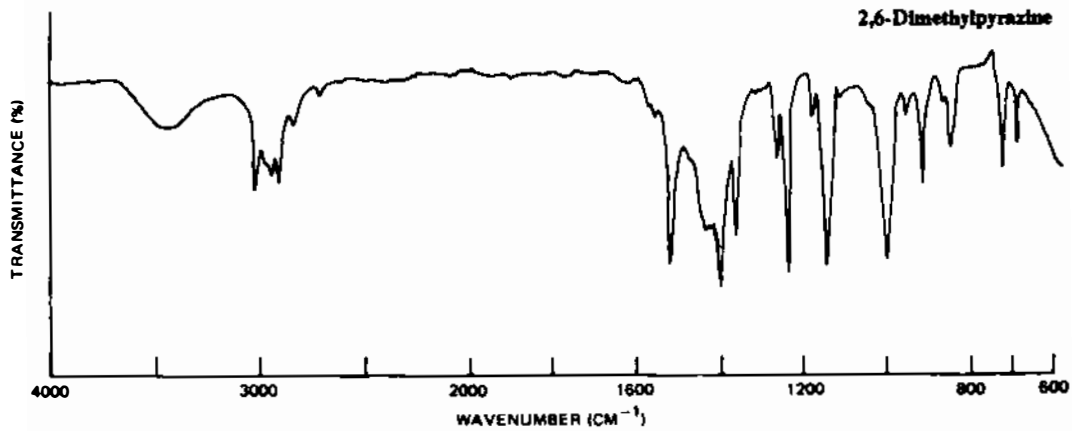
2,3-Dimethylpyrazine



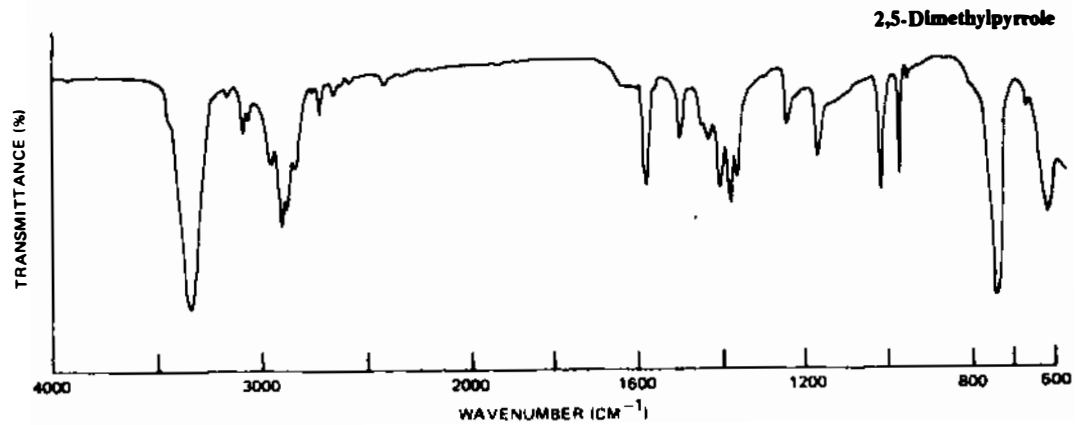
2,5-Dimethylpyrazine



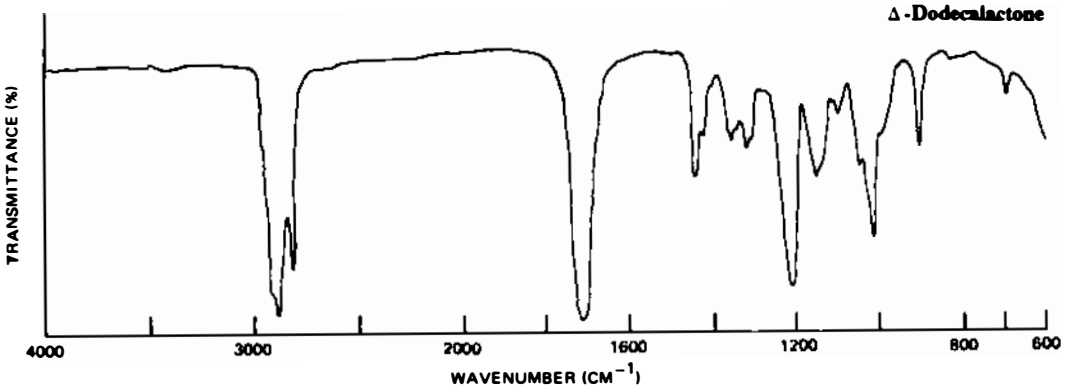
2,6-Dimethylpyrazine



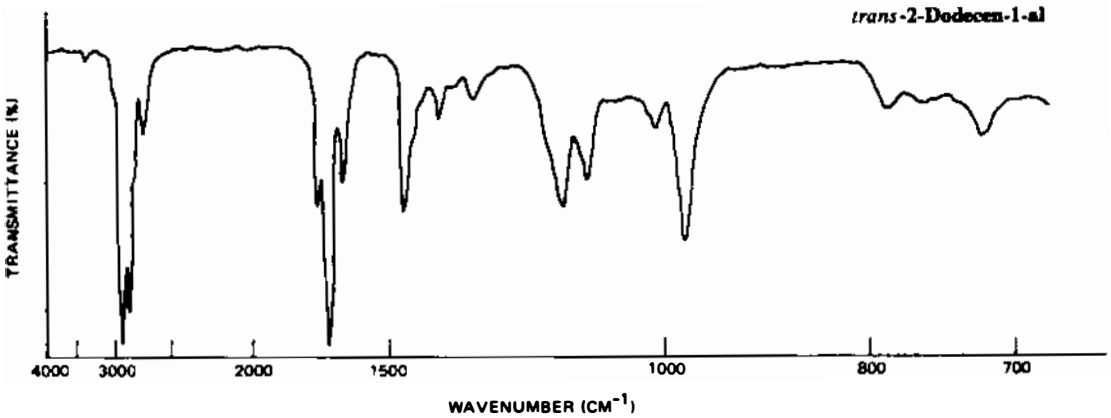
2,5-Dimethylpyrrole



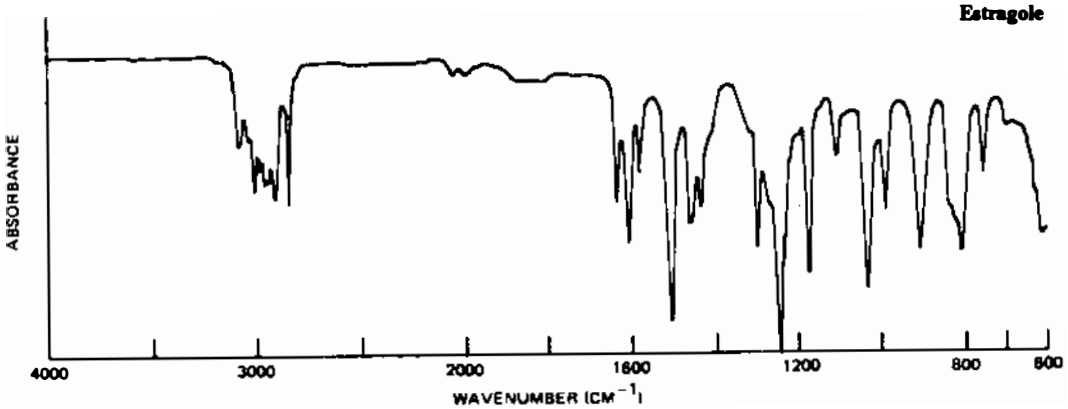
Δ-Dodecalactone



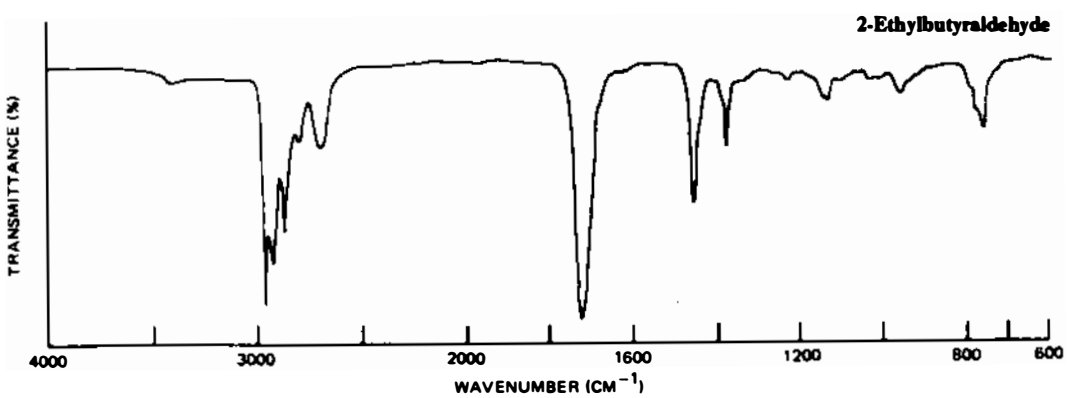
***trans*-2-Dodecen-1-al**



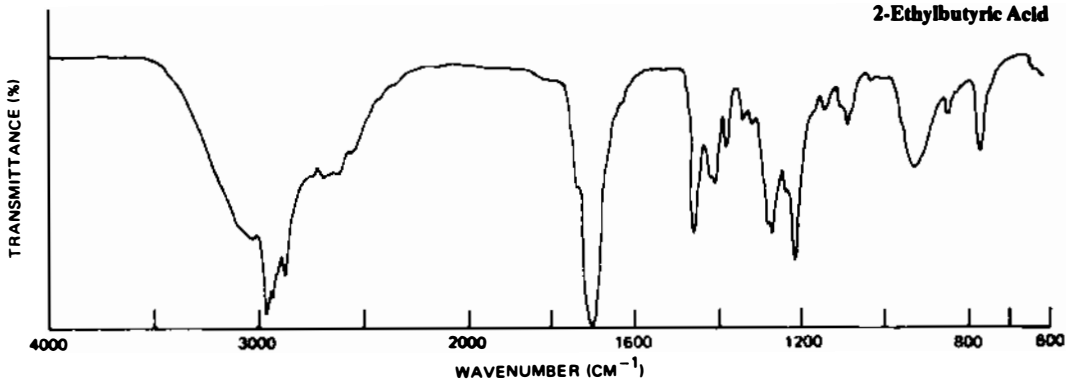
Estragole



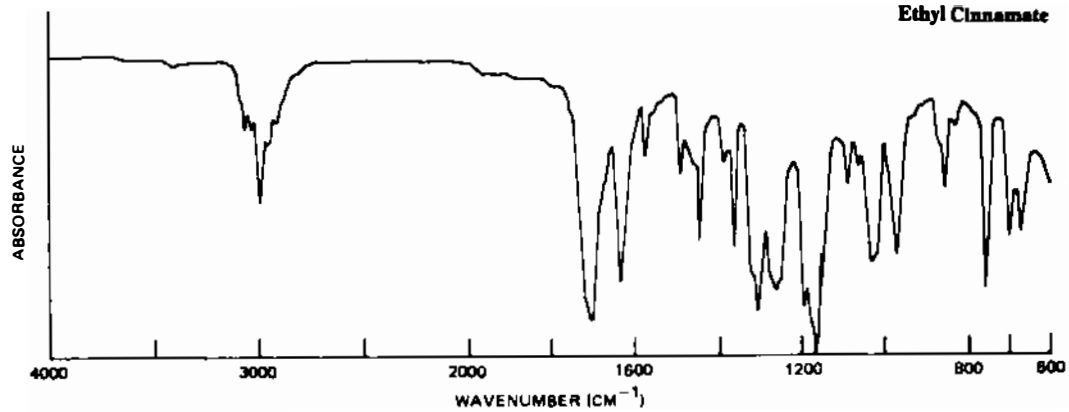
2-Ethylbutyraldehyde



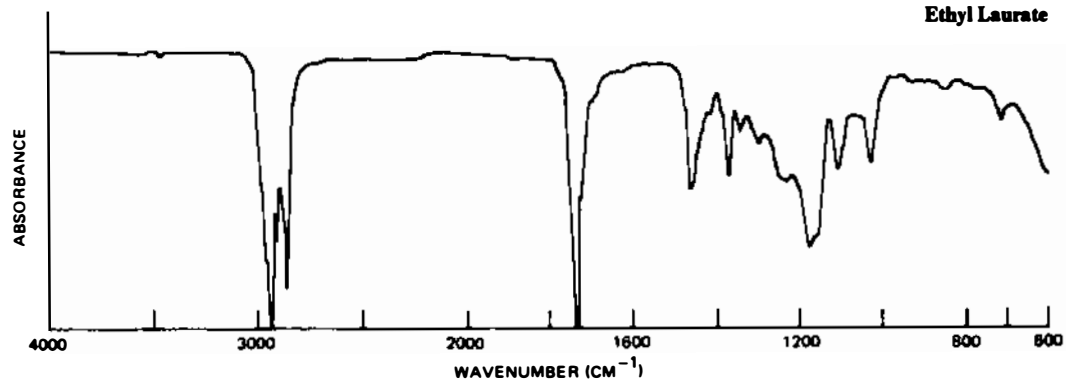
2-Ethylbutyric Acid



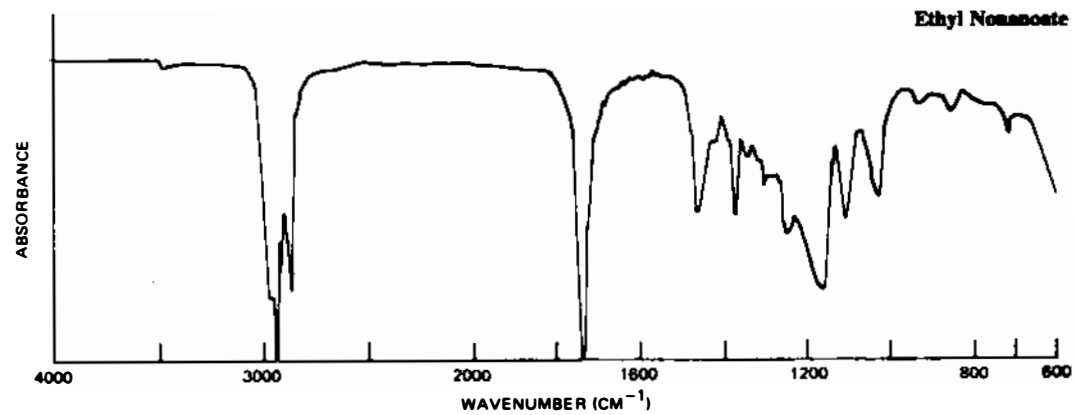
Ethyl Cinnamate



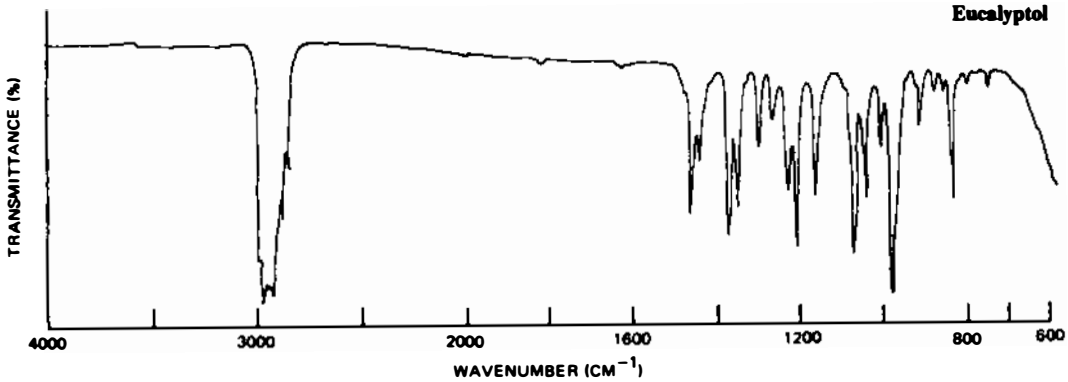
Ethyl Laurate



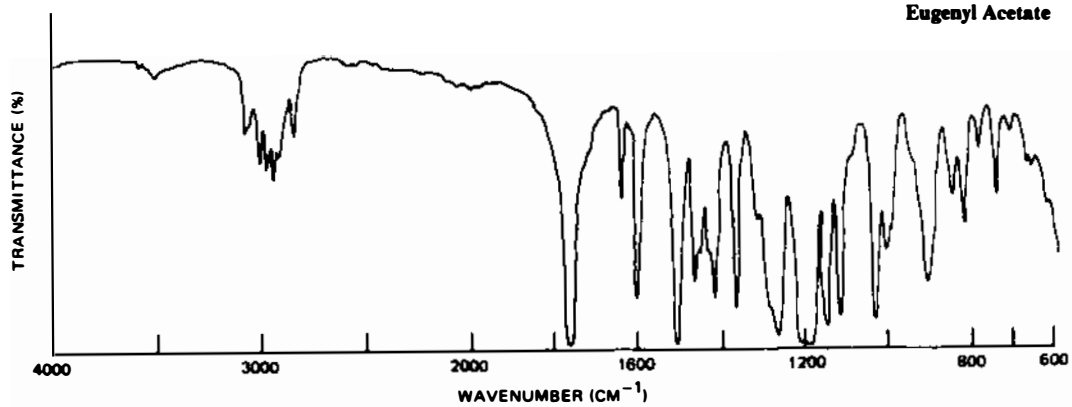
Ethyl Nonanoate



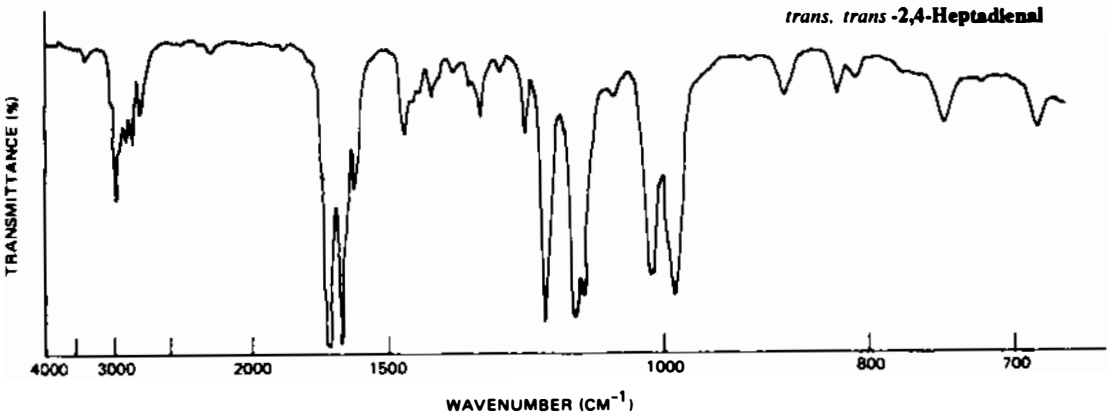
Eucalyptol



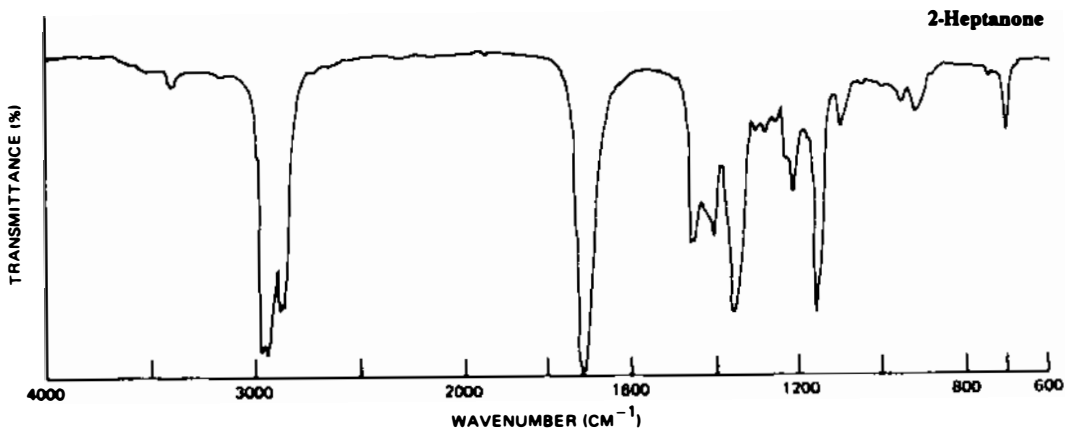
Eugenyl Acetate



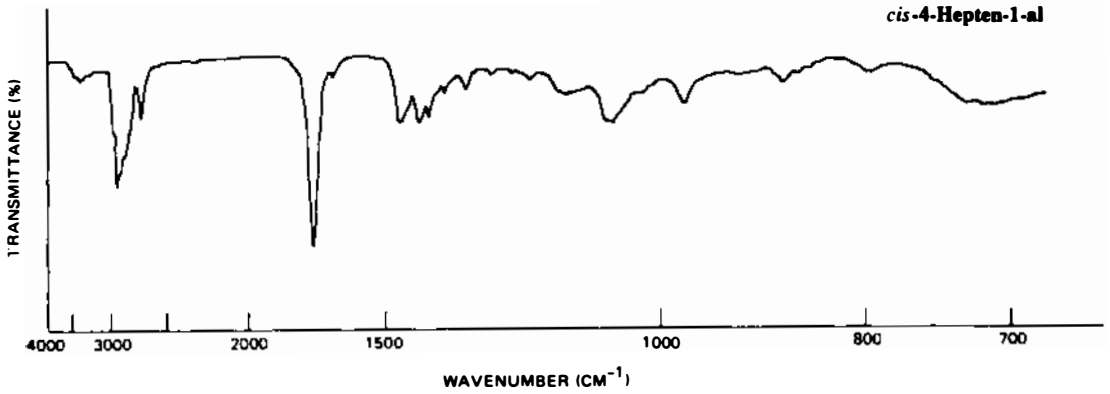
***trans, trans*-2,4-Heptadienal**



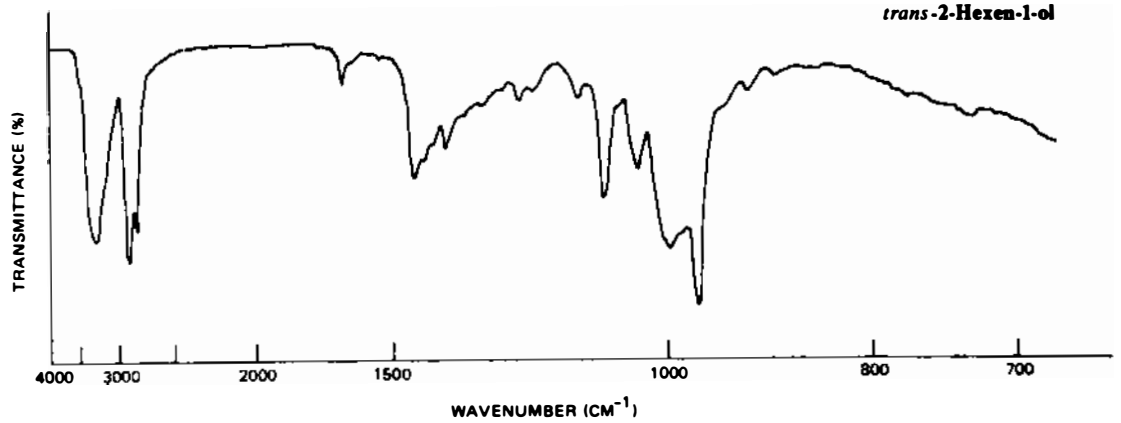
2-Heptanone



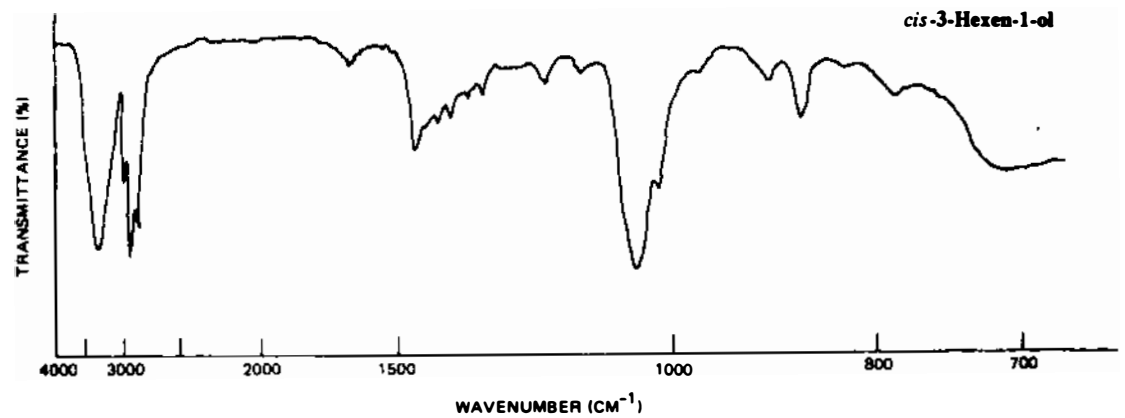
cis-4-Hepten-1-al



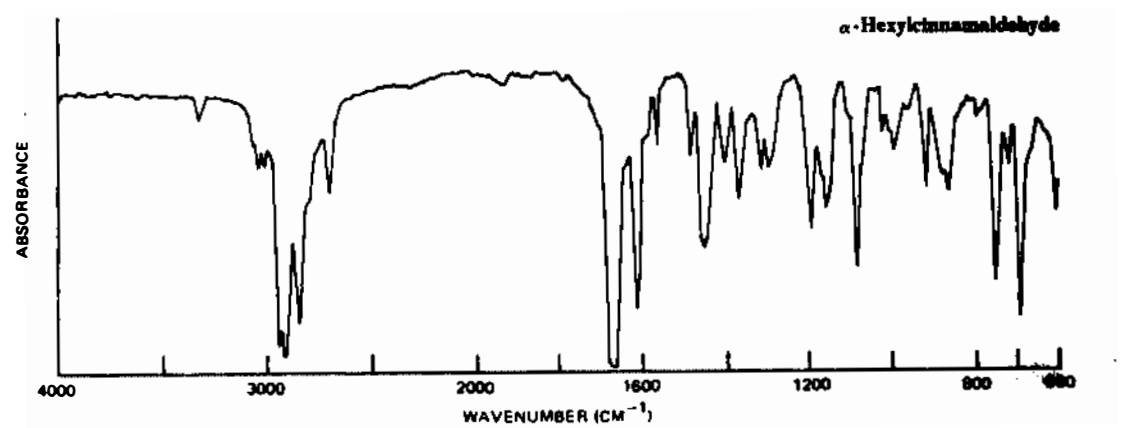
trans-2-Hexen-1-ol



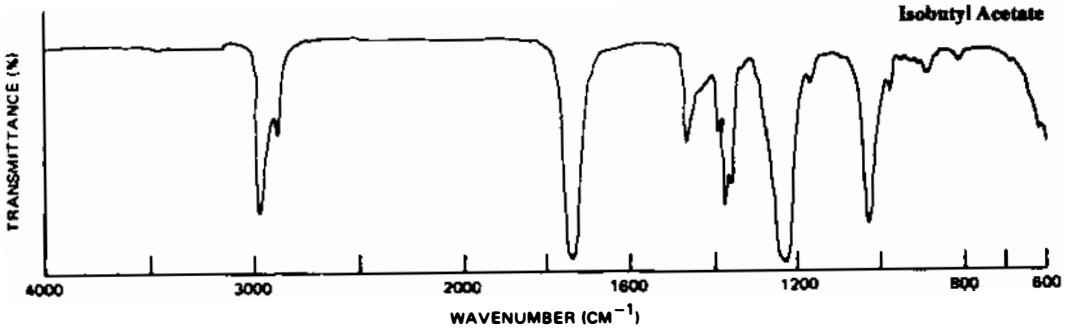
cis-3-Hexen-1-ol



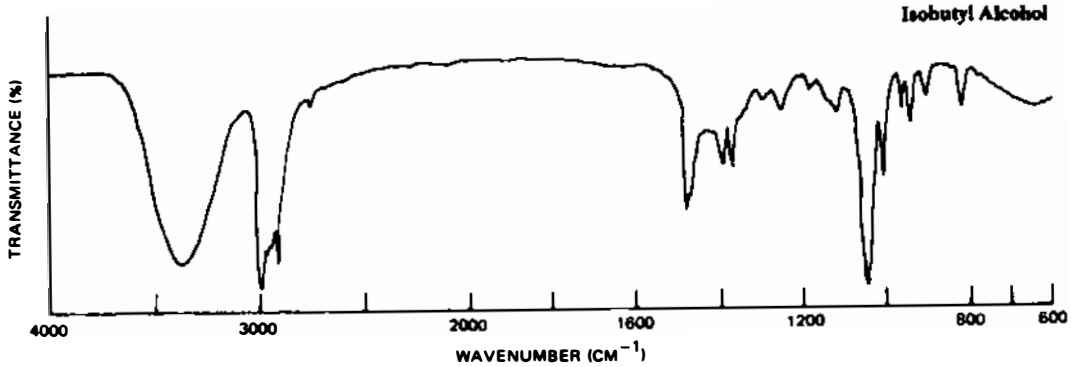
α-Hexylcinnamaldehyde



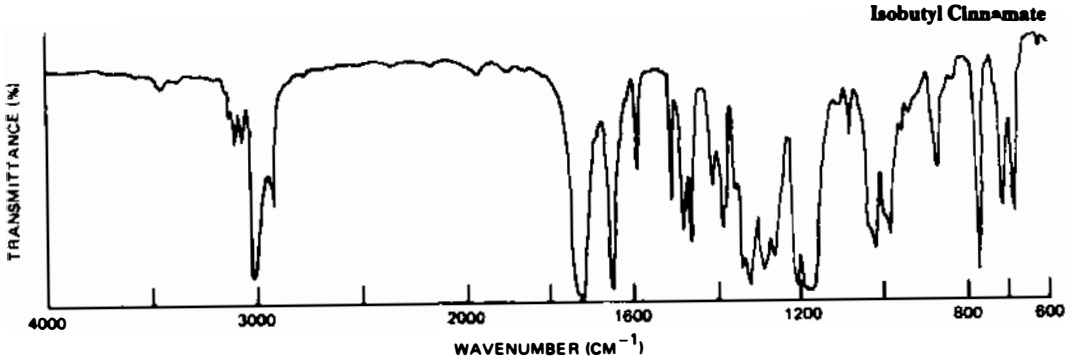
Isobutyl Acetate



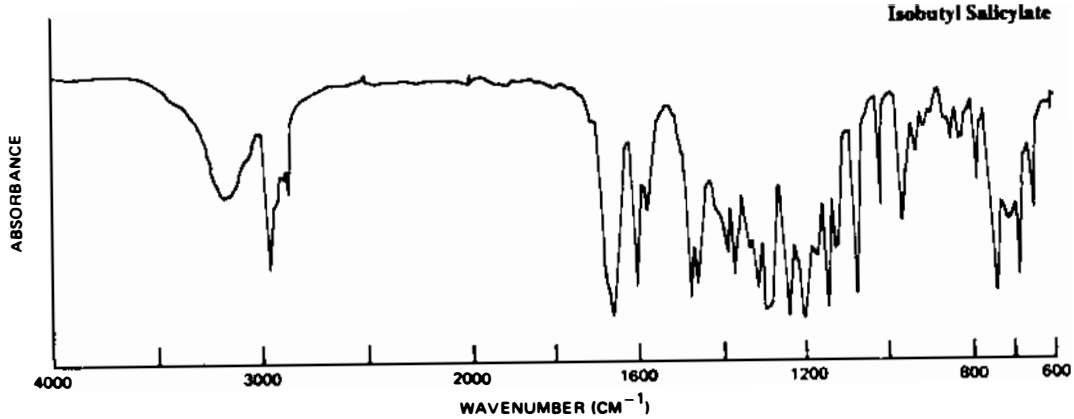
Isobutyl Alcohol



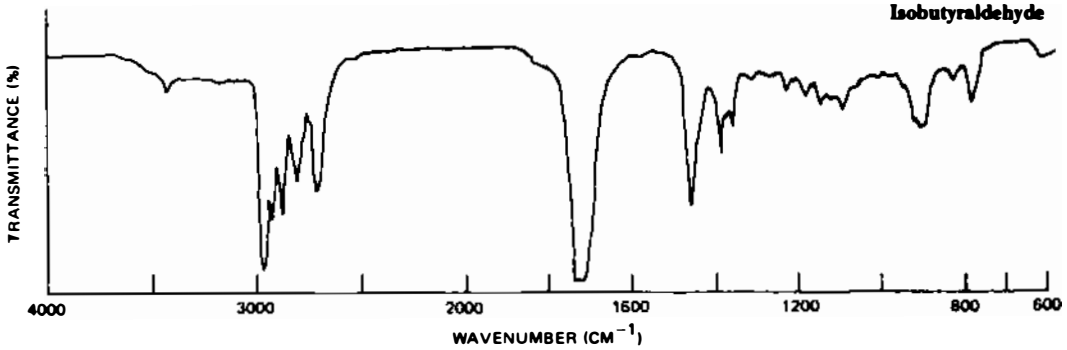
Isobutyl Cinnamate



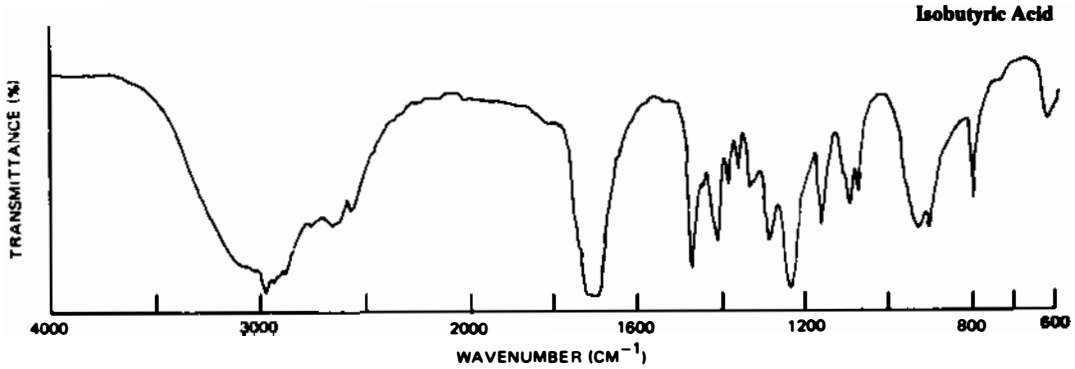
Isobutyl Salicylate



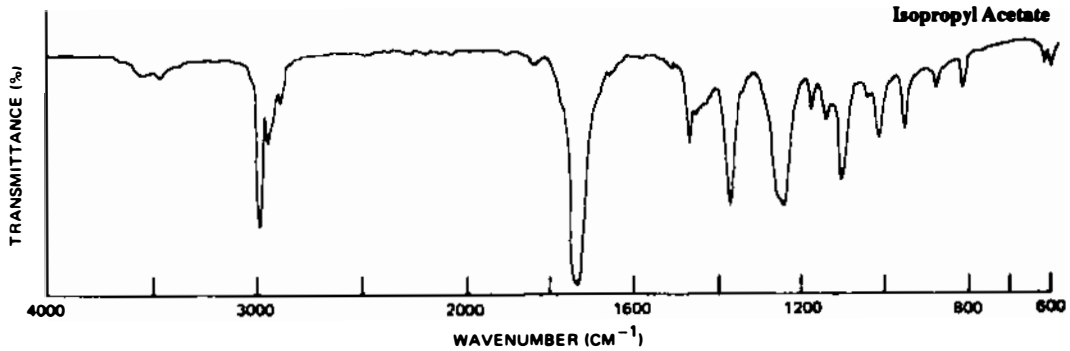
Isobutyraldehyde



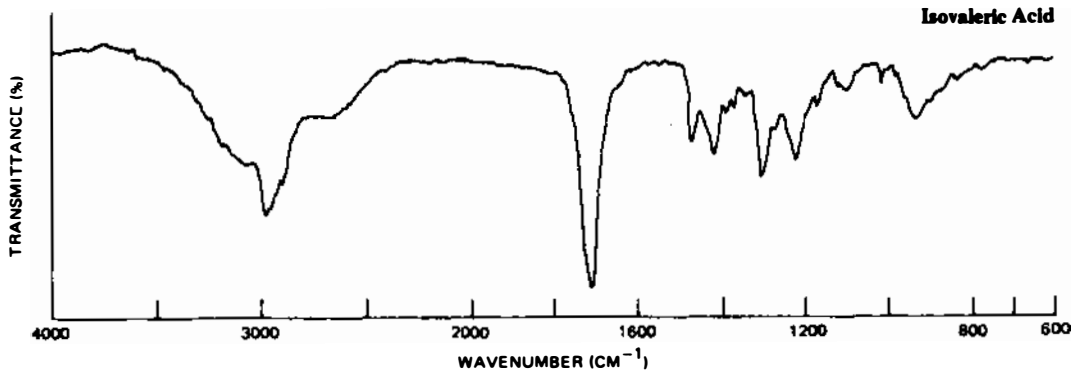
Isobutyric Acid



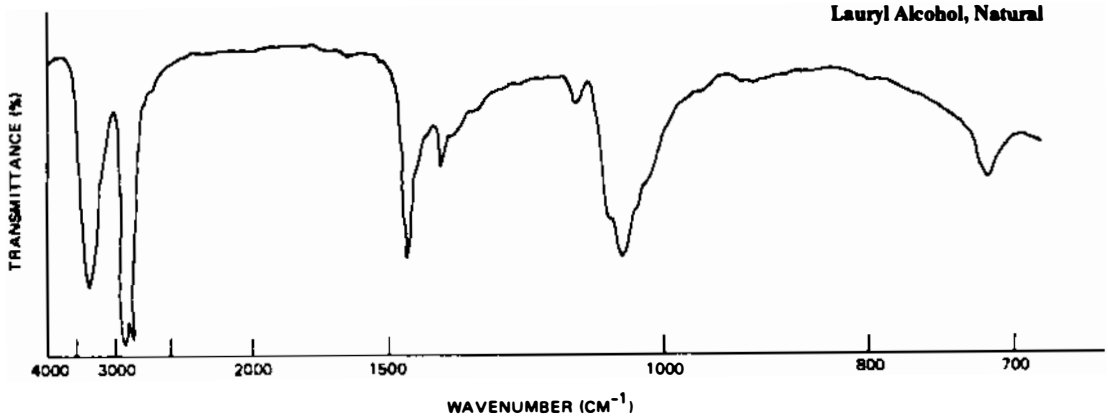
Isopropyl Acetate



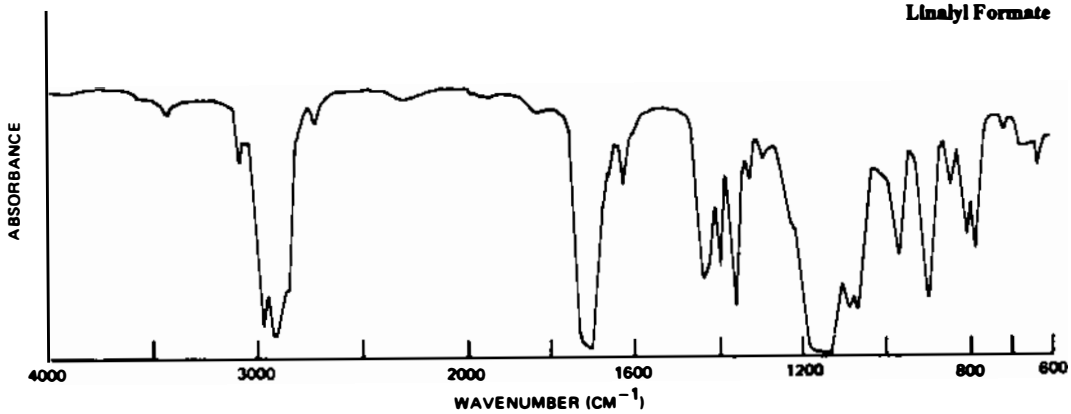
Isovaleric Acid



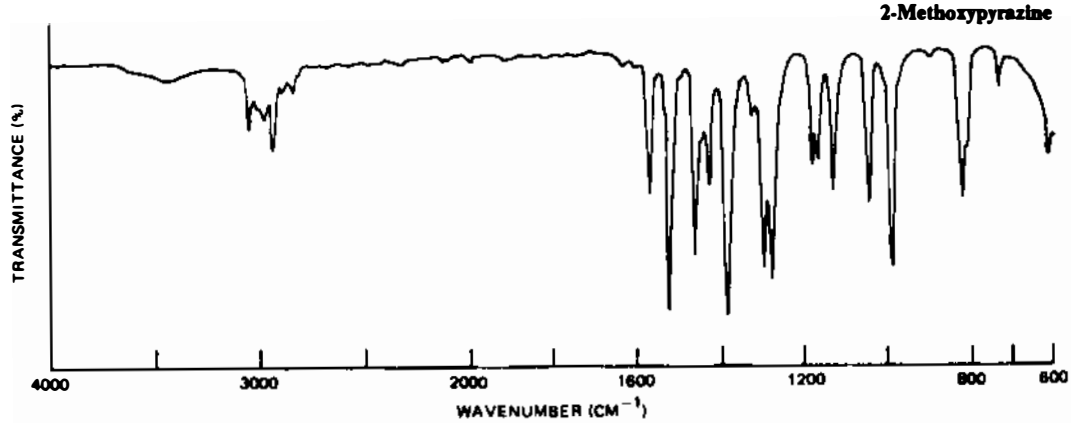
Lauryl Alcohol, Natural



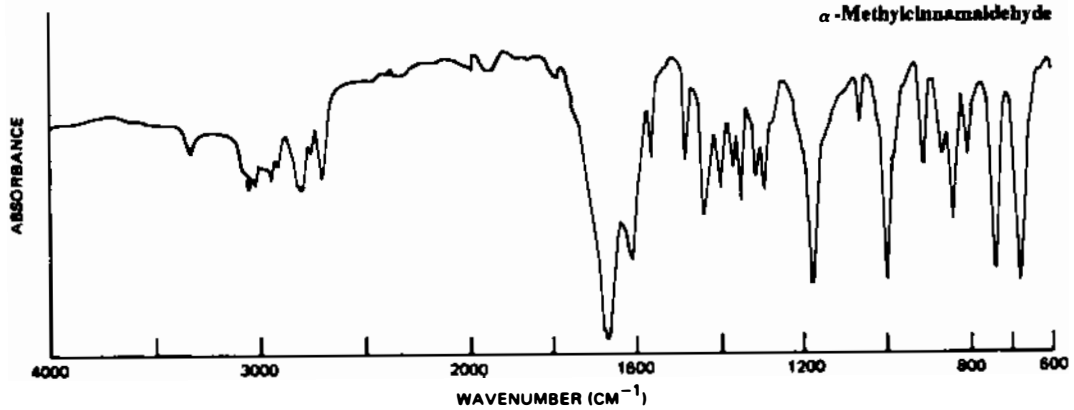
Linalyl Formate



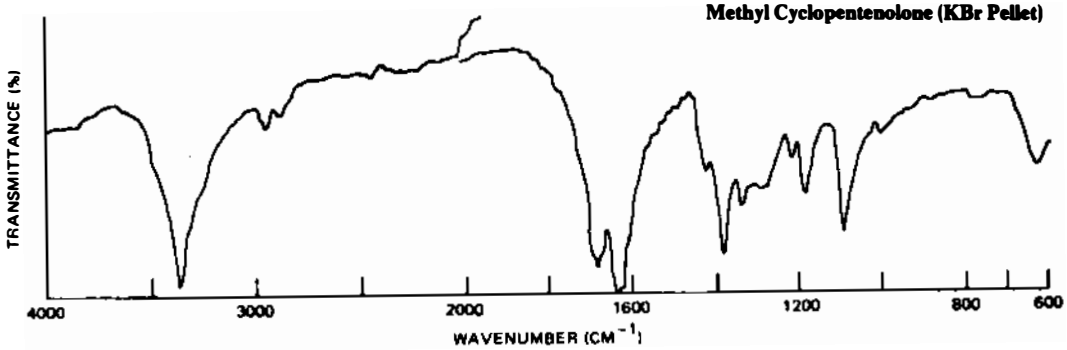
2-Methoxypyrazine



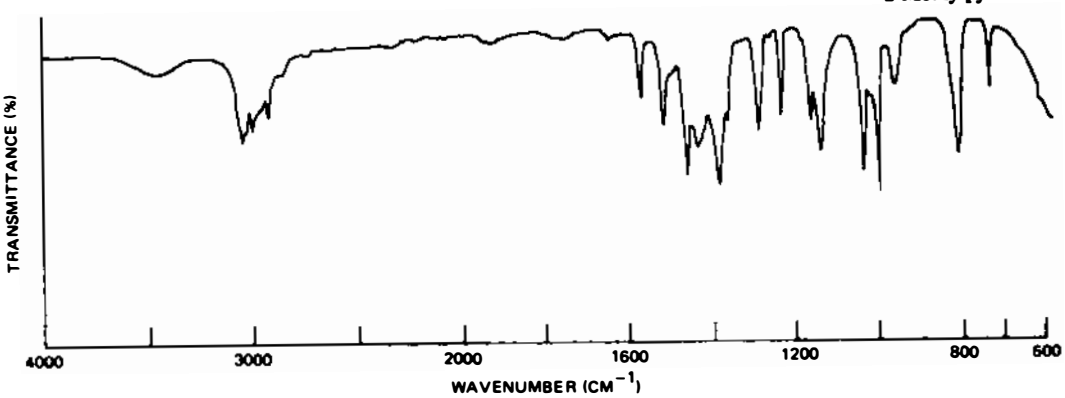
α-Methylcinnamaldehyde



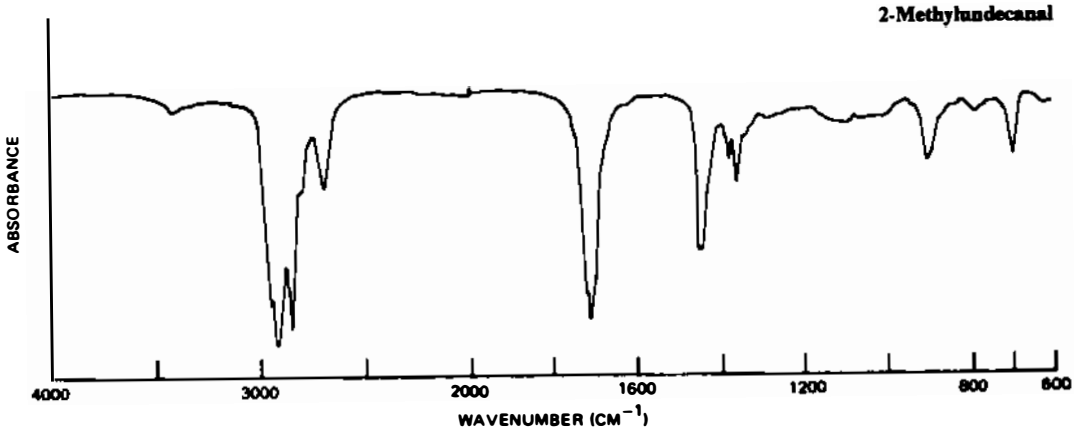
Methyl Cyclopentenolone (KBr Pellet)



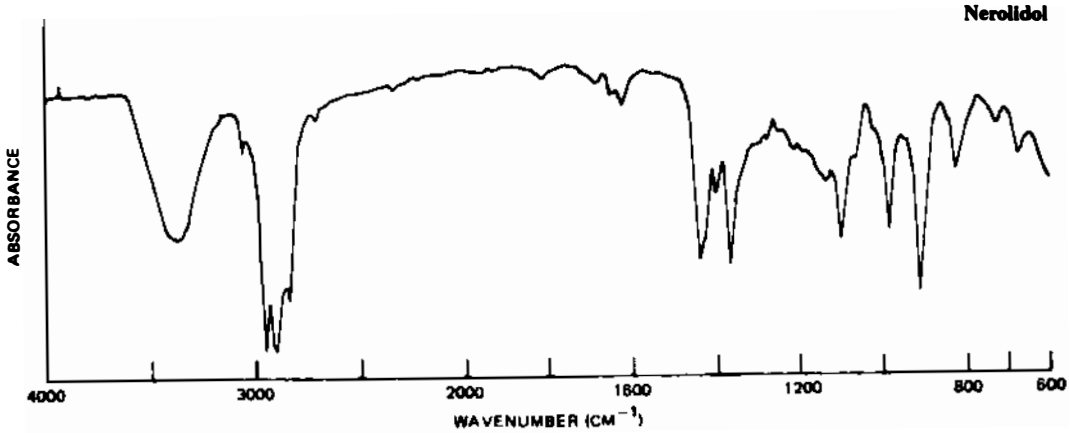
2-Methylpyrazine



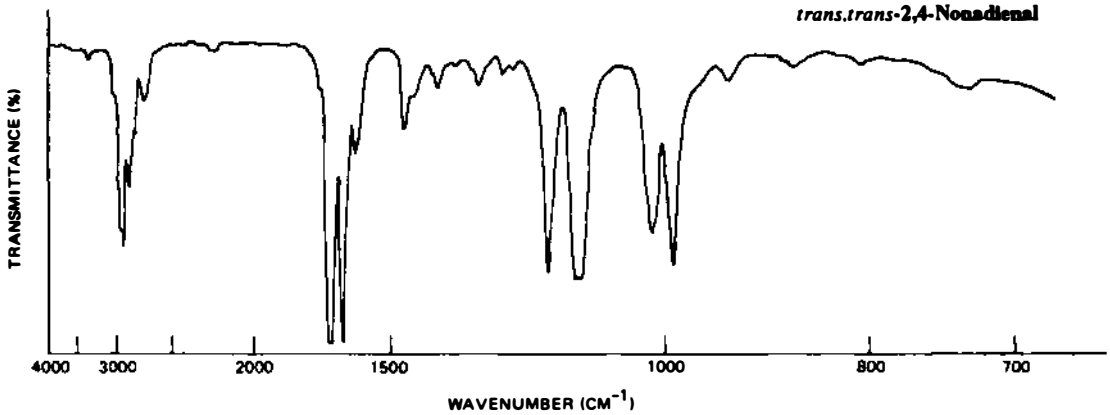
2-Methylundecanal



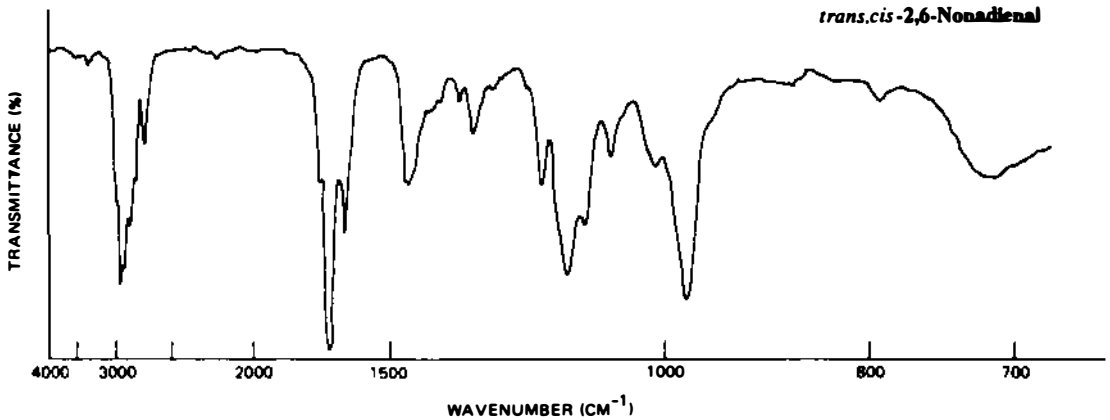
Nerolidol



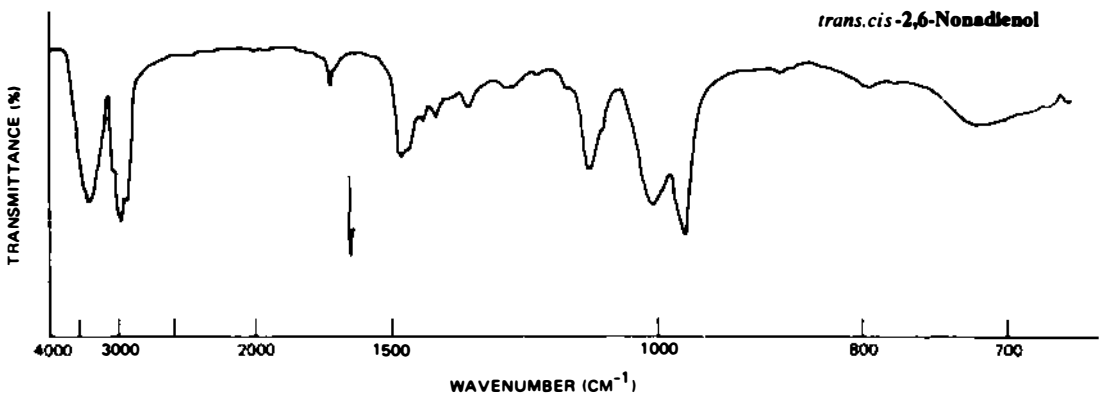
trans,trans-2,4-Nonadienal



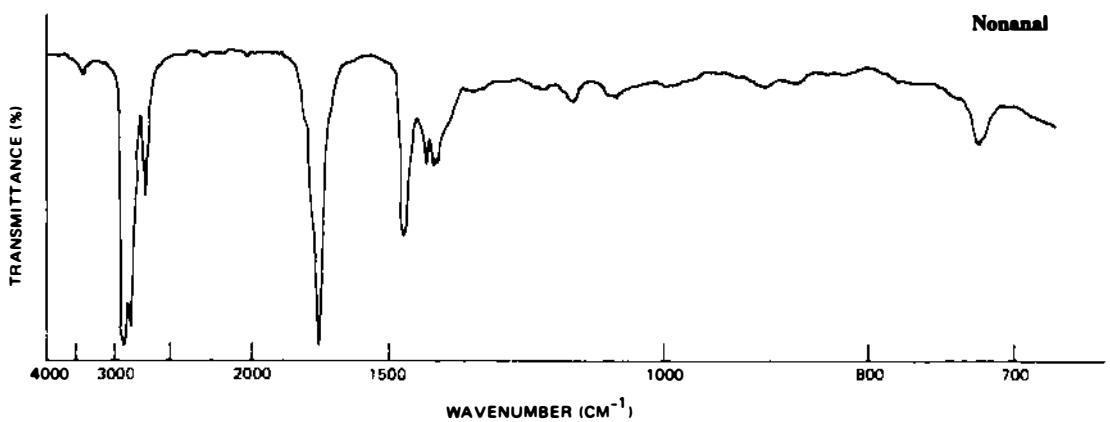
trans,cis-2,6-Nonadienal



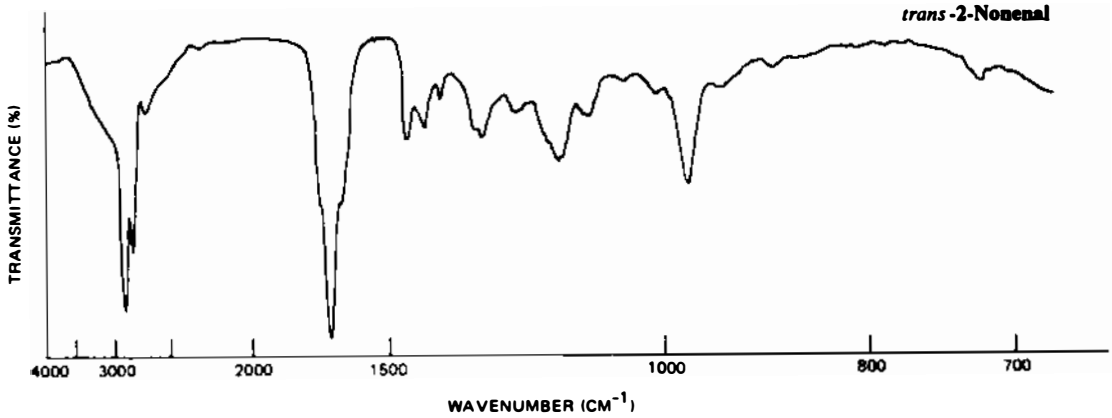
trans,cis-2,6-Nonadienol



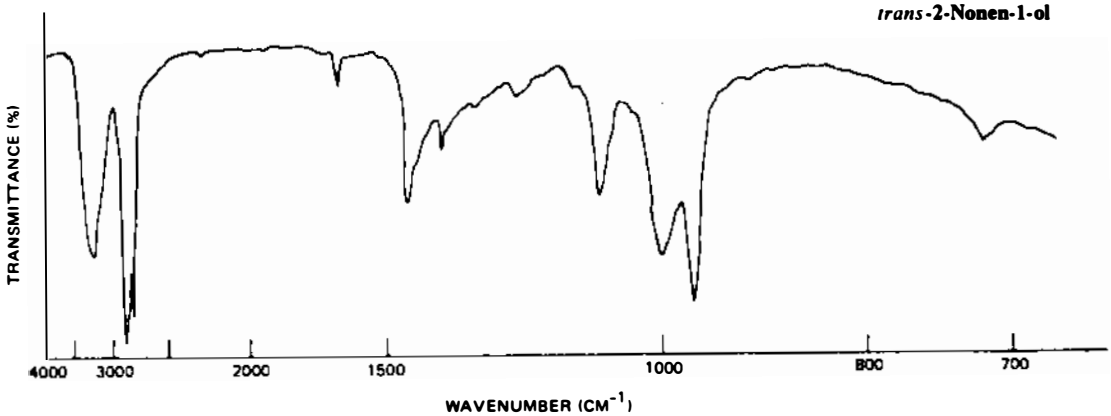
Nonanal



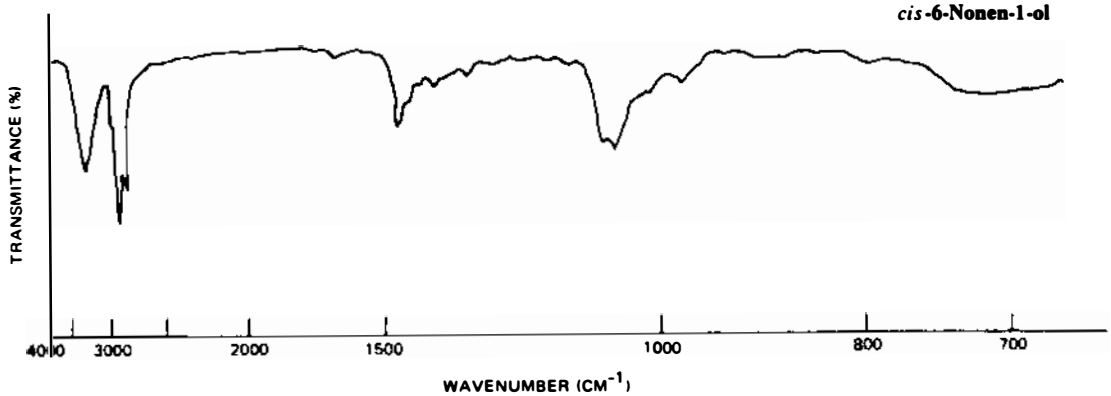
trans-2-Nonenal



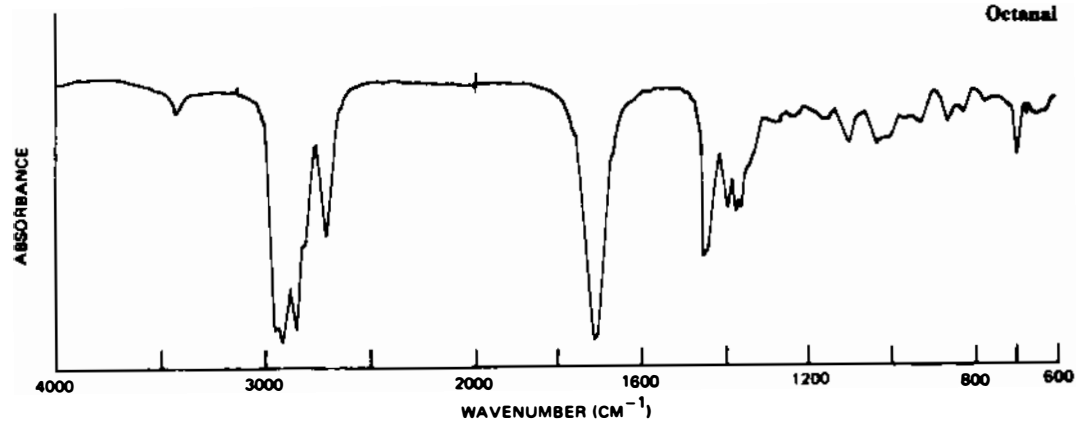
trans-2-Nonen-1-ol



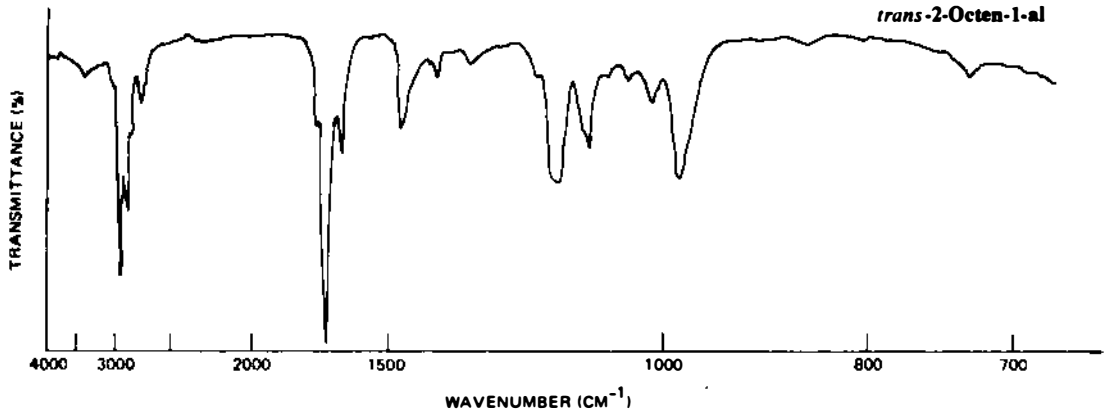
cis-6-Nonen-1-ol



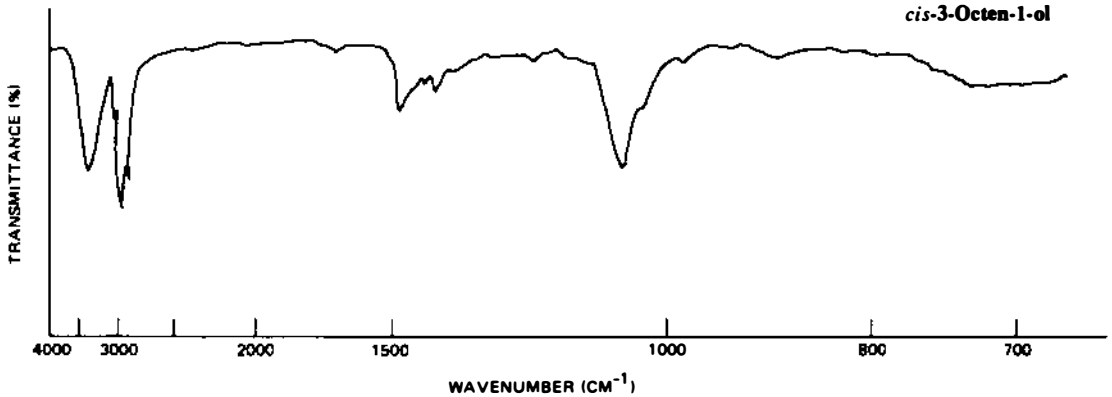
Octanal



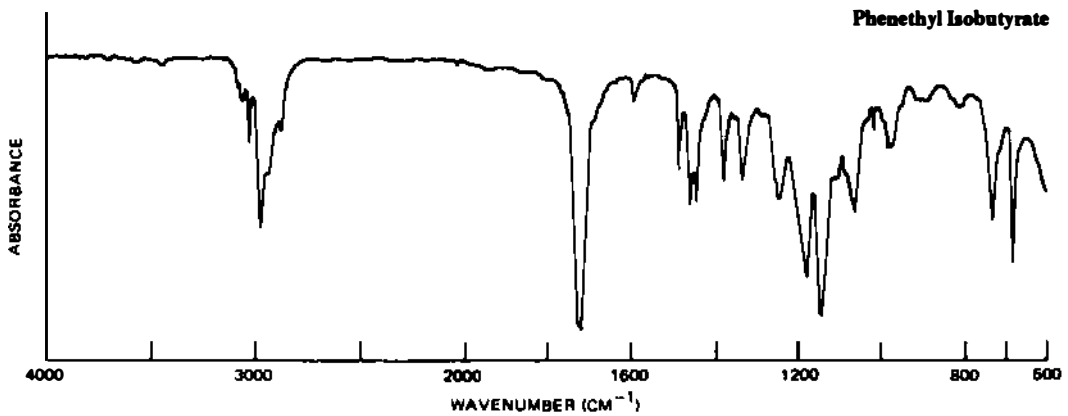
trans-2-Octen-1-al



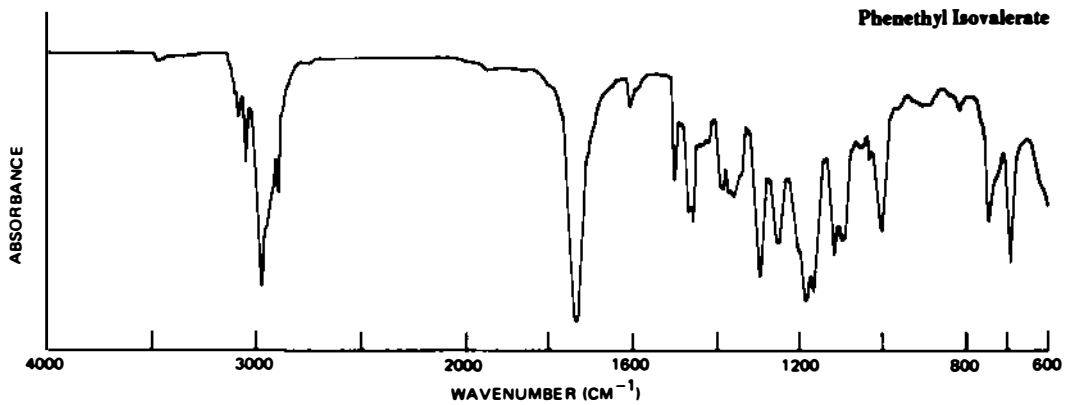
cis-3-Octen-1-ol



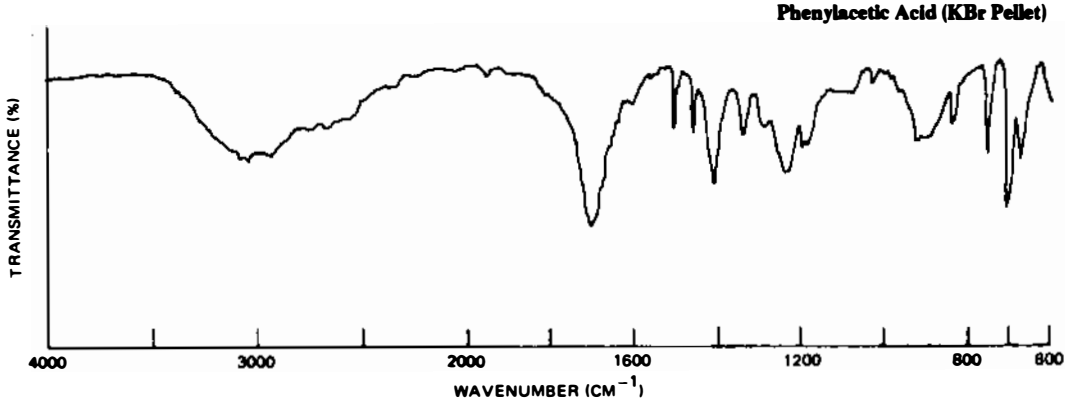
Phenethyl Isobutyrate



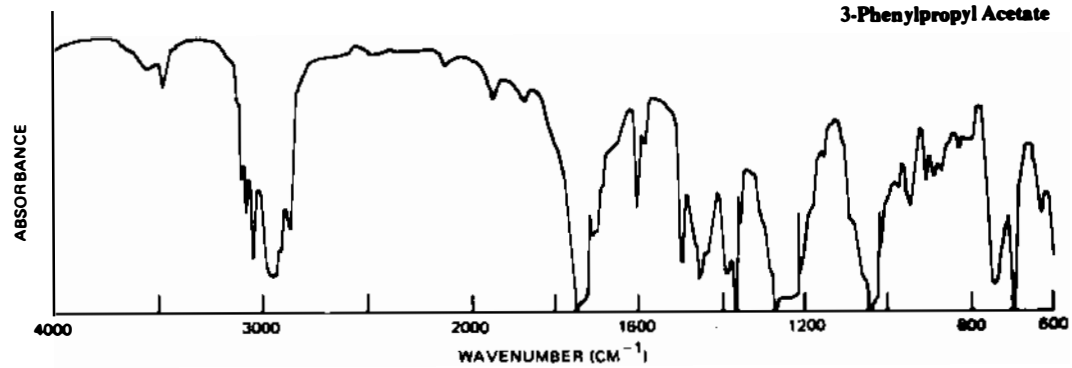
Phenethyl Isovalerate



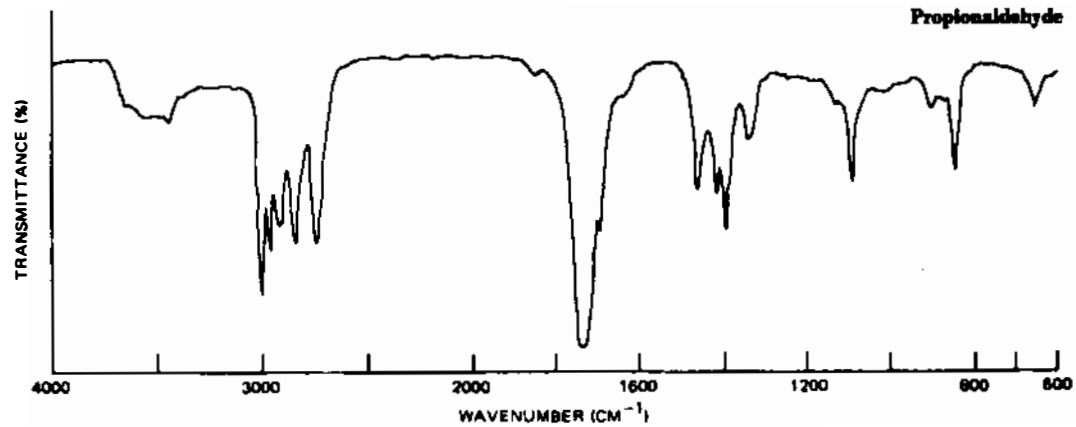
Phenylacetic Acid (KBr Pellet)



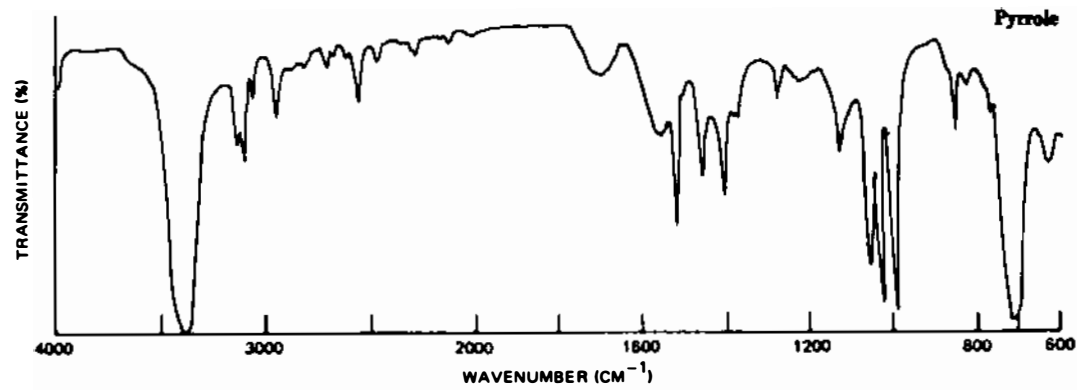
3-Phenylpropyl Acetate



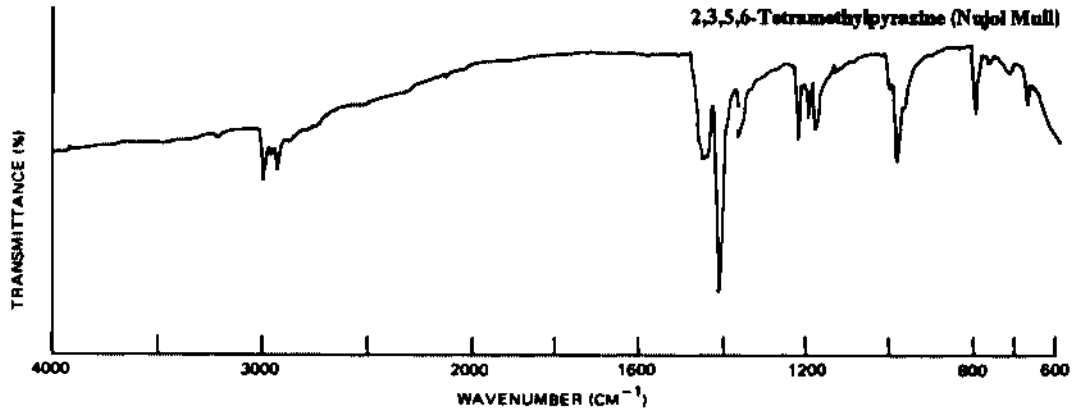
Propionaldehyde



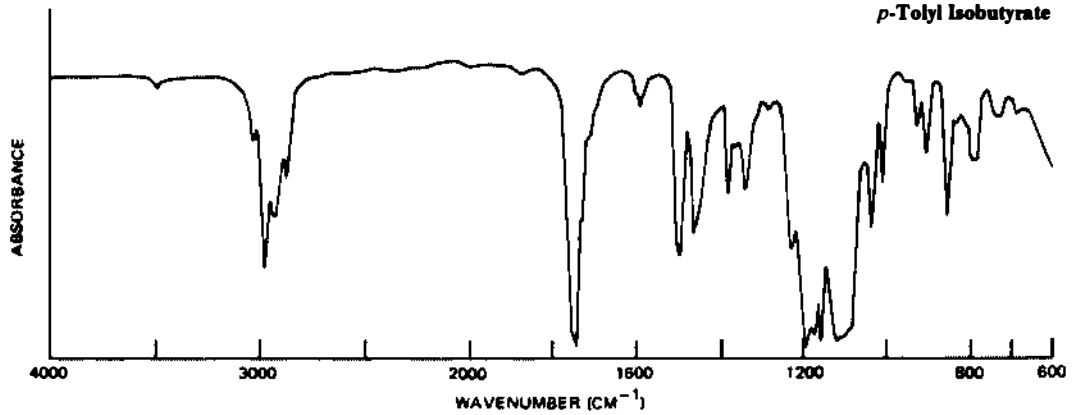
Pyrrole



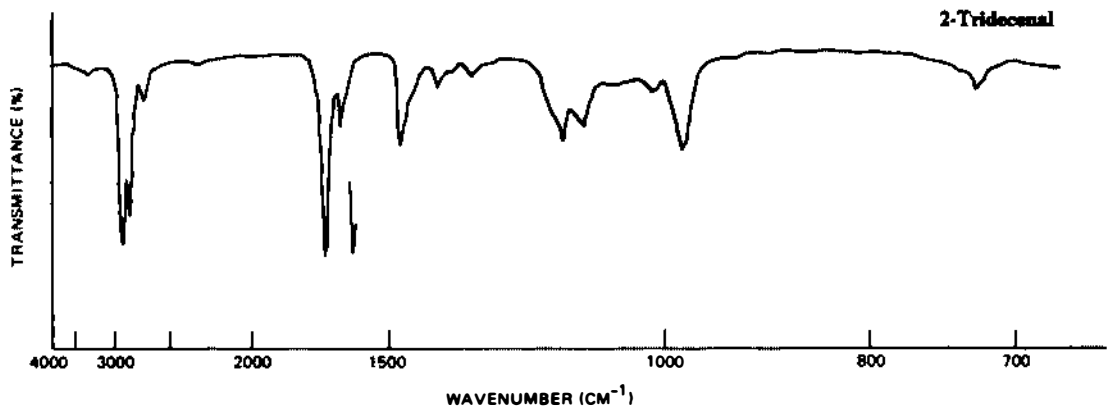
2,3,5,6-Tetramethylpyrazine (Nujol Mull)



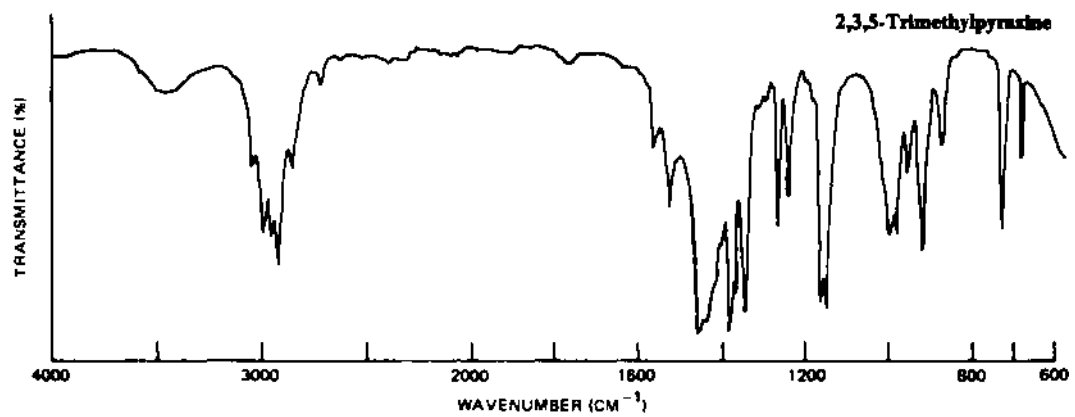
***p*-Tolyl Isobutyrate**



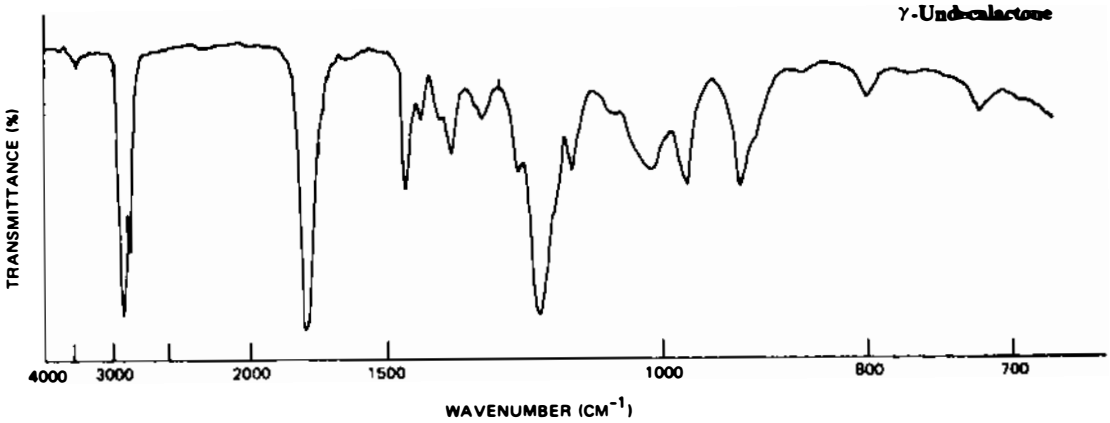
2-Tridecenal



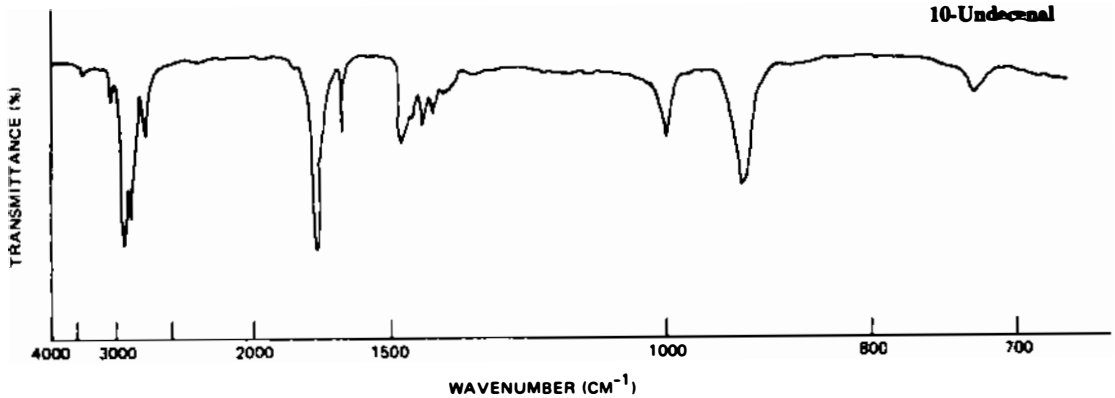
2,3,5-Trimethylpyrazine



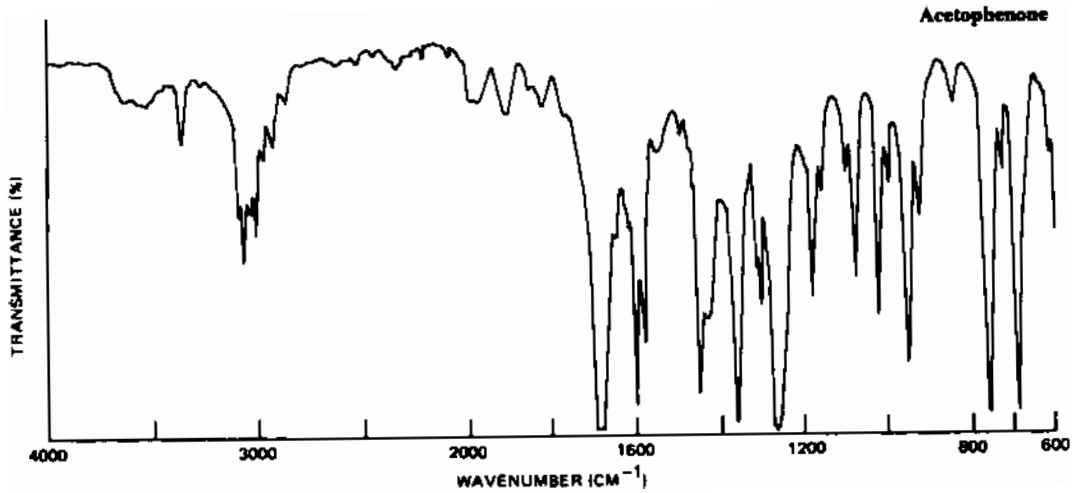
γ-Undecalactone

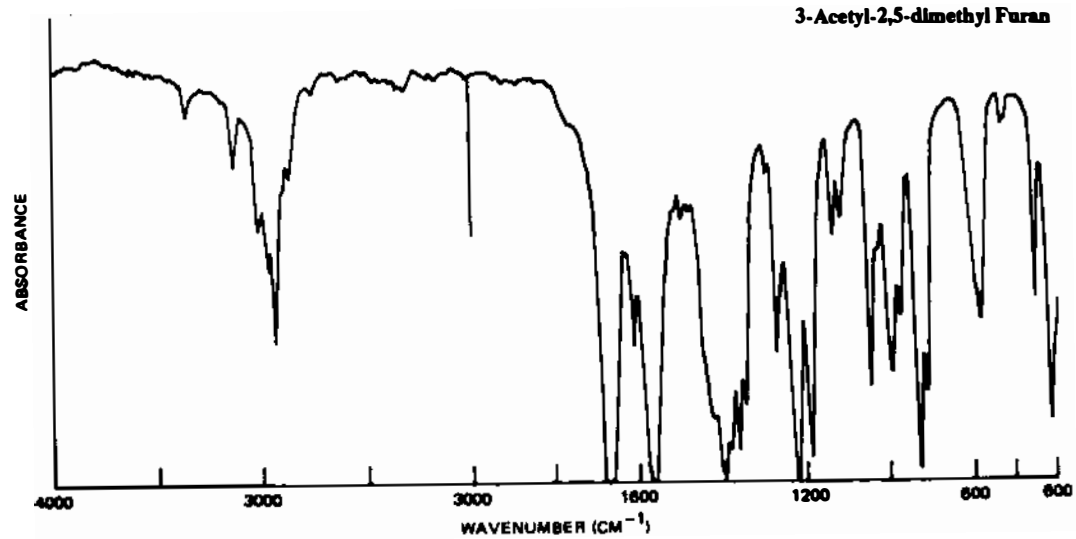


10-Undecenal

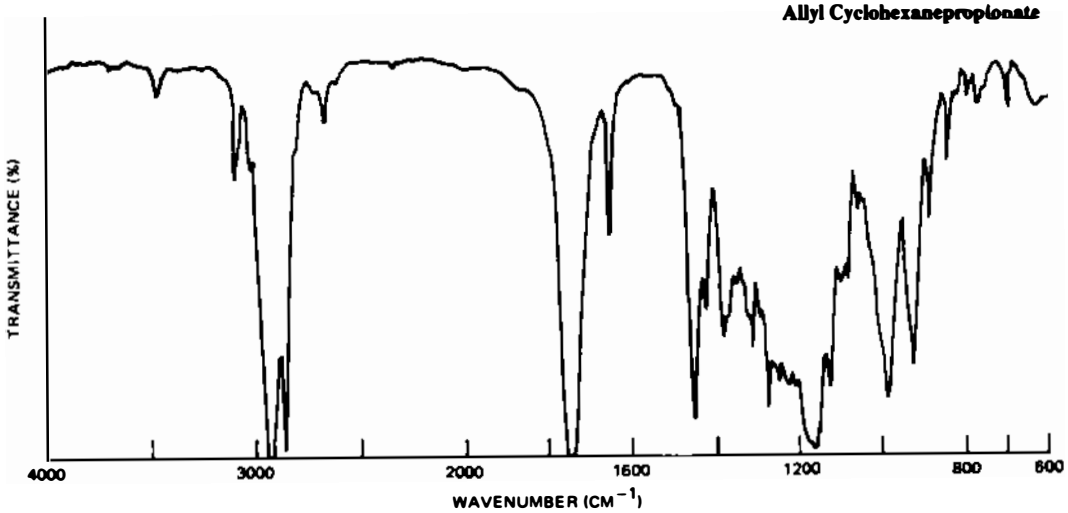


SERIES B-2

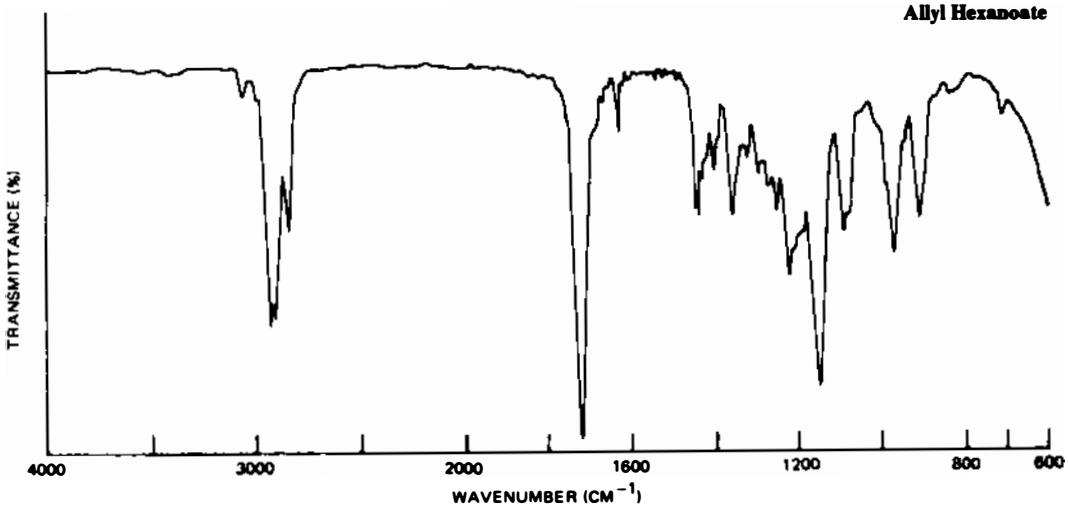




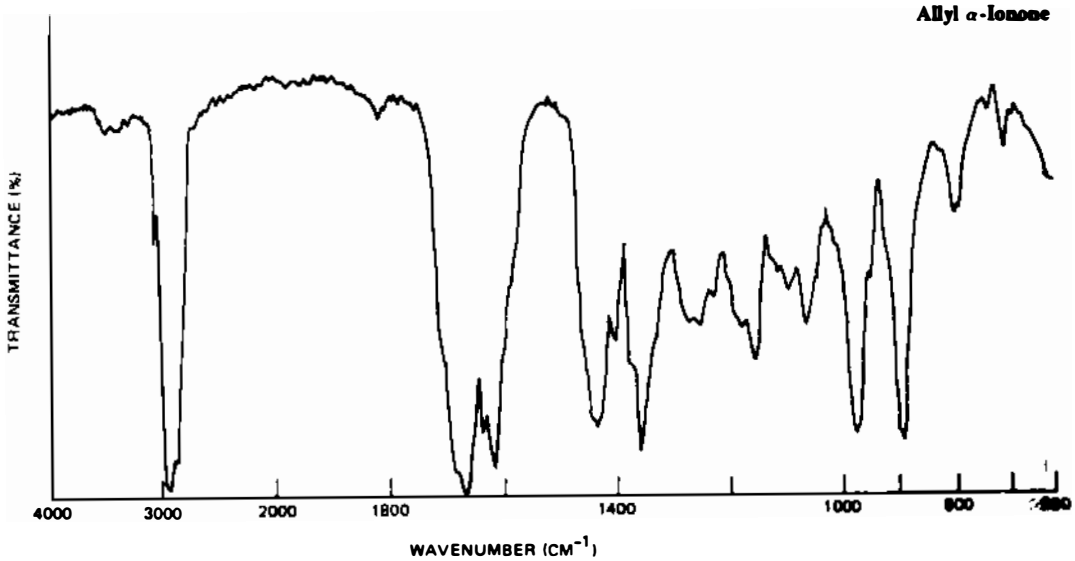
Allyl Cyclohexanepropionate



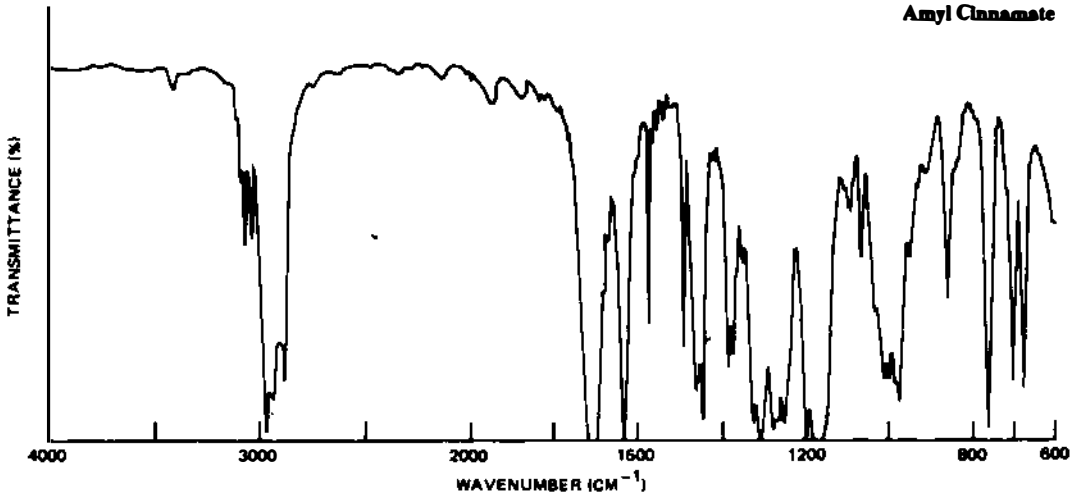
Allyl Hexanoate



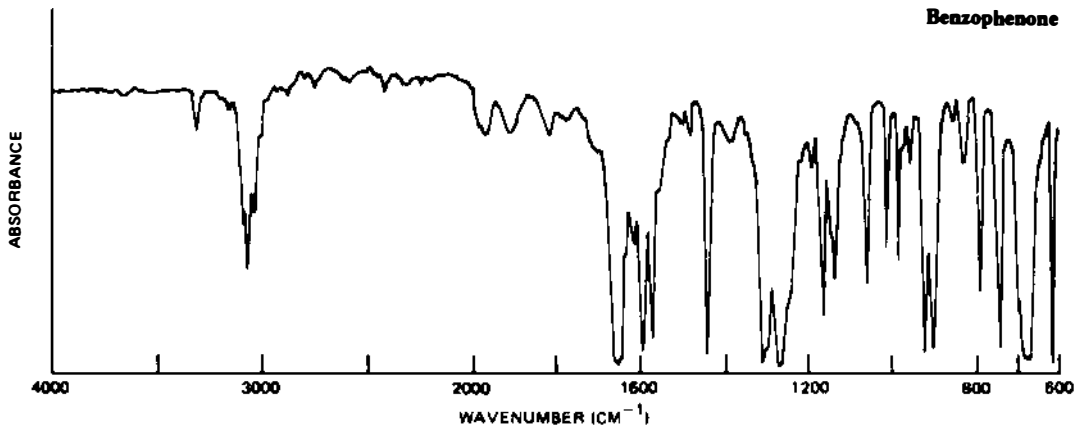
Allyl α-Ionone



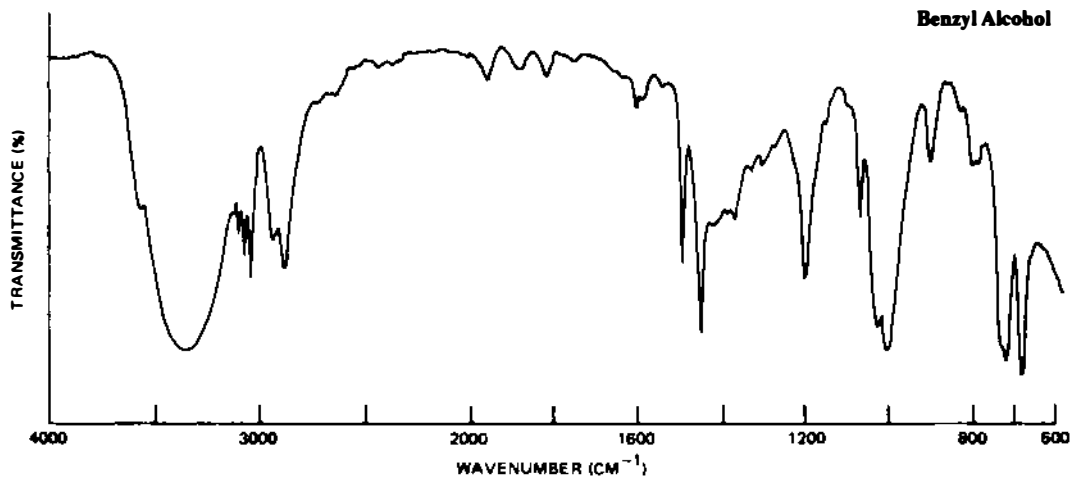
Amyl Cinnamate

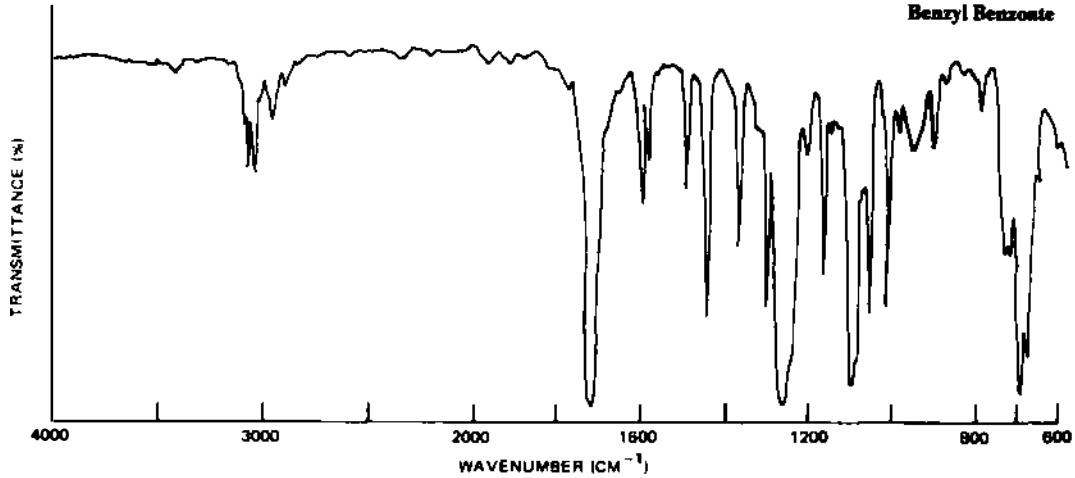


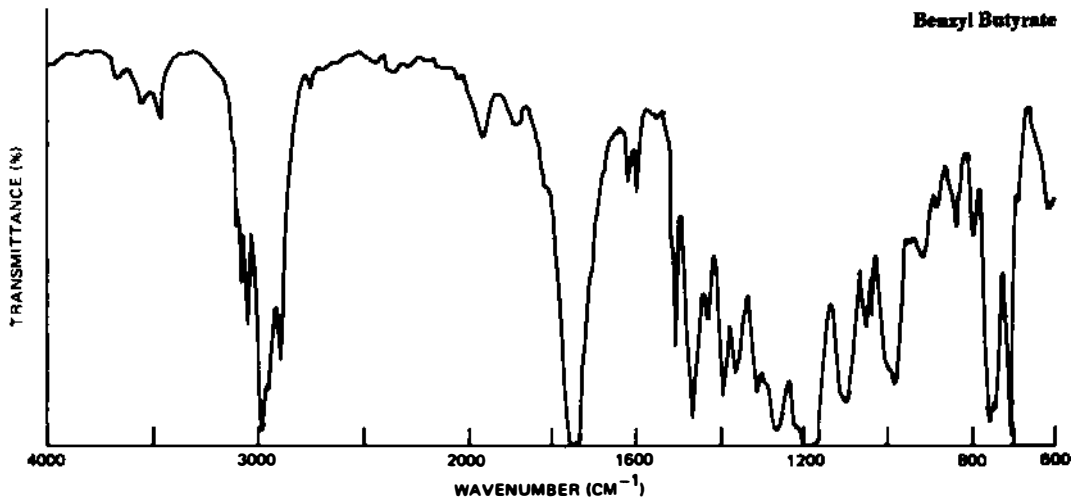
Benzophenone

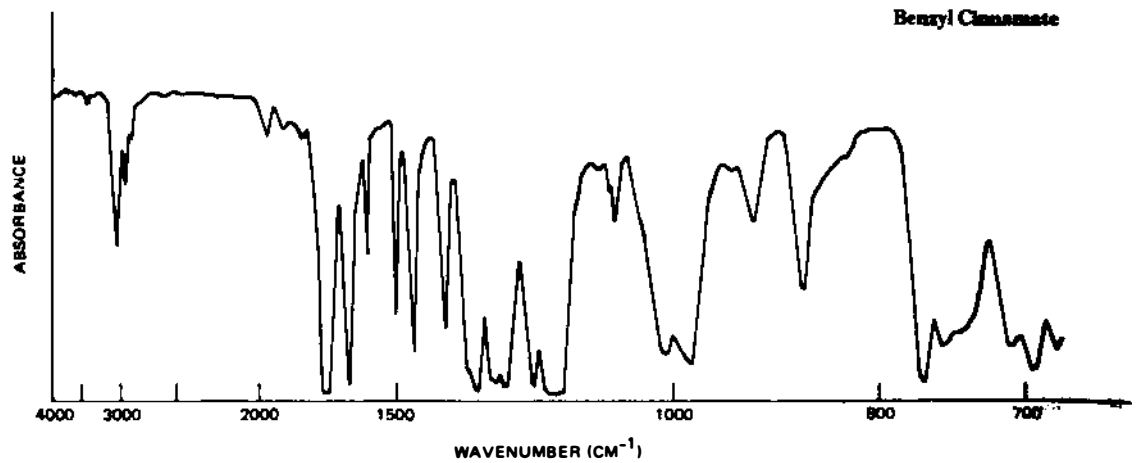


Benzyl Alcohol

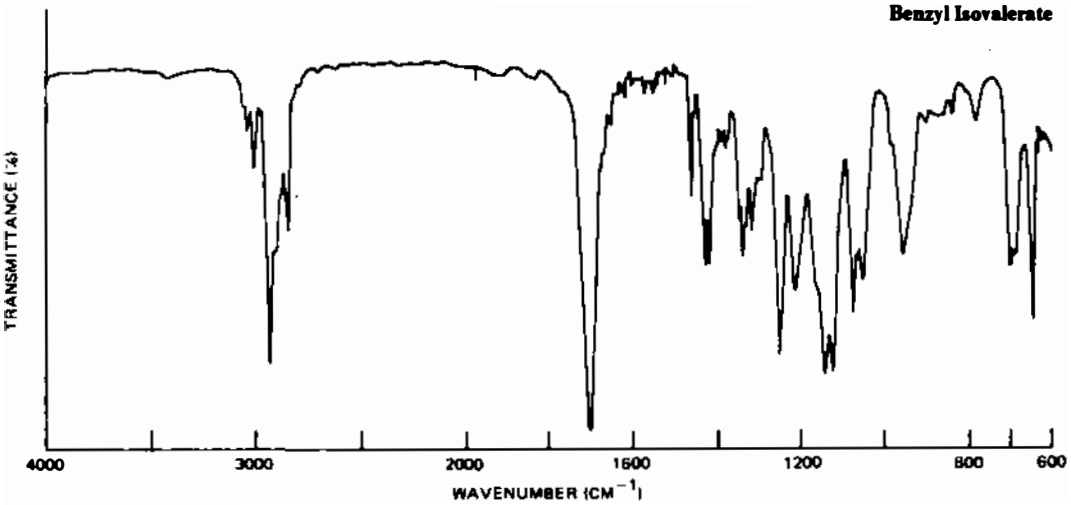



Benzyl Benzoate
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM⁻¹)

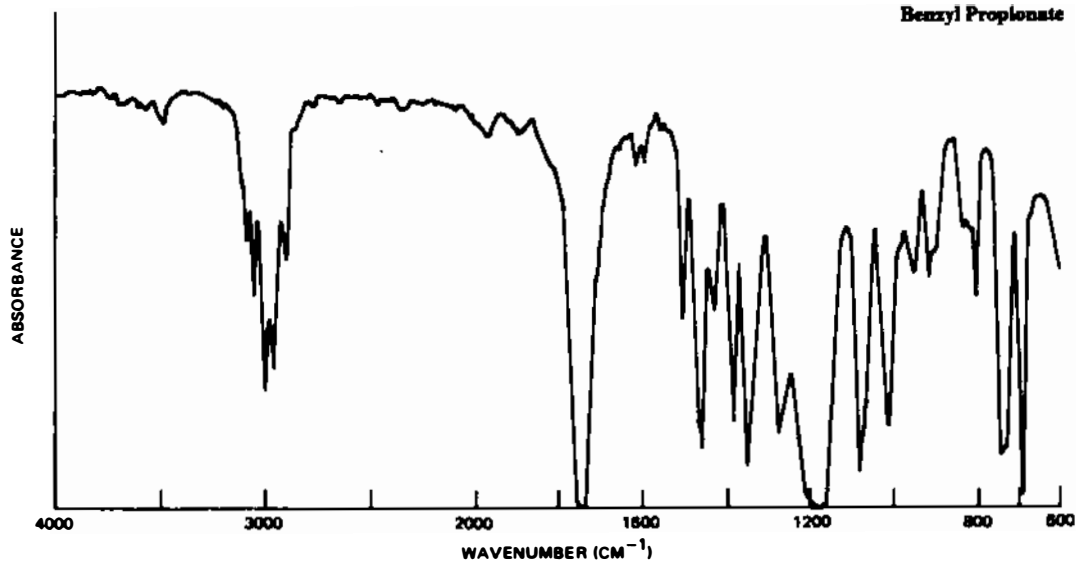

Benzyl Butyrate
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM⁻¹)


Benzyl Cinnamate
ABSORBANCE
4000
3000
2000
1500
1000
800
700
WAVENUMBER (CM⁻¹)

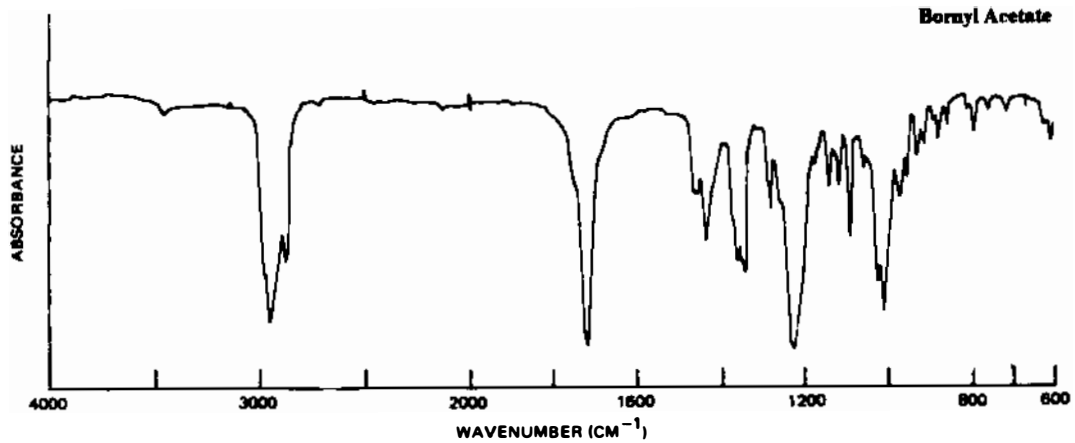
Benzyl Isovalerate



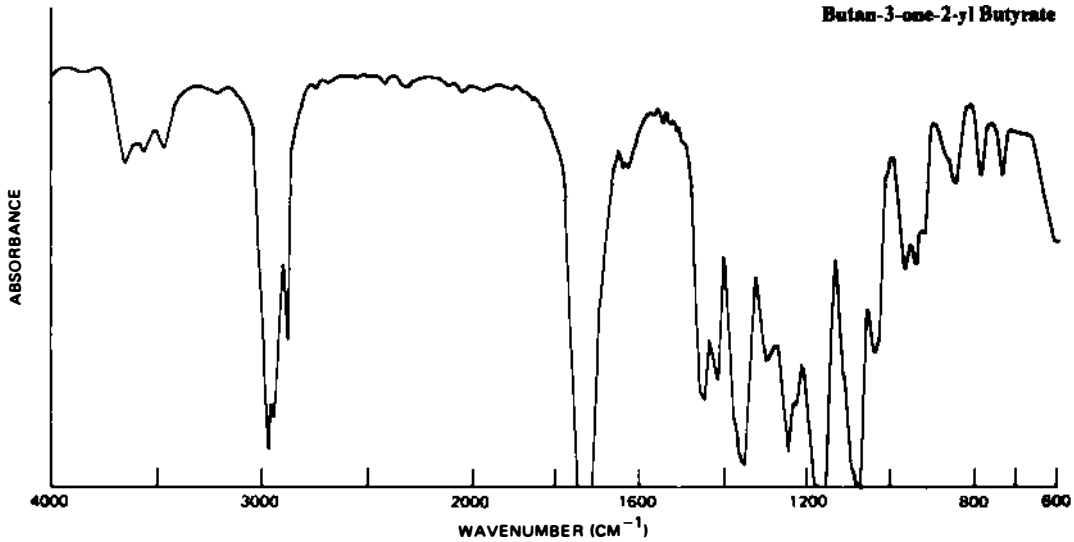
Benzyl Propionate



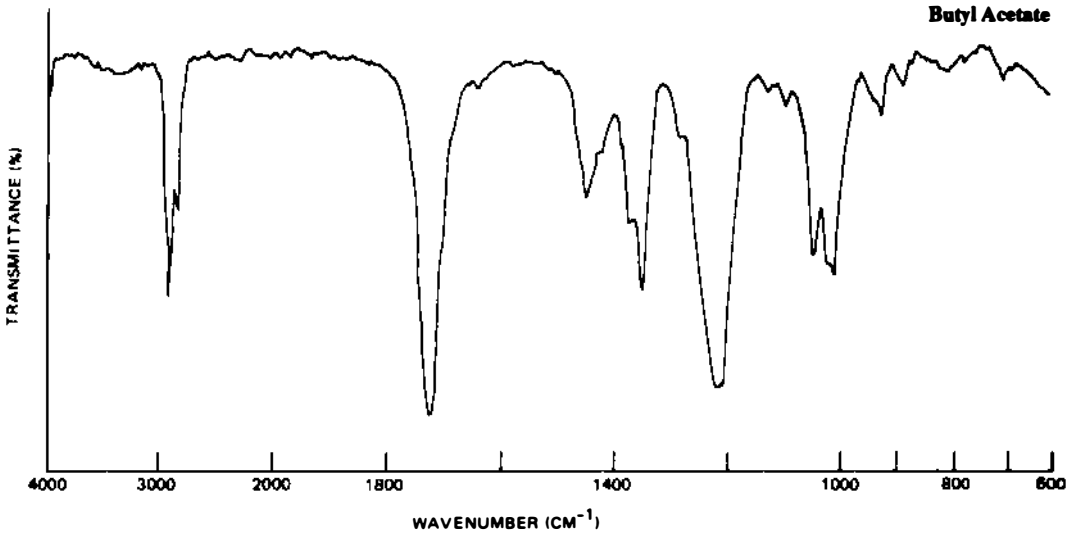
Bornyl Acetate



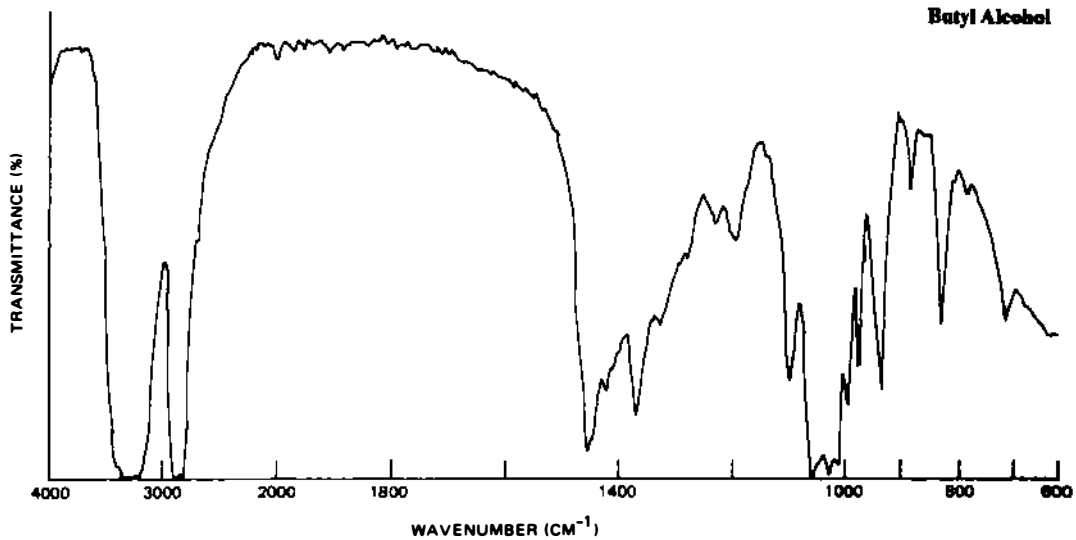
Butan-3-one-2-yl Butyrate



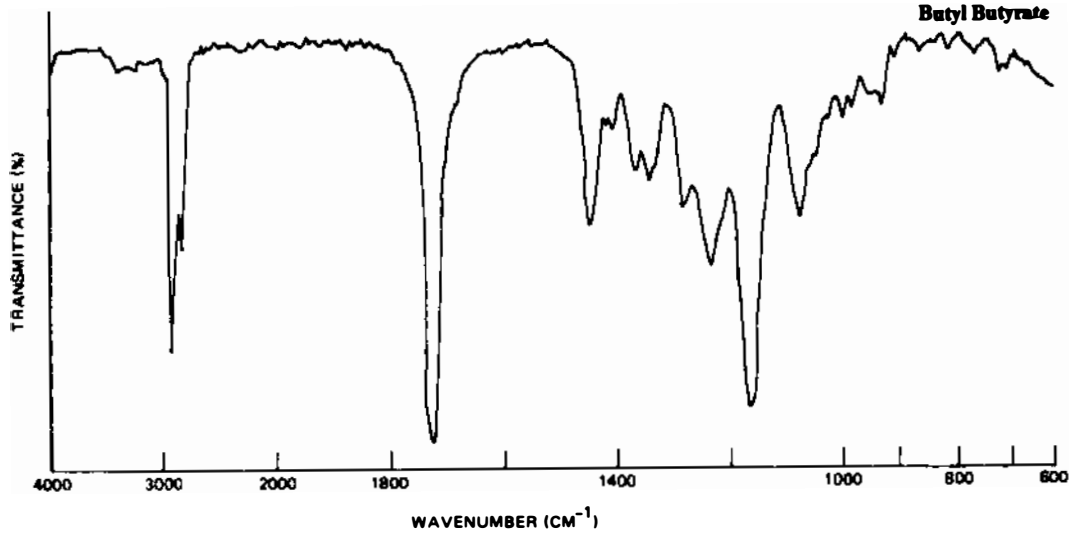
Butyl Acetate



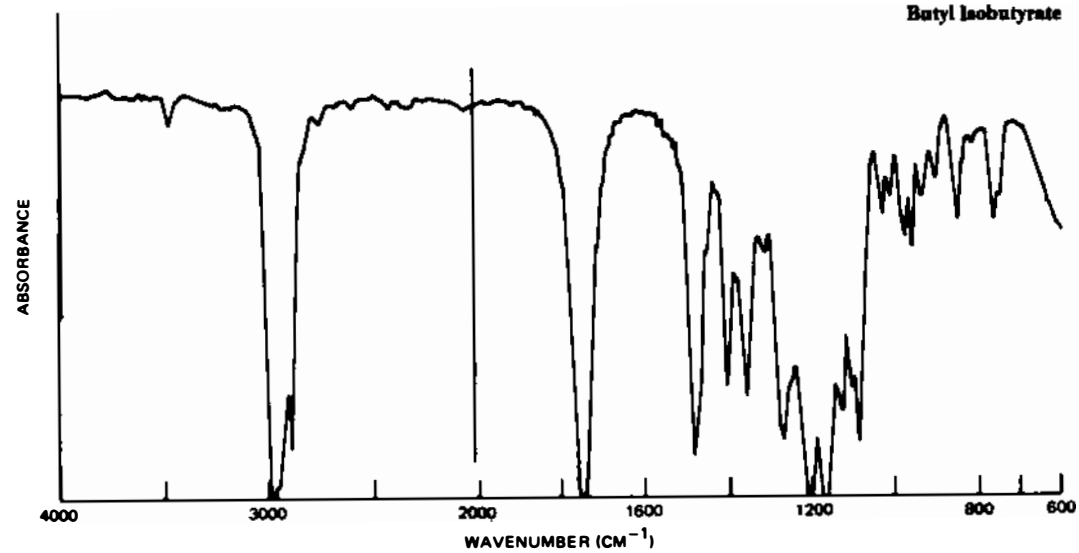
Butyl Alcohol



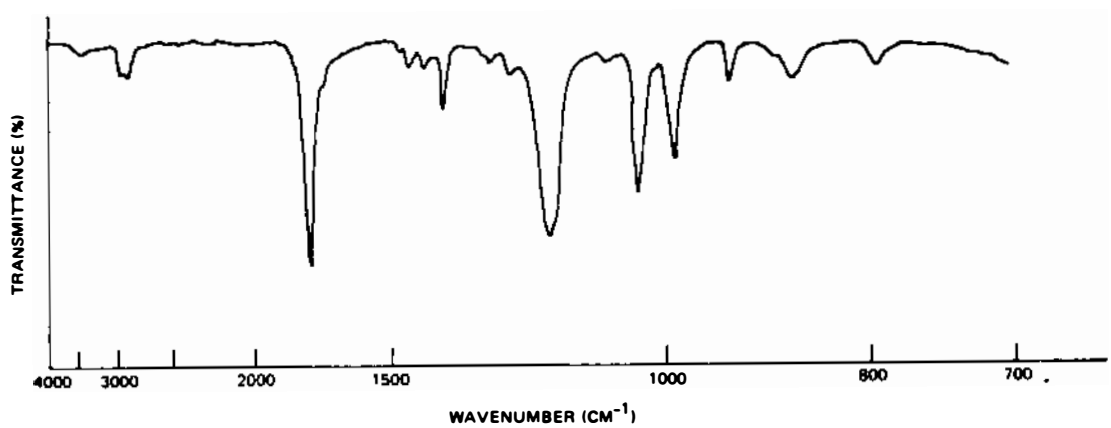
Butyl Butyrate



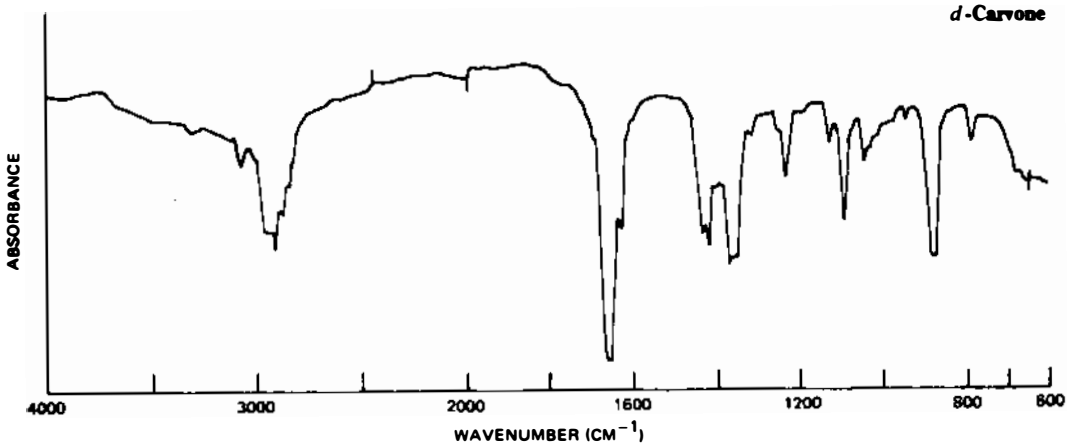
Butyl Isobutyrate



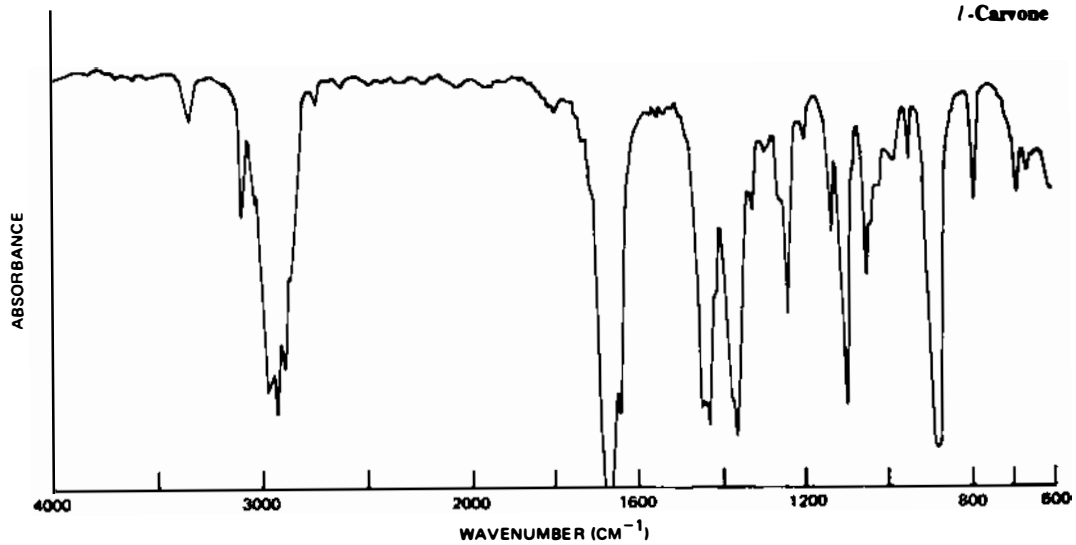
γ-Butyrolactone



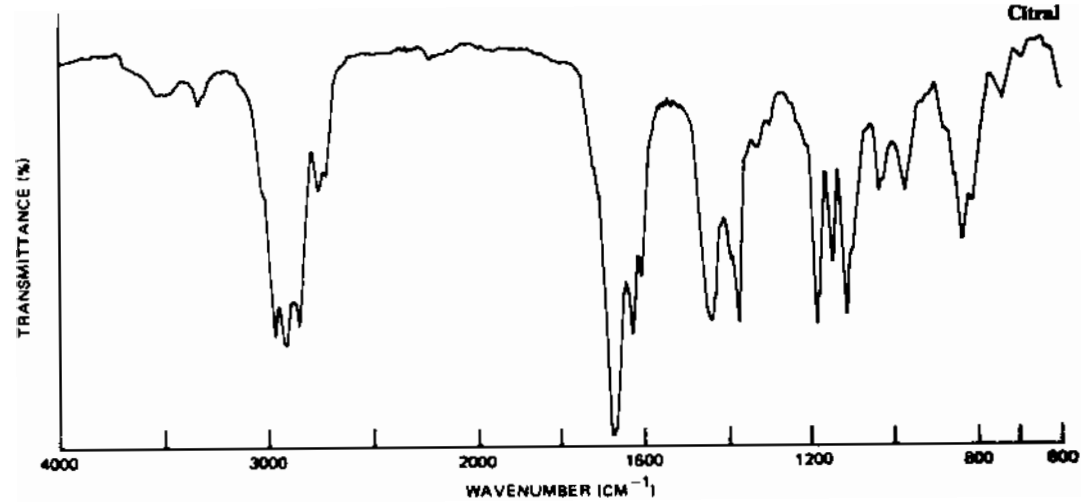
d-Carvone



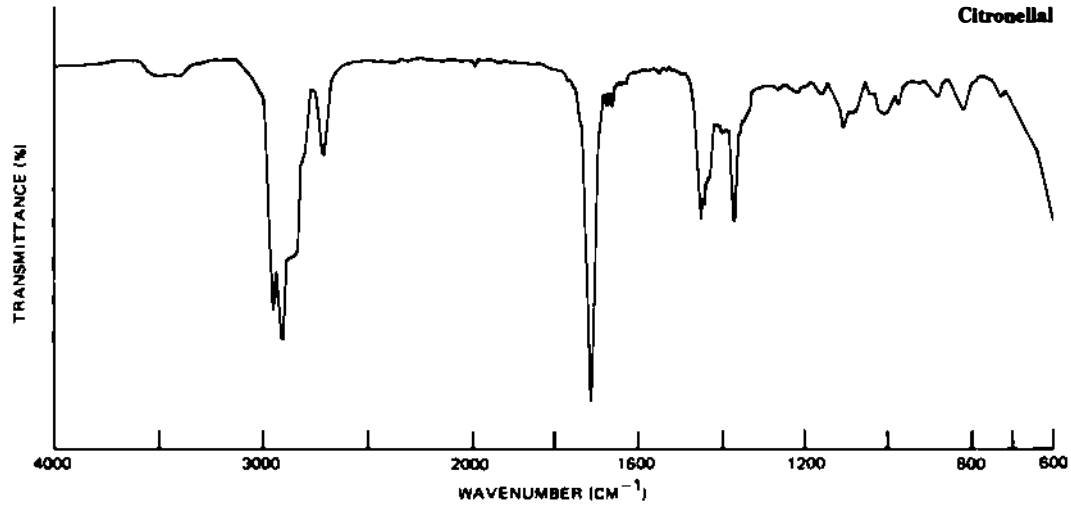
l-Carvone



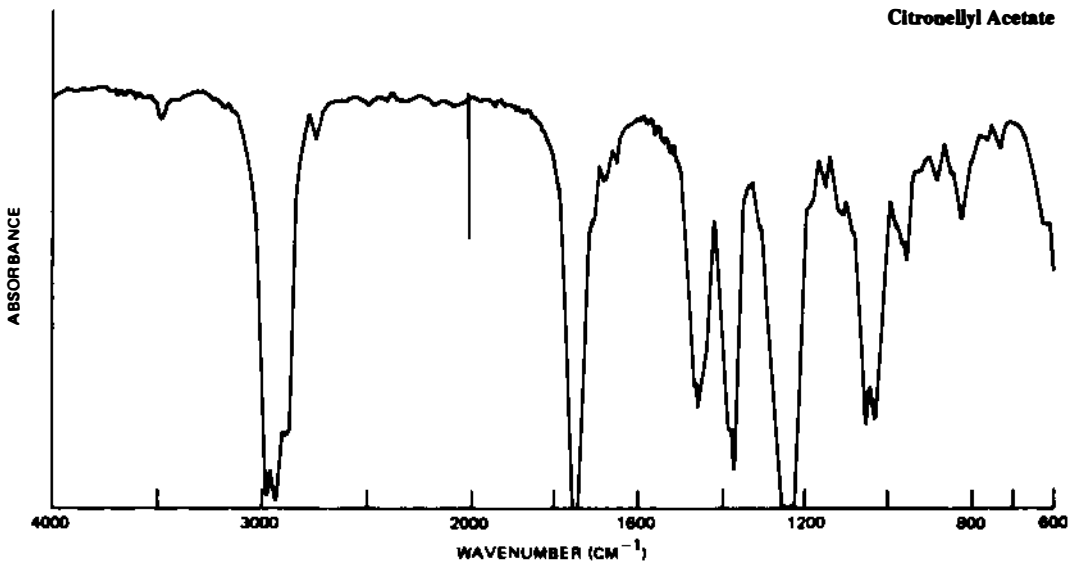
Citral



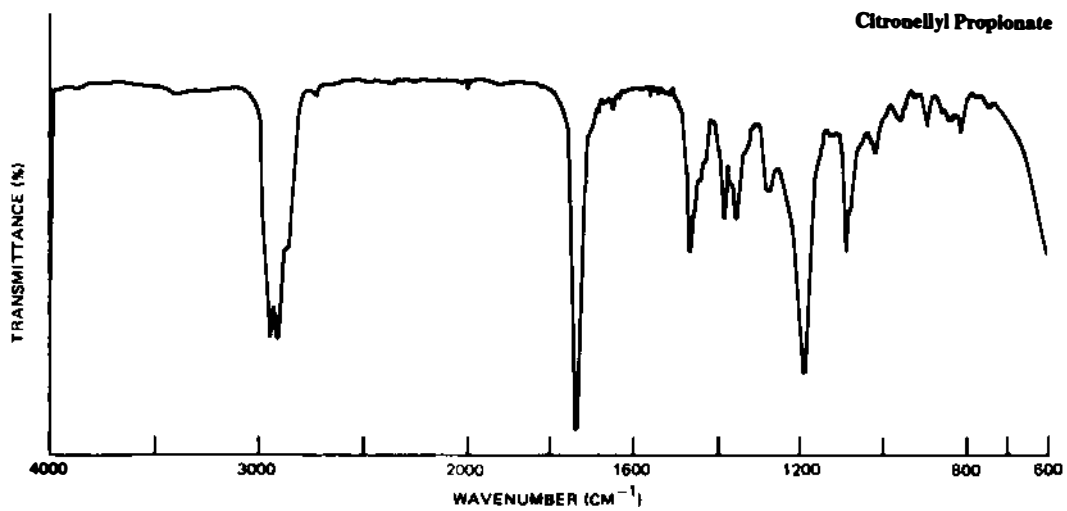
Citronellal



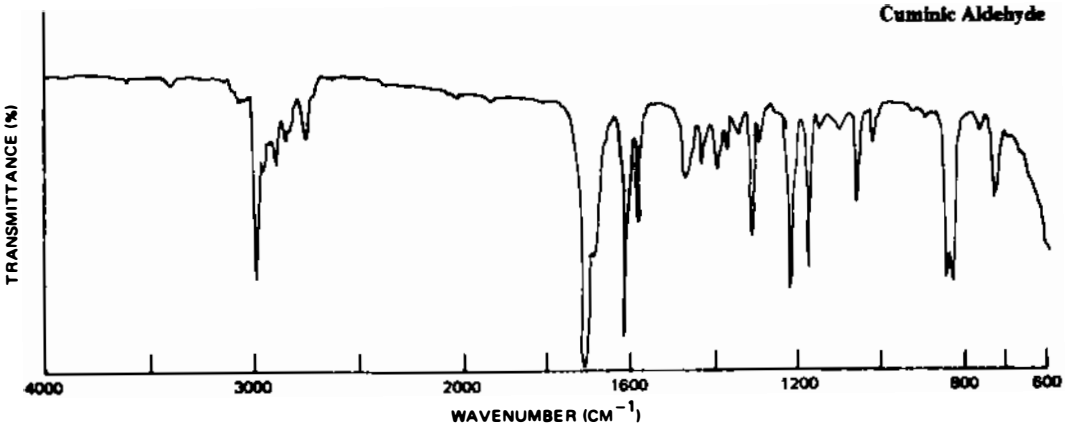
Citronellyl Acetate



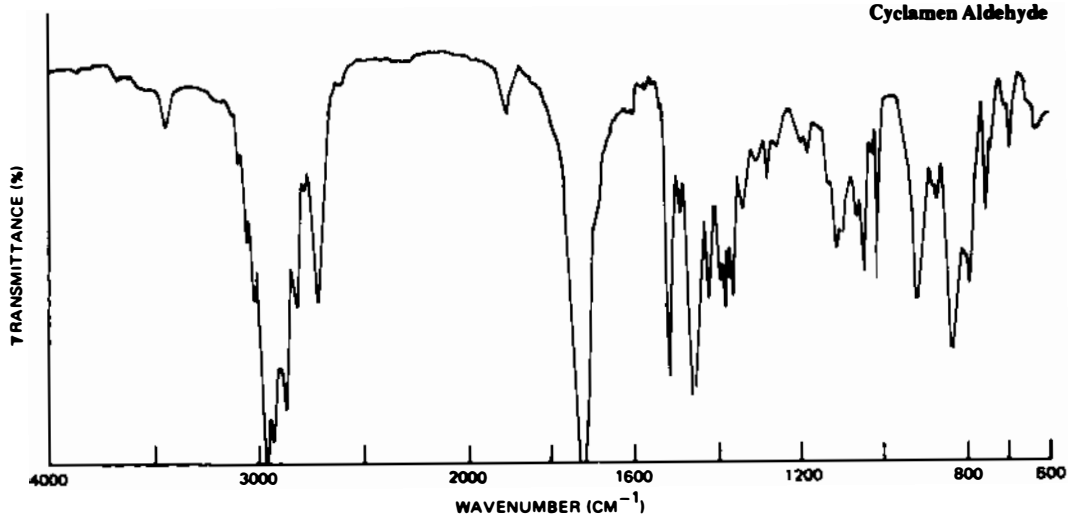
Citronellyl Propionate



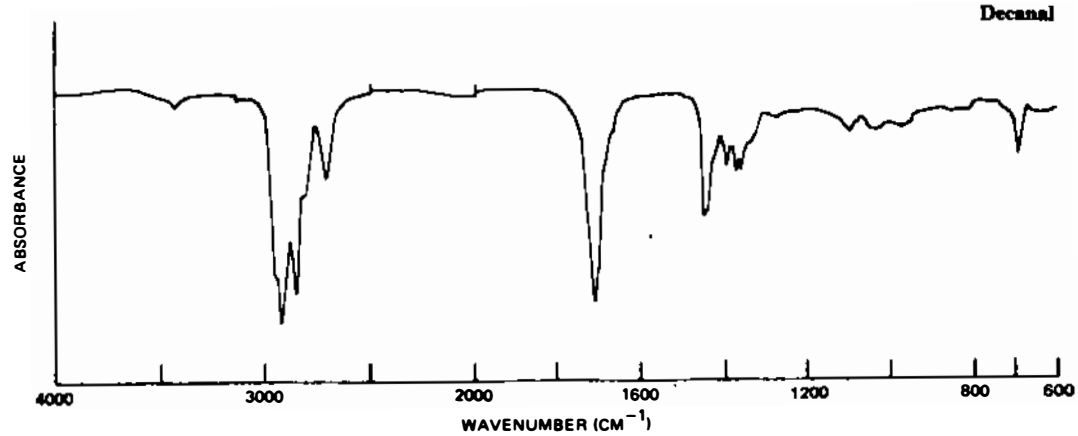
Cuminic Aldehyde



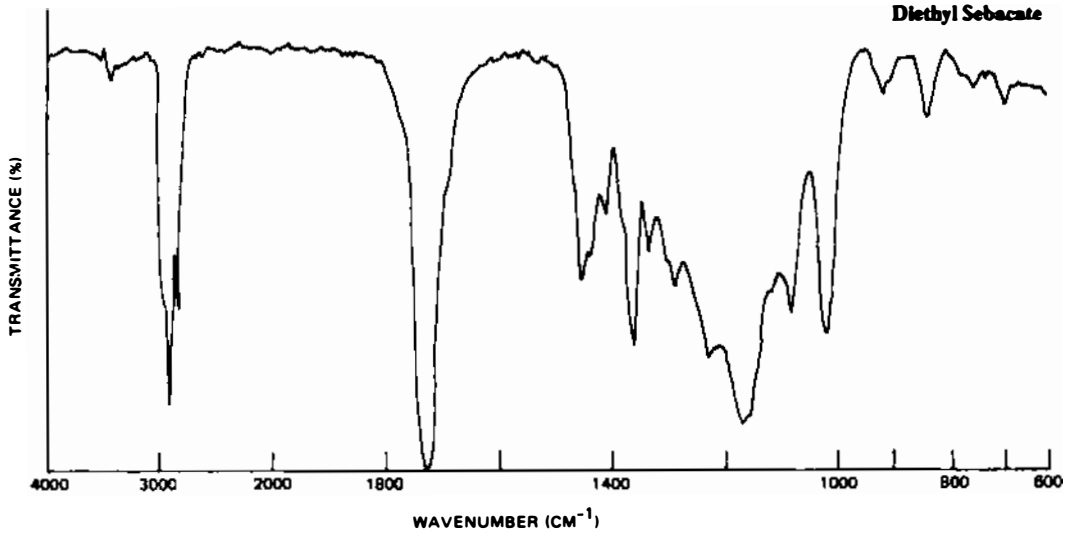
Cyclamen Aldehyde



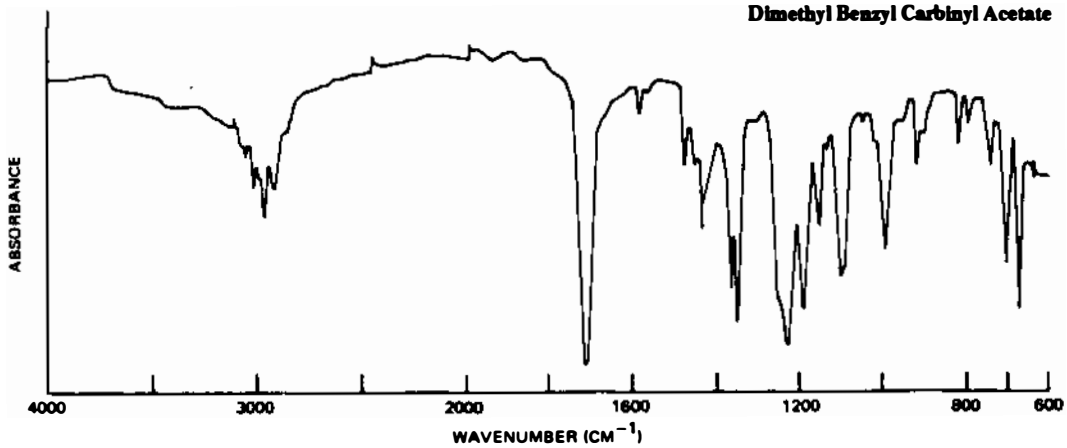
Decanal



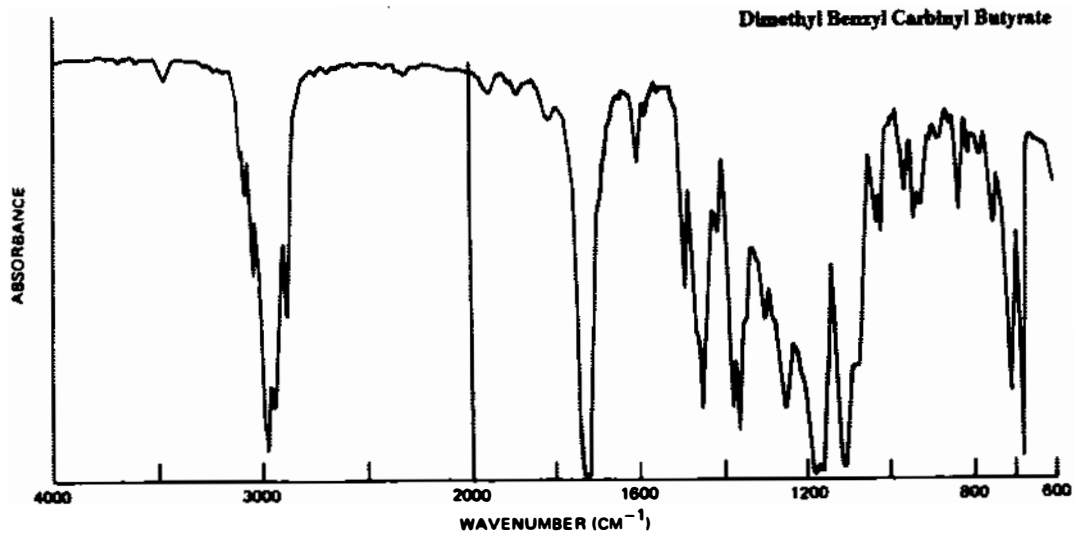
Diethyl Sebacate



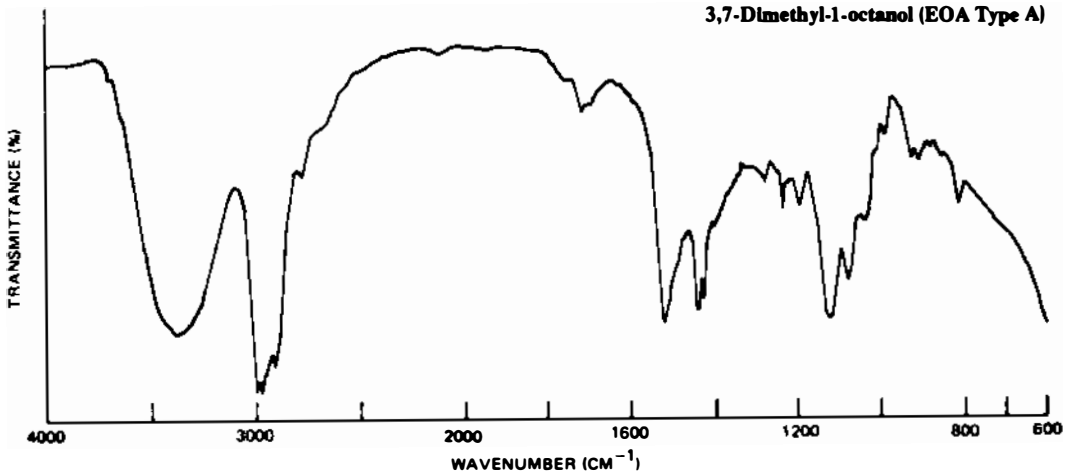
Dimethyl Benzyl Carbinyl Acetate



Dimethyl Benzyl Carbinyl Butyrate



3,7-Dimethyl-1-octanol (EOA Type A)



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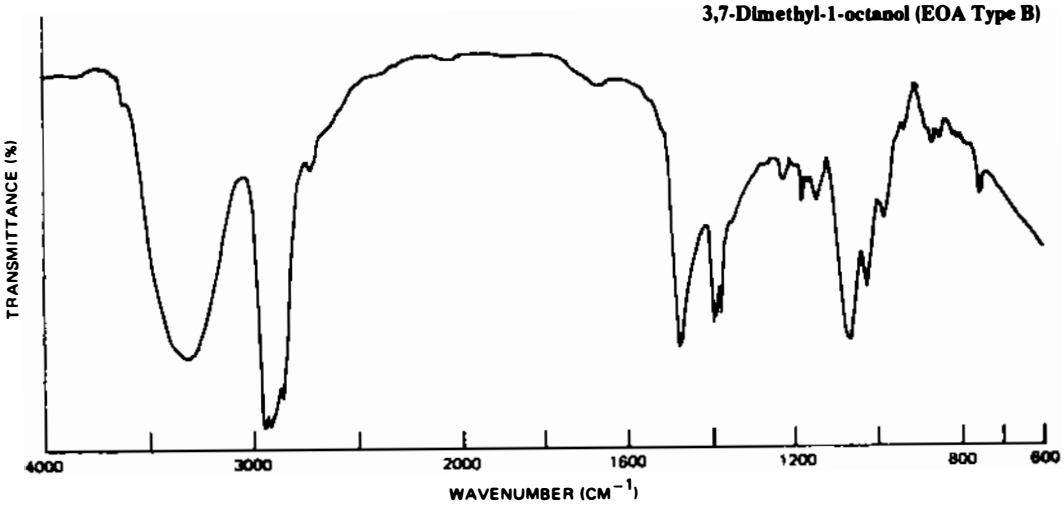
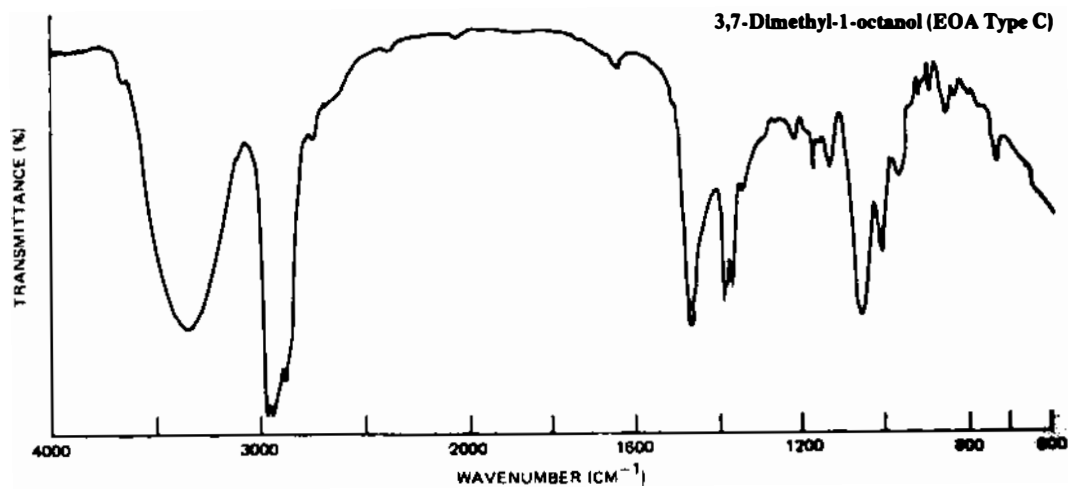
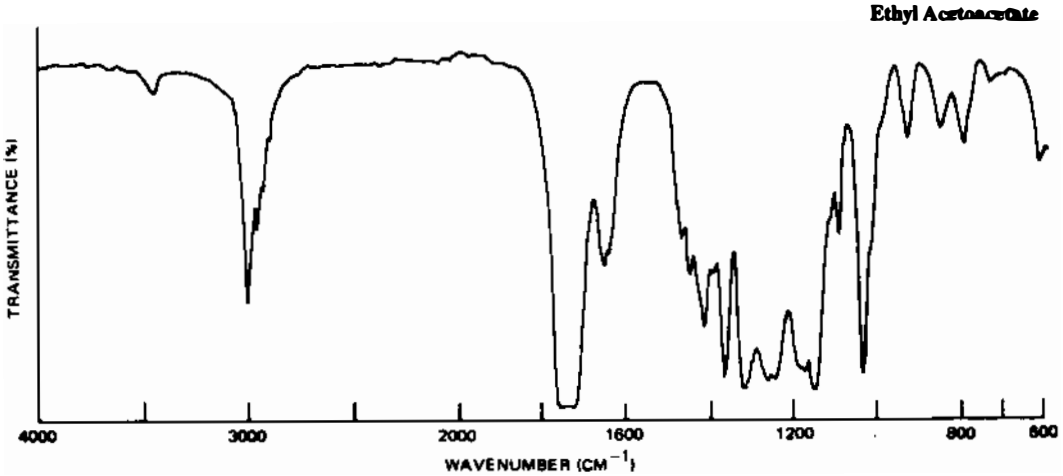


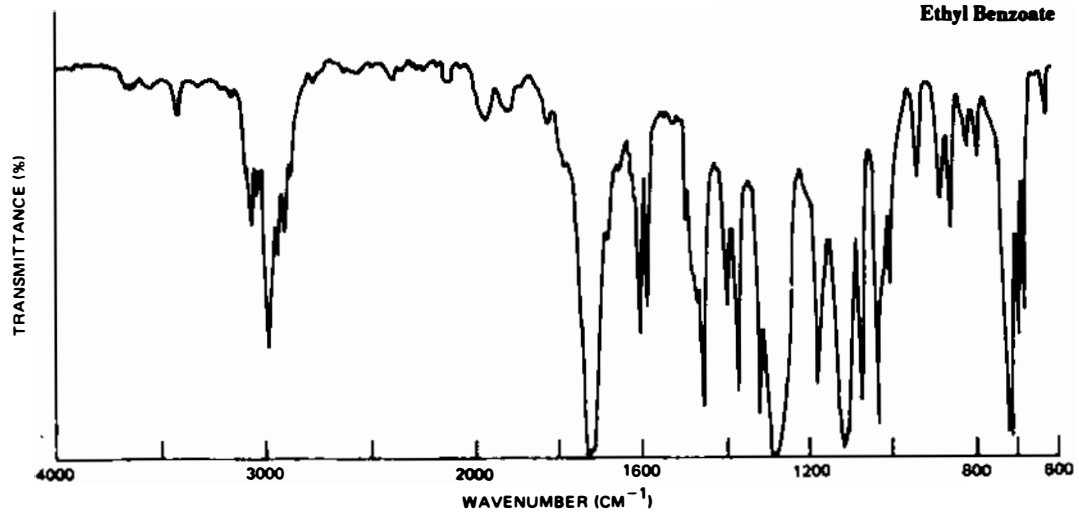
3,7-Dimethyl-1-octanol (EOA Type C)



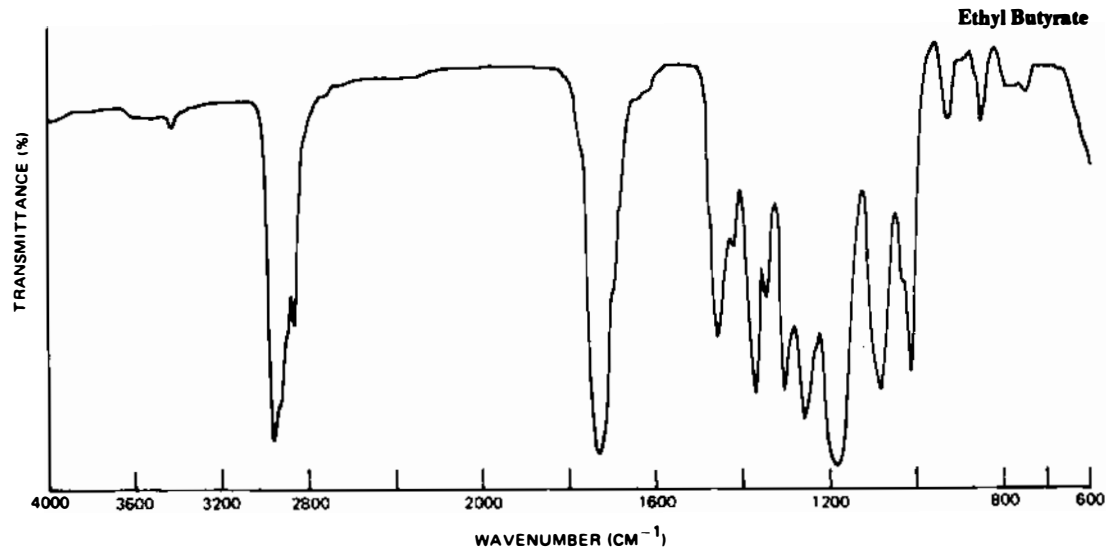
Ethyl Acetoacetate



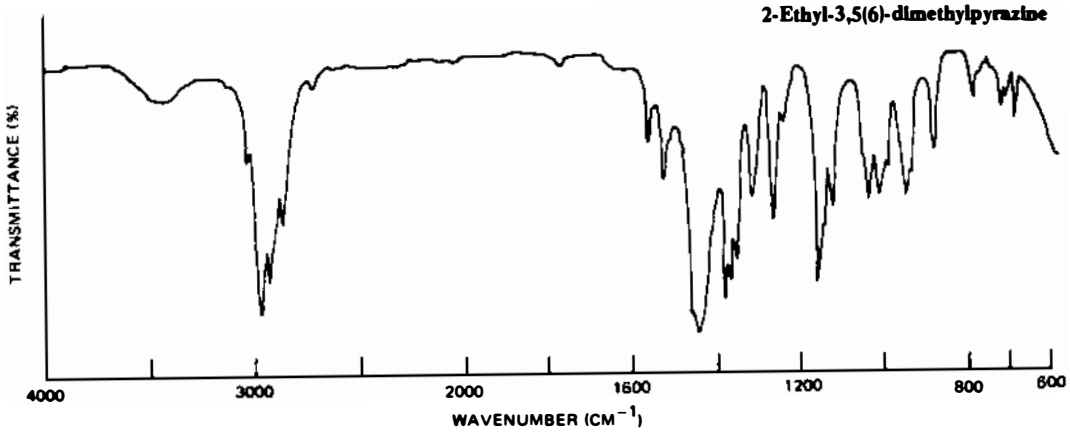
Ethyl Benzoate



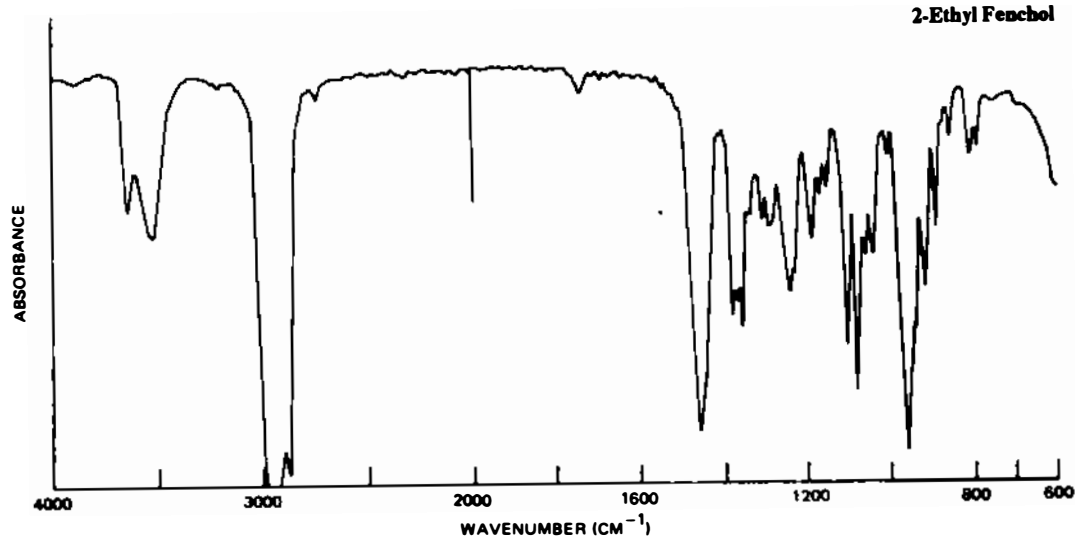
Ethyl Butyrate



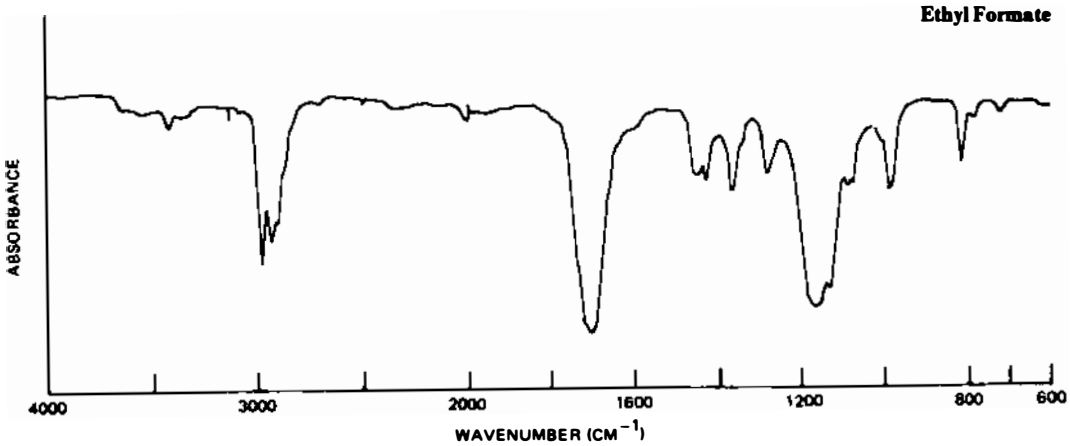
2-Ethyl-3,5(6)-dimethylpyrazine



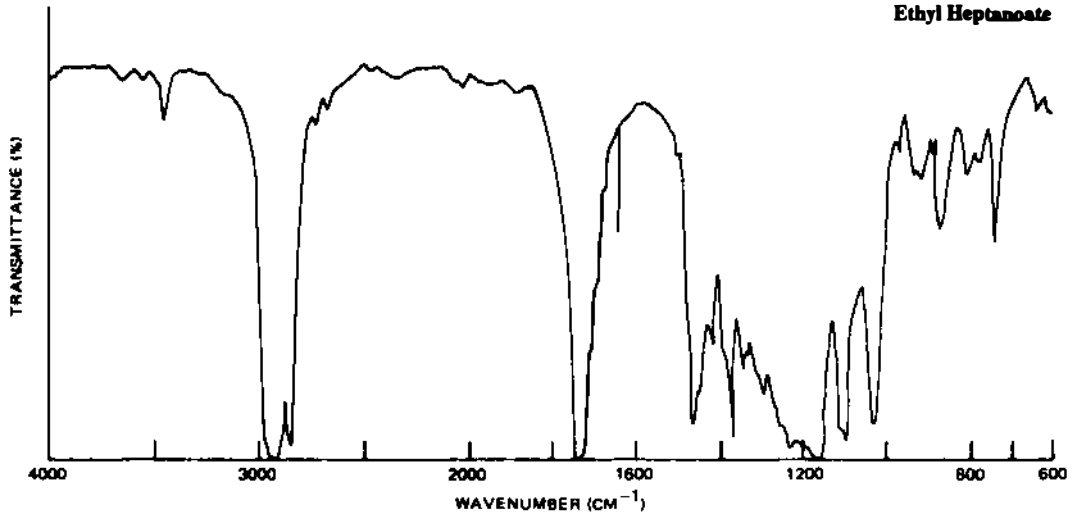
2-Ethyl Fenchol



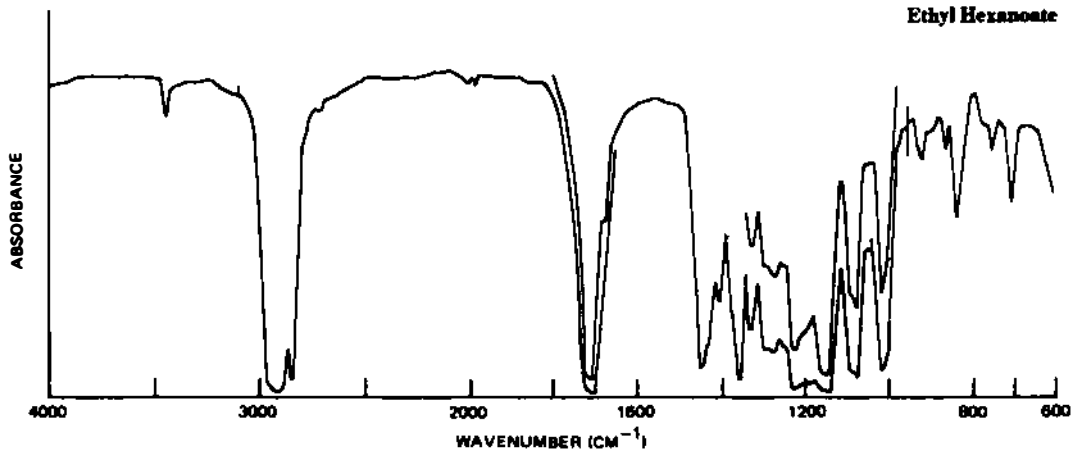
Ethyl Formate



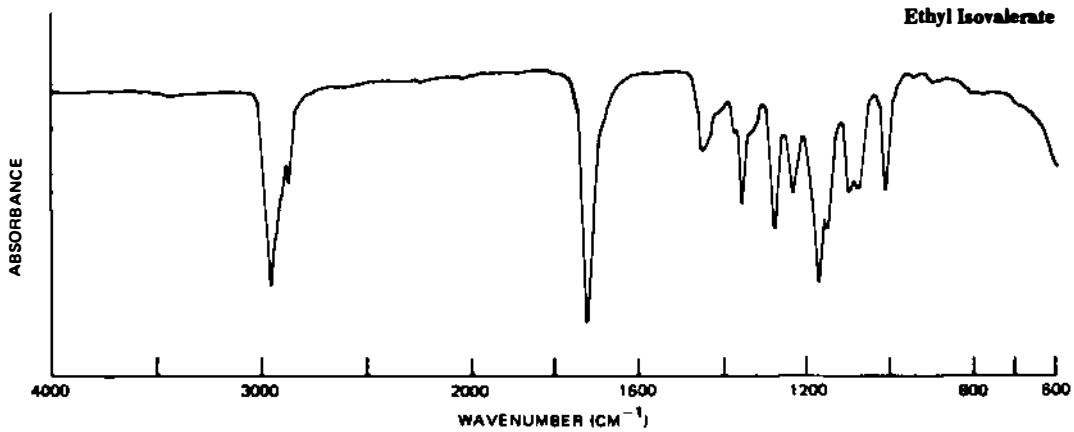
Ethyl Heptanoate



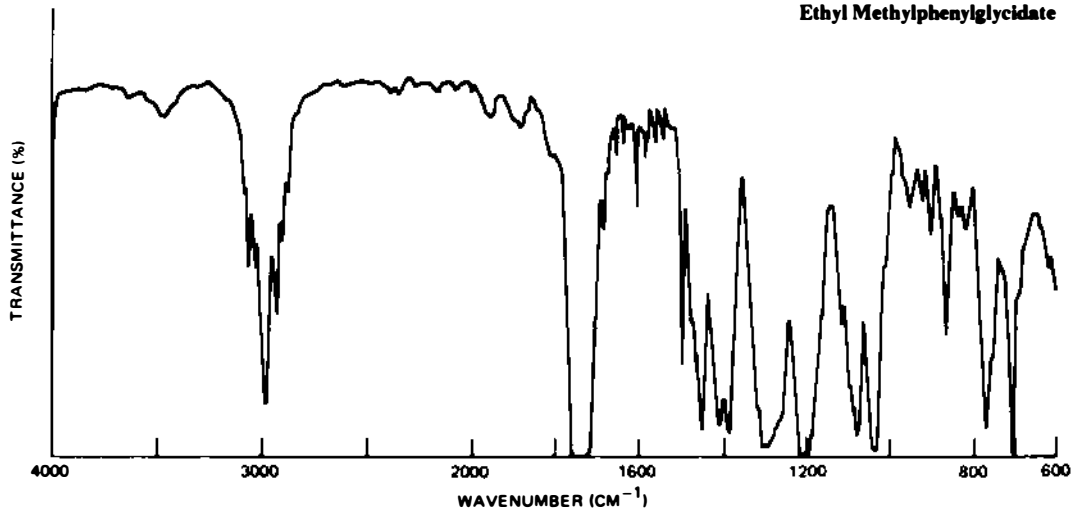
Ethyl Hexanoate



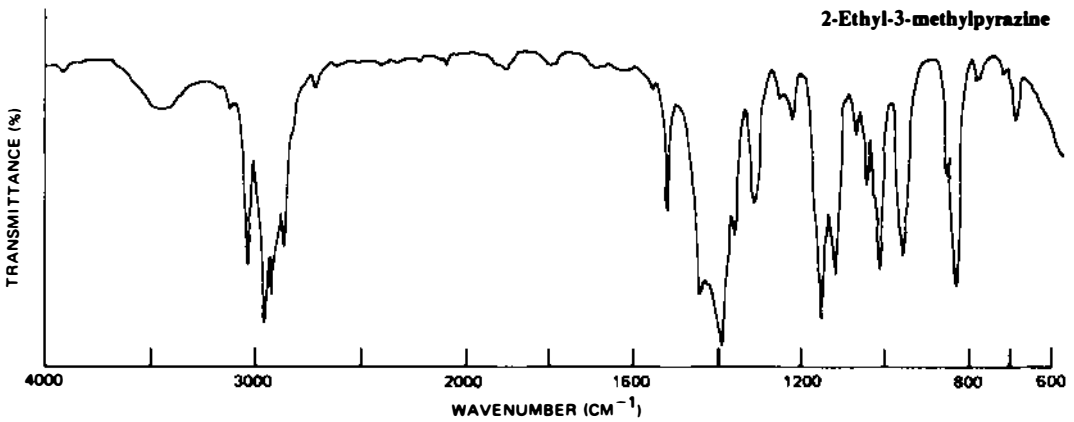
Ethyl Isovalerate



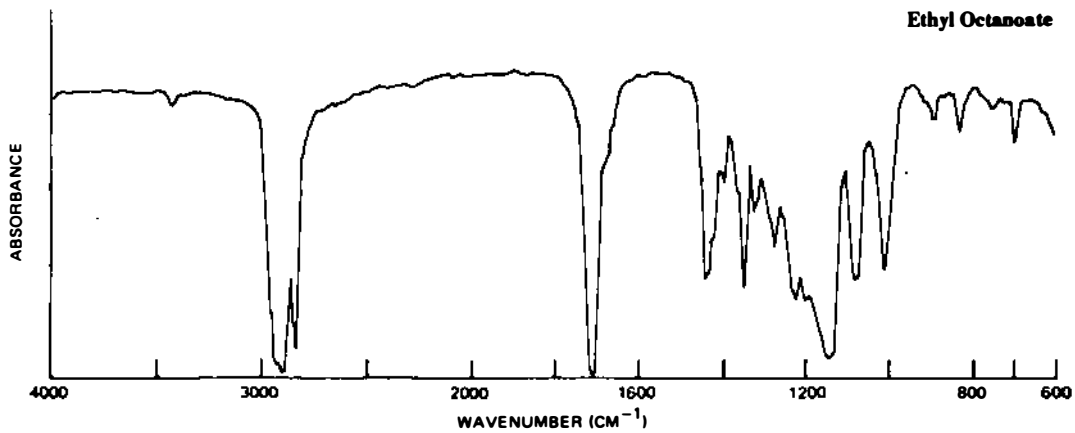
Ethyl Methylphenylglycidate



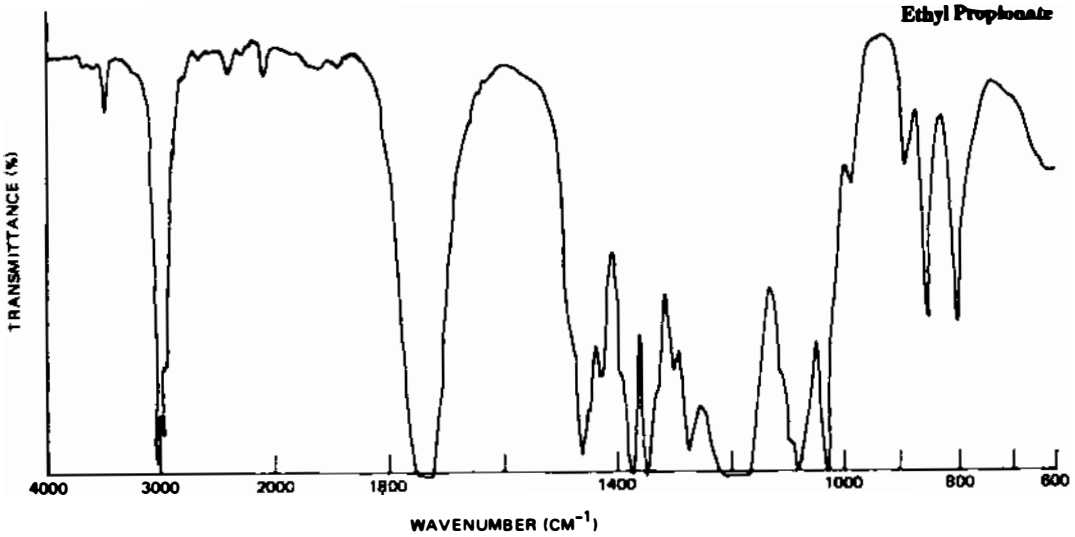
2-Ethyl-3-methylpyrazine



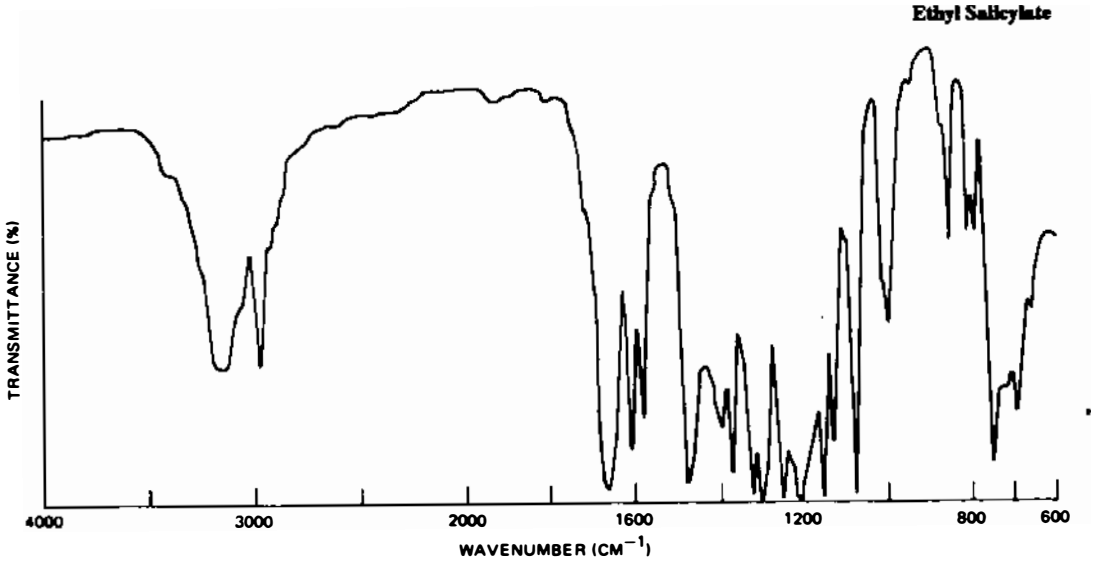
Ethyl Octanoate



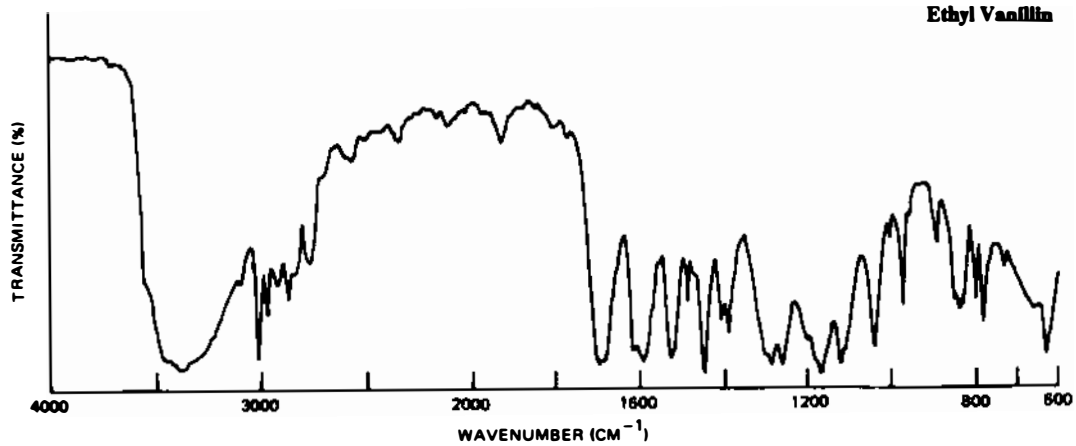
Ethyl Propionate



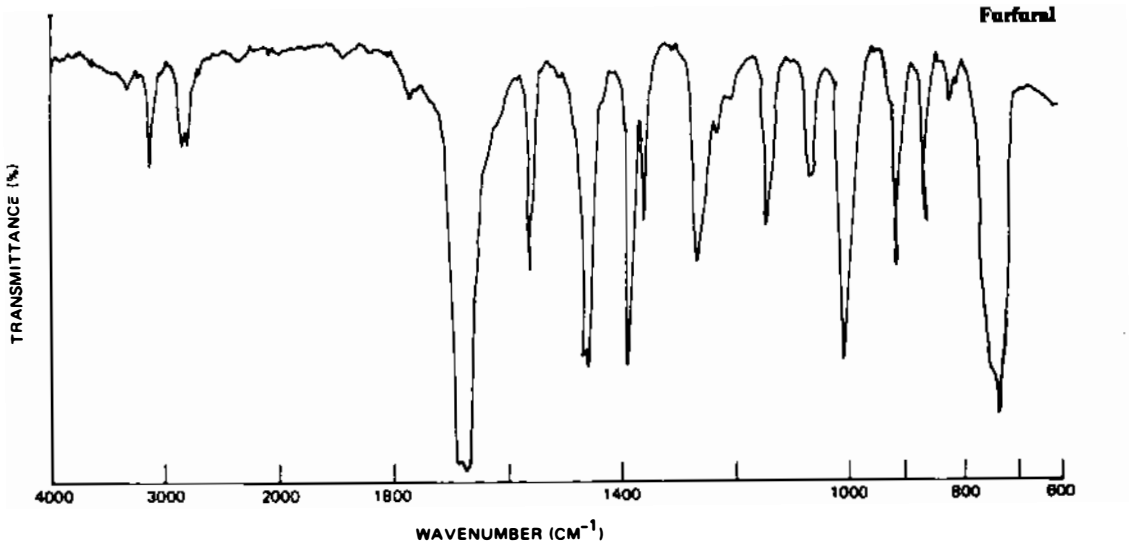
Ethyl Salicylate



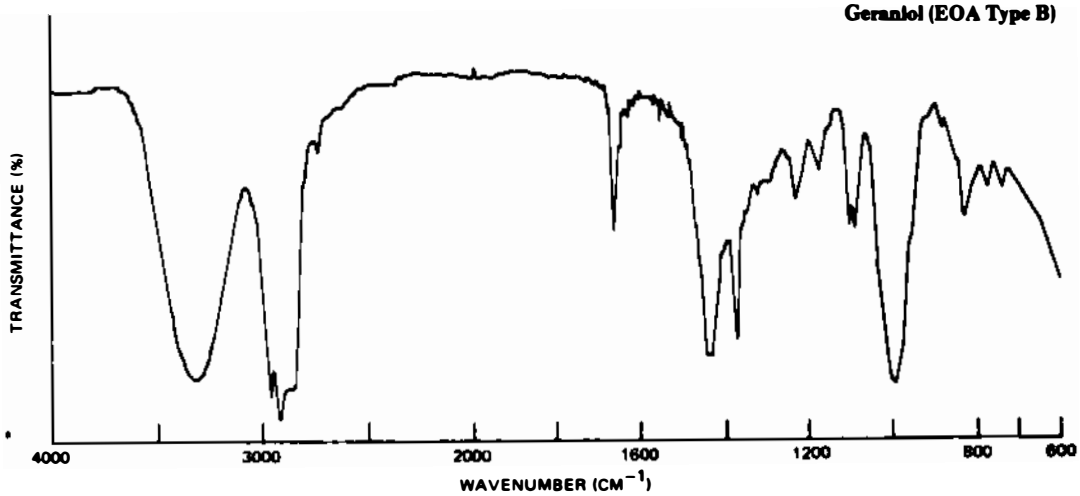
Ethyl Vanillin



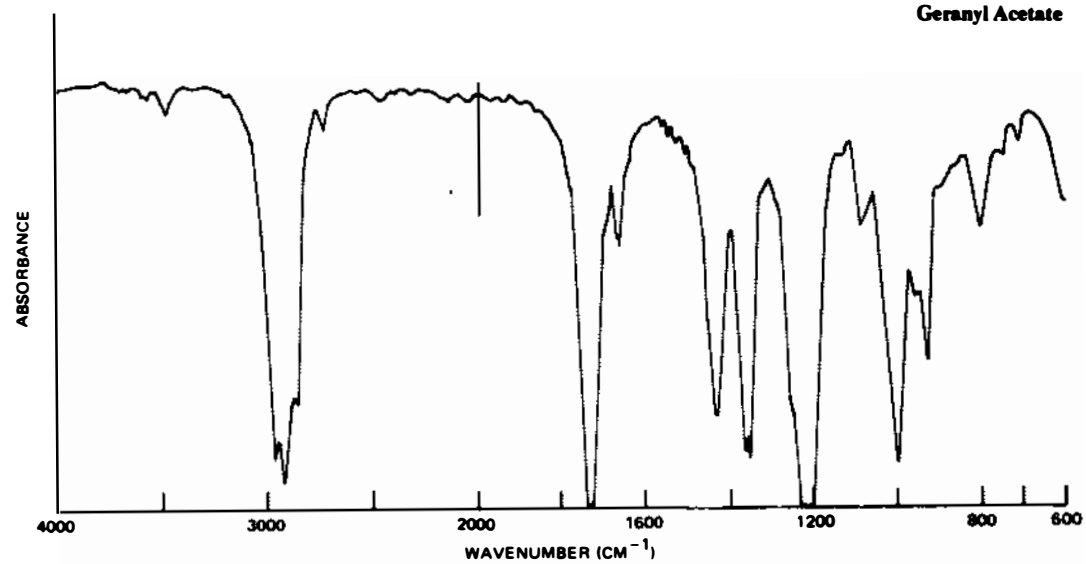
Furfural



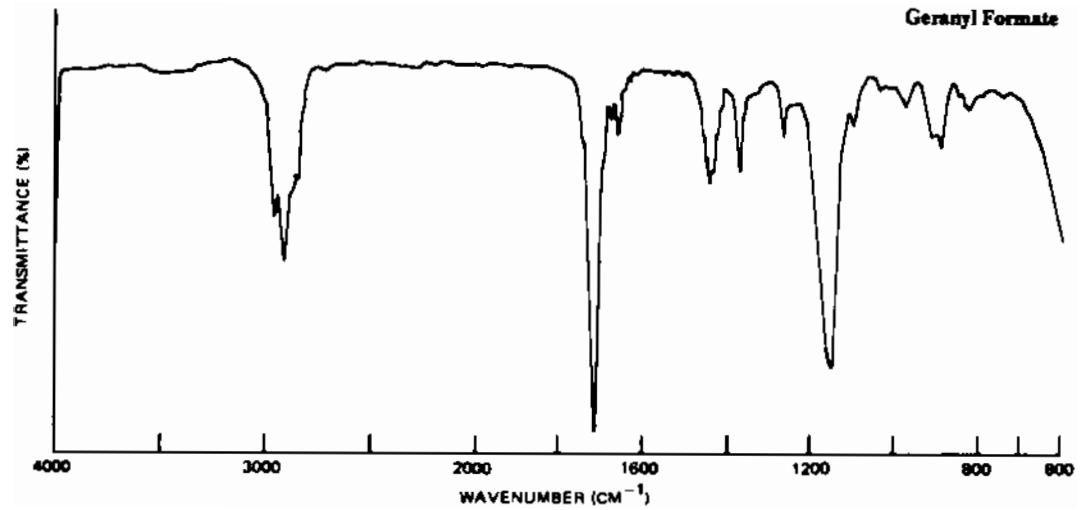
Geraniol (EOA Type B)



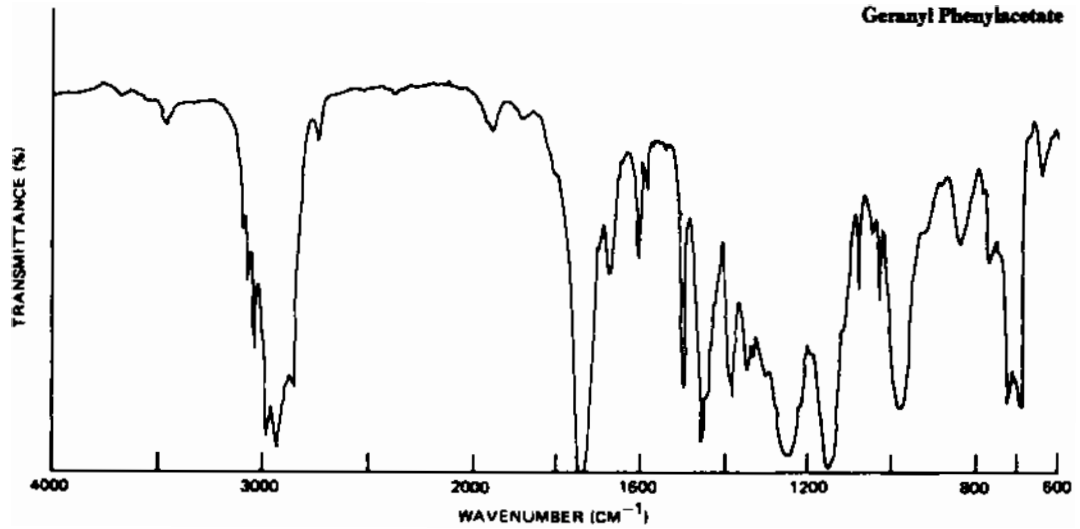
Geranyl Acetate



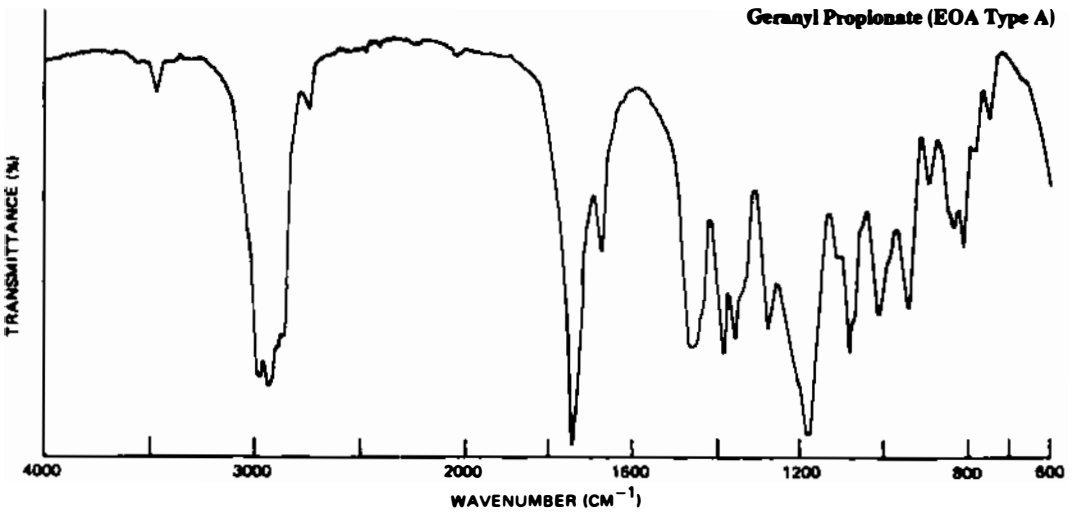
Geranyl Formate



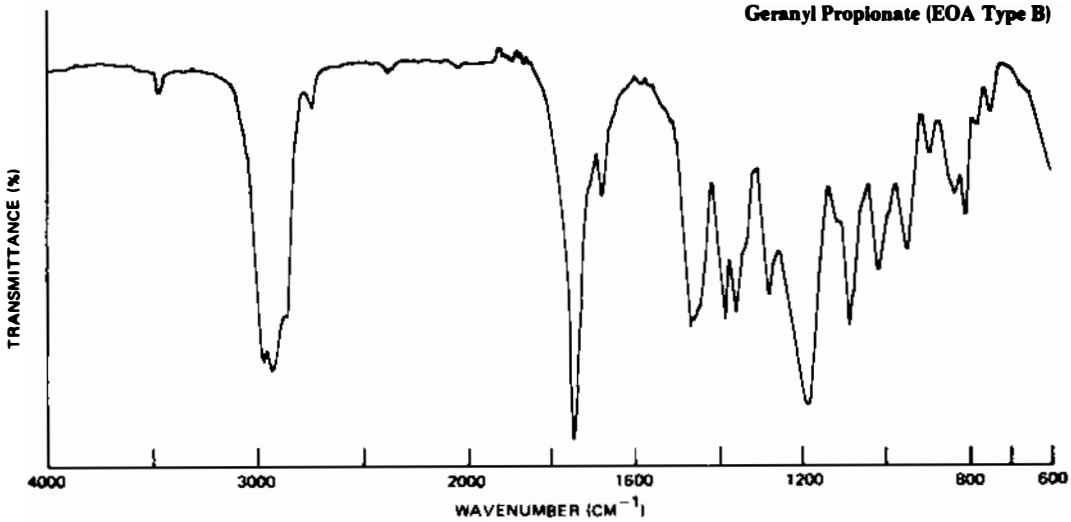
Geranyl Phenylacetate



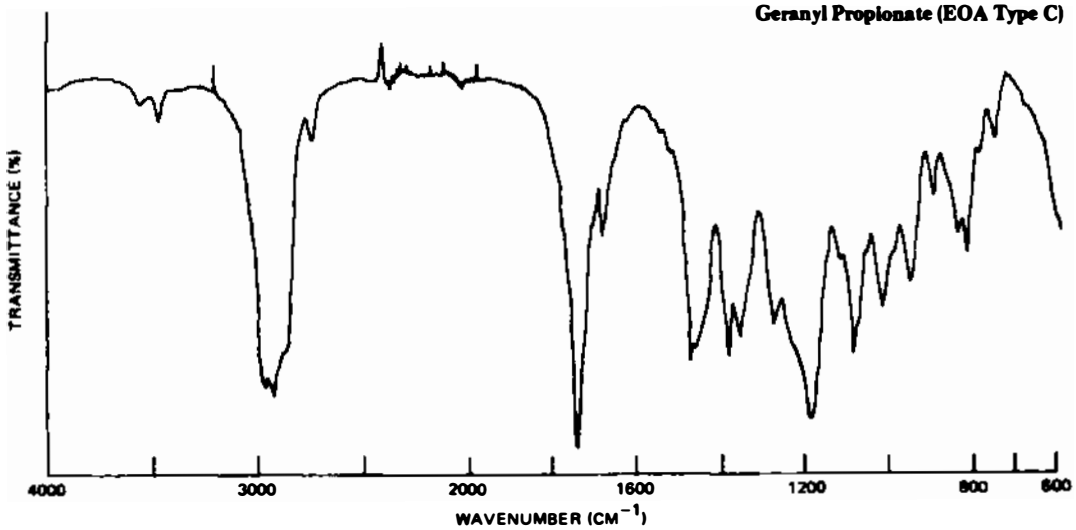
Geranyl Propionate (EOA Type A)



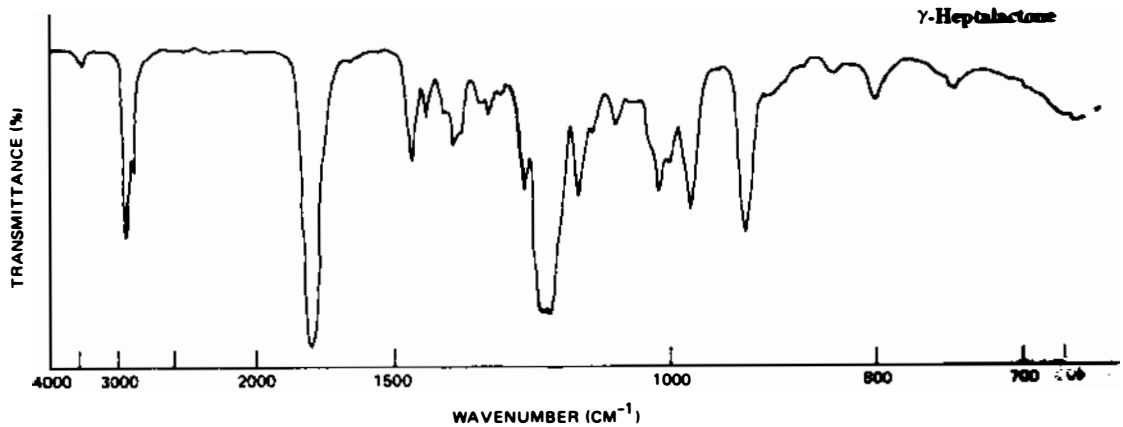
Geranyl Propionate (EOA Type B)



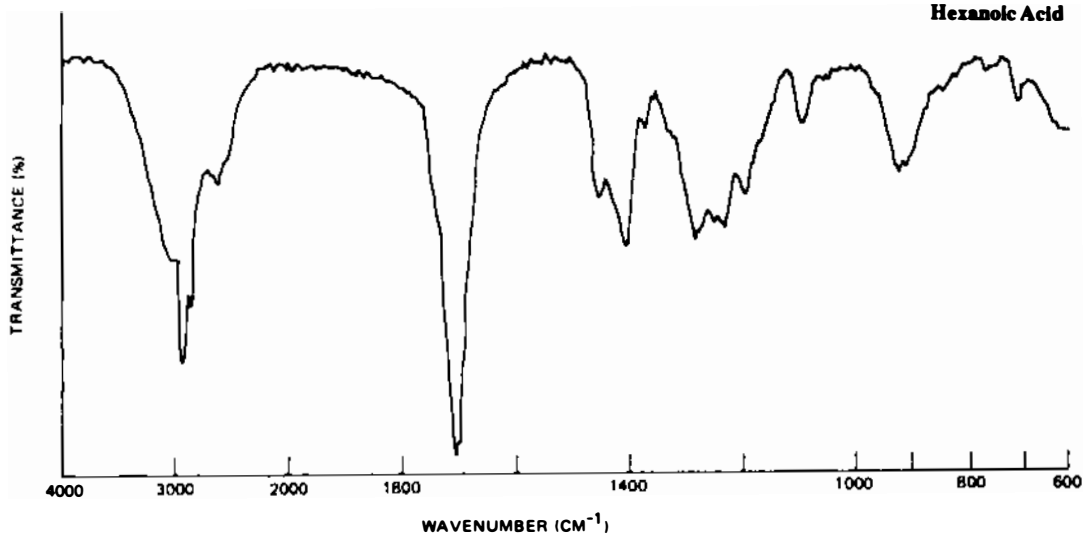
Geranyl Propionate (EOA Type C)



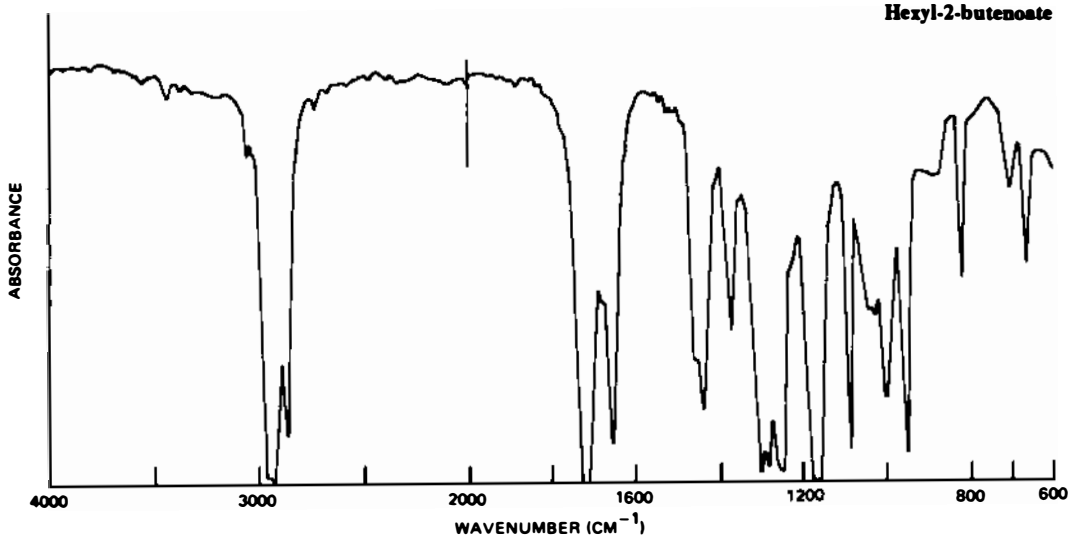
γ-Heptalactone



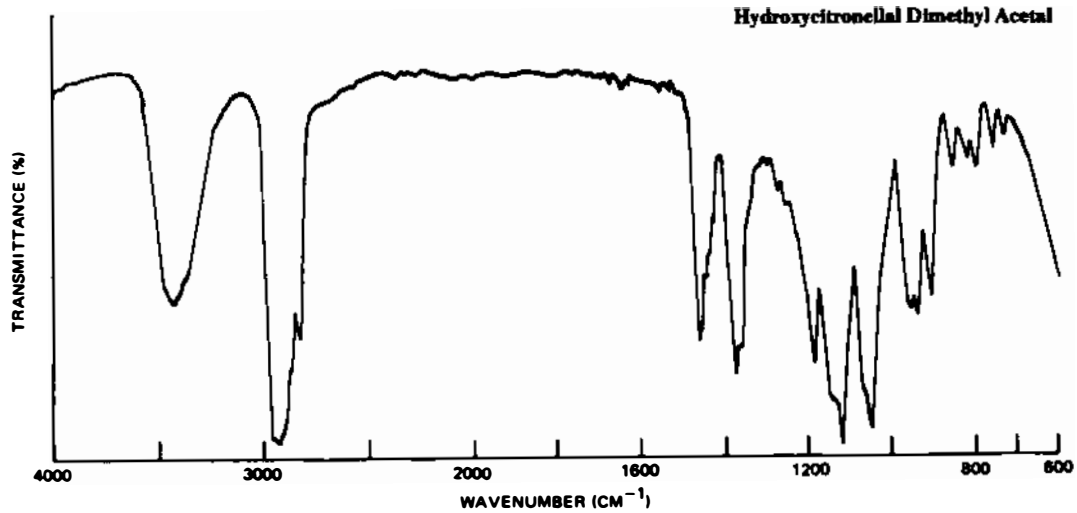
Hexanoic Acid



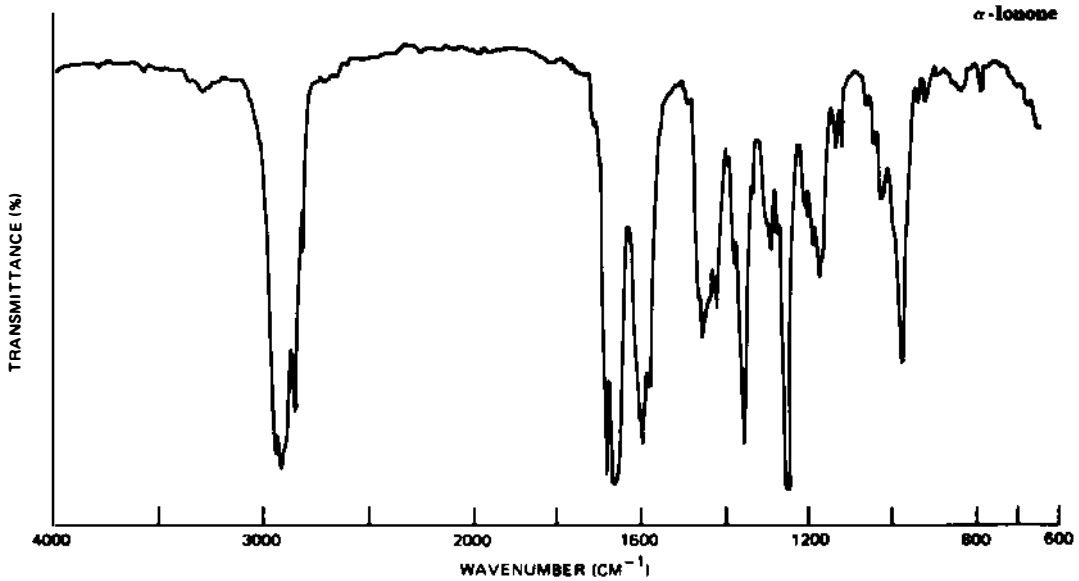
Hexyl-2-butenoate



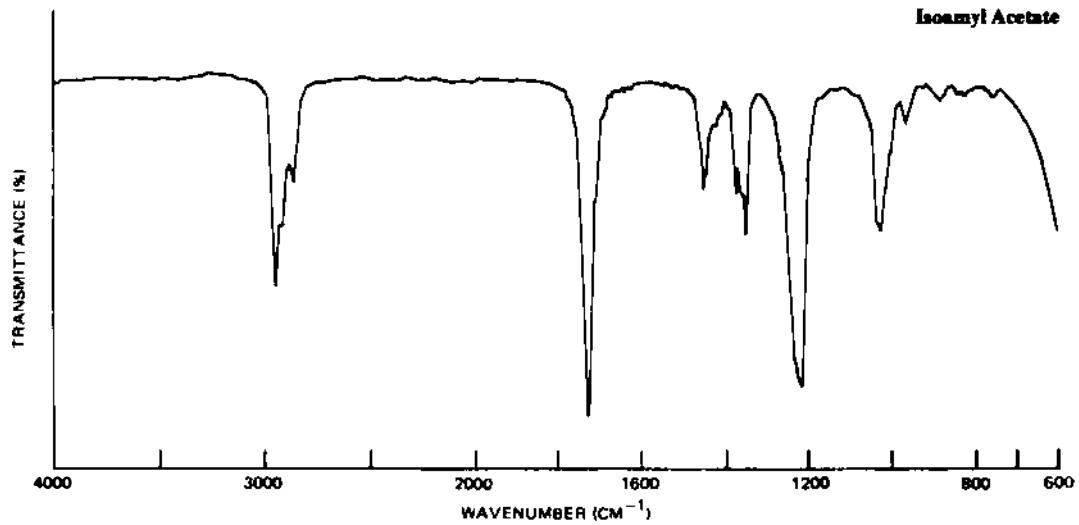
Hydroxycitronellal Dimethyl Acetal



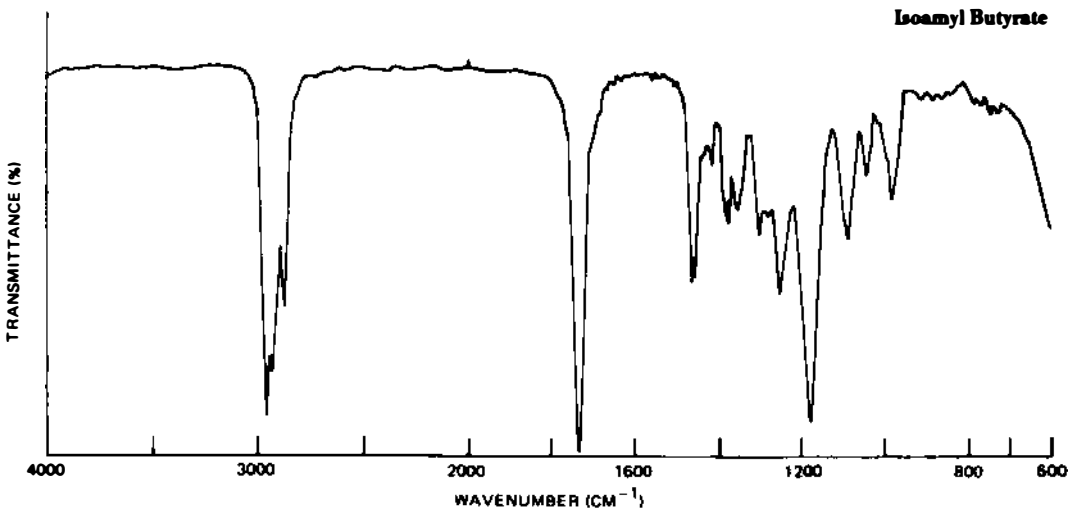
α-Ionone



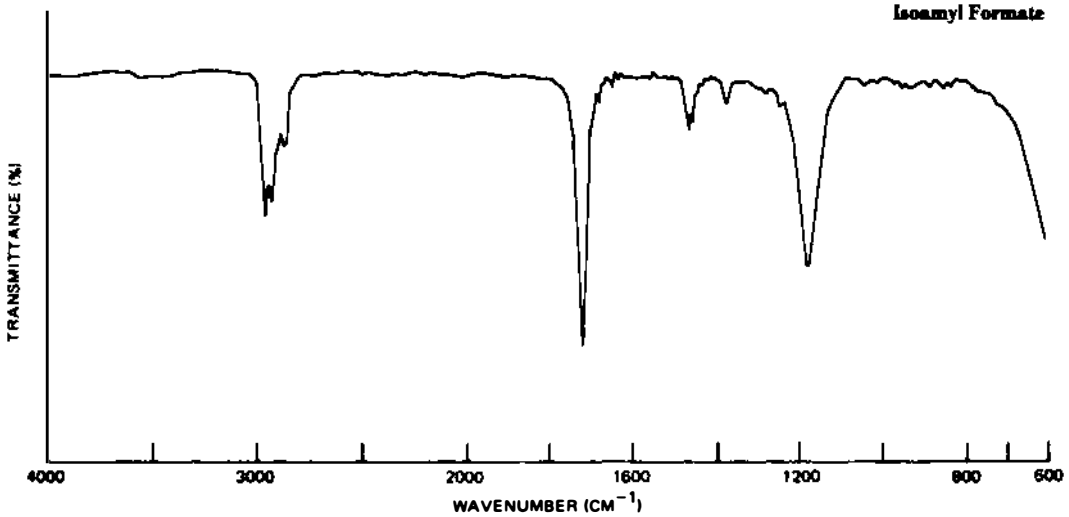
Isoamyl Acetate



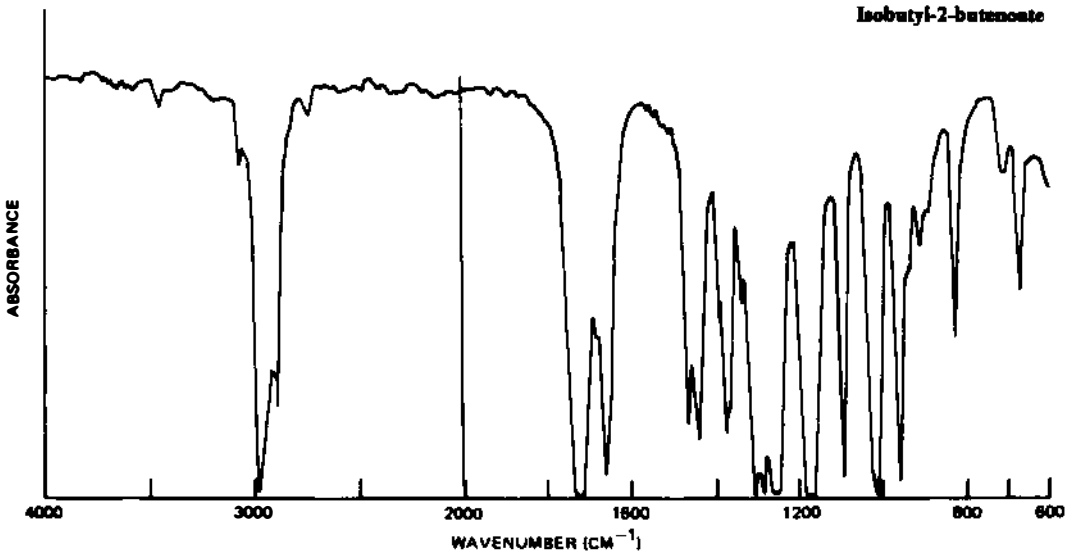
Isoamyl Butyrate



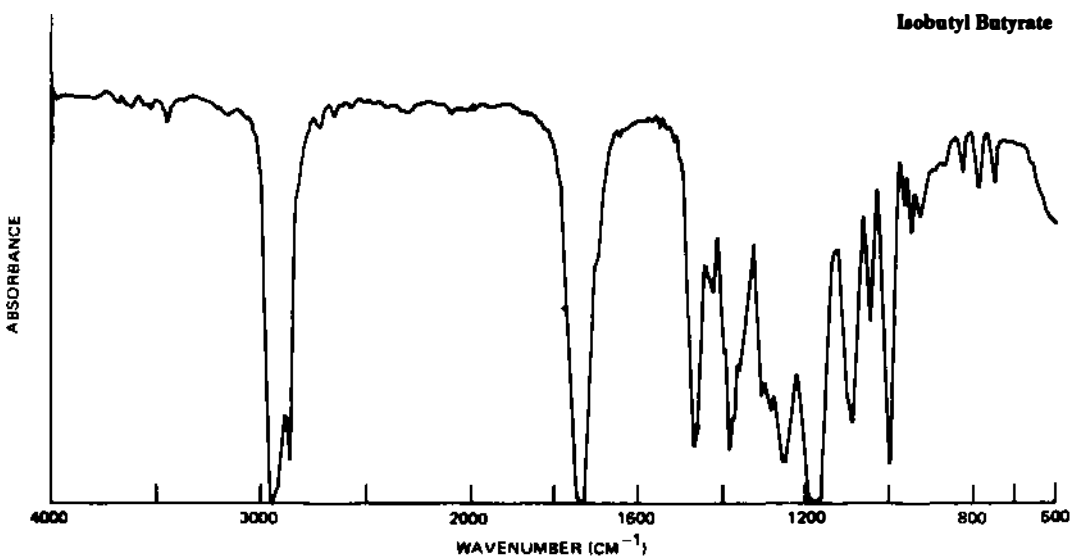
Isoamyl Formate



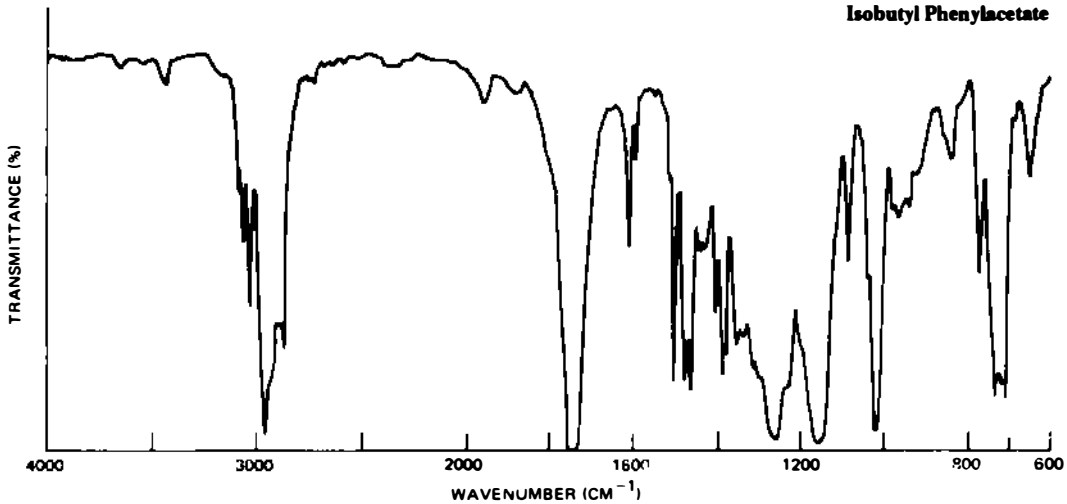
Isobutyl-2-butenoate



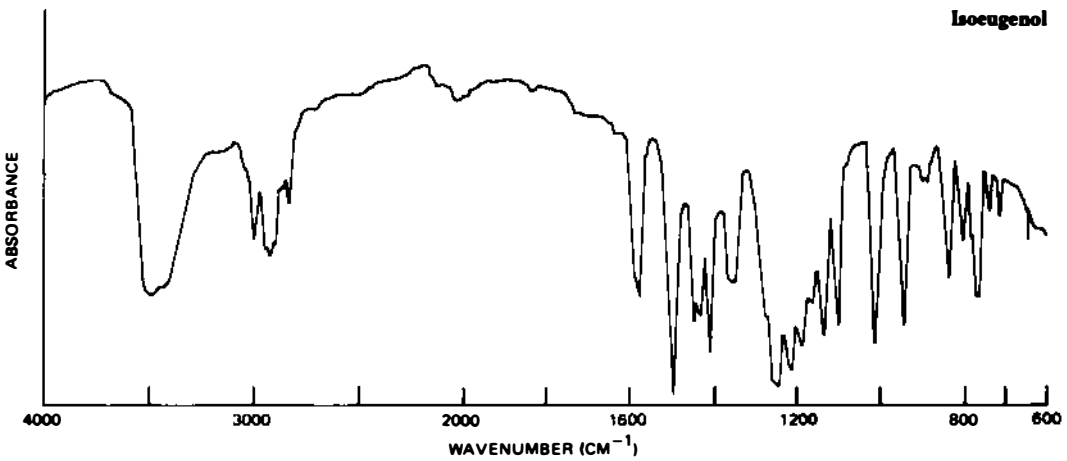
Isobutyl Butyrate



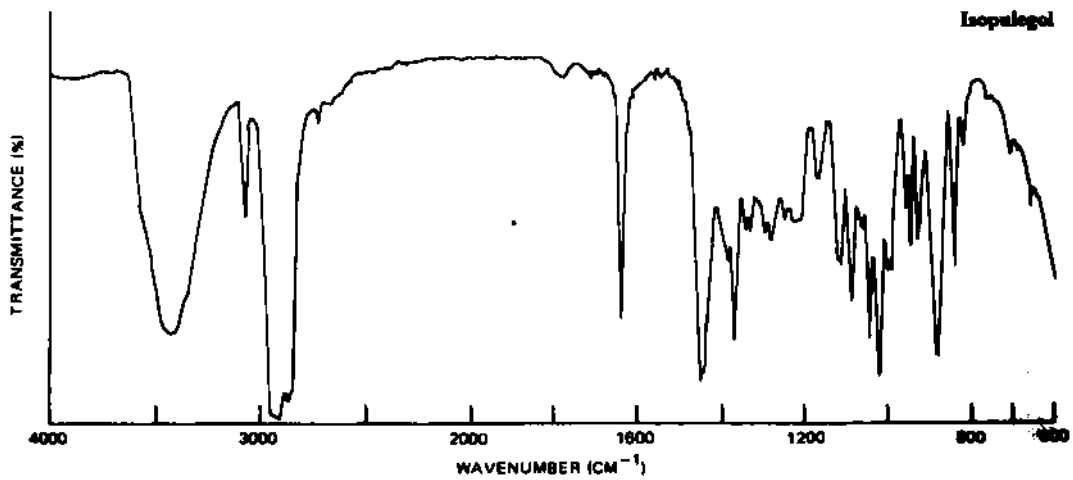
Isobutyl Phenylacetate



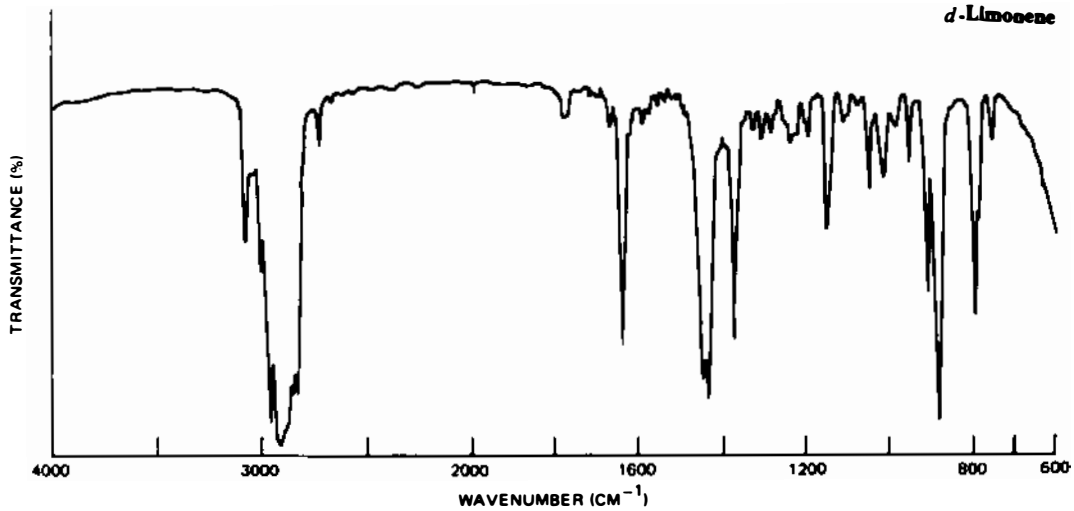
Isoeugenol



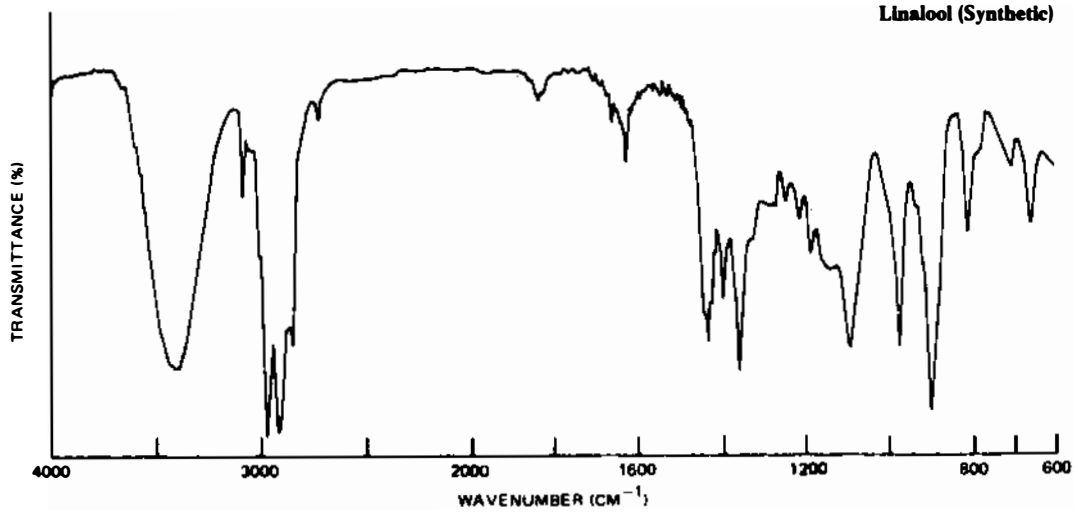
Isopulegol



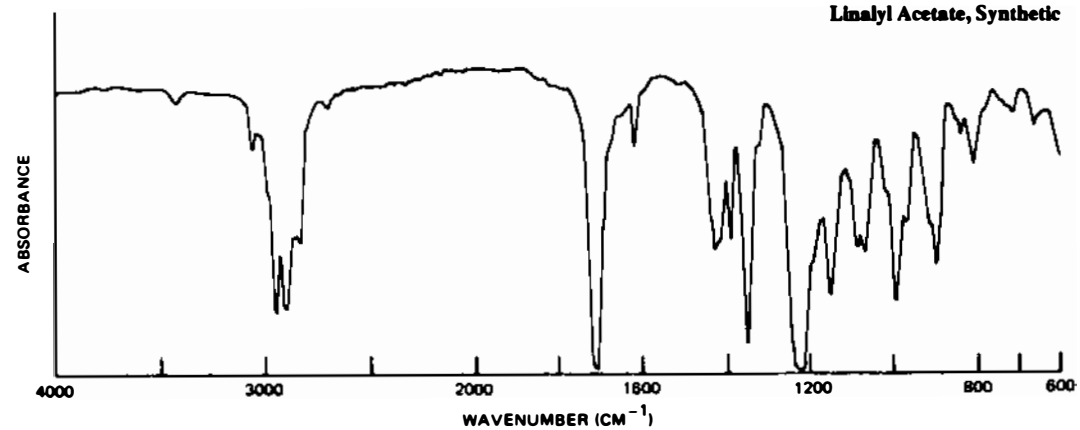
d-Limonene

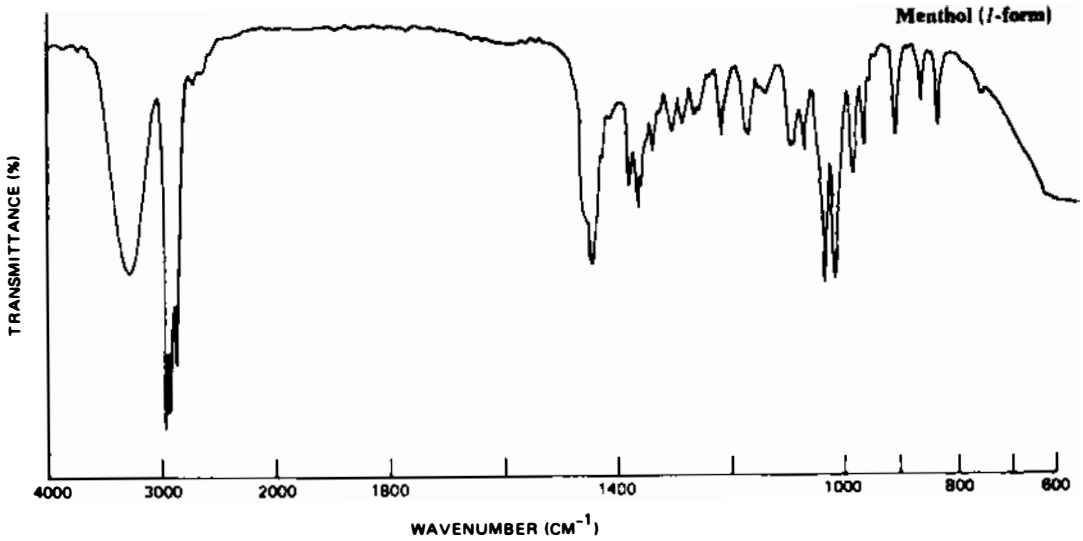


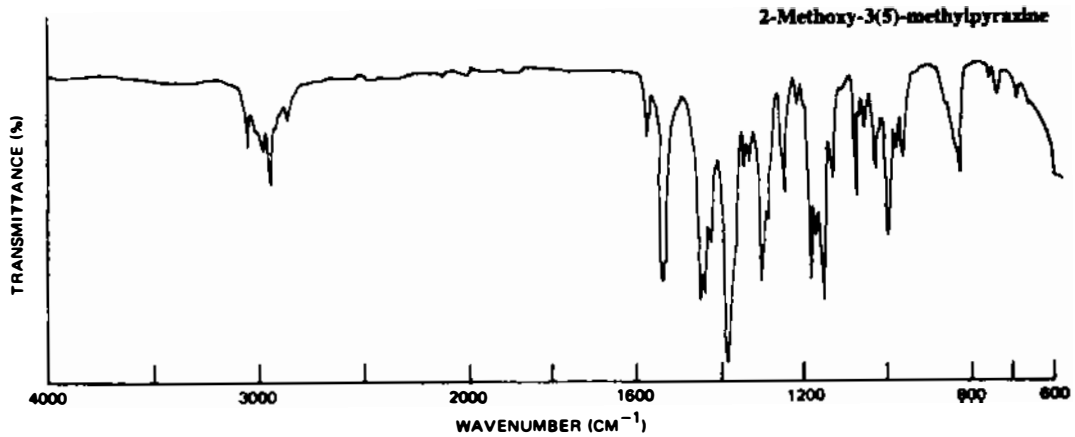
Linalool (Synthetic)

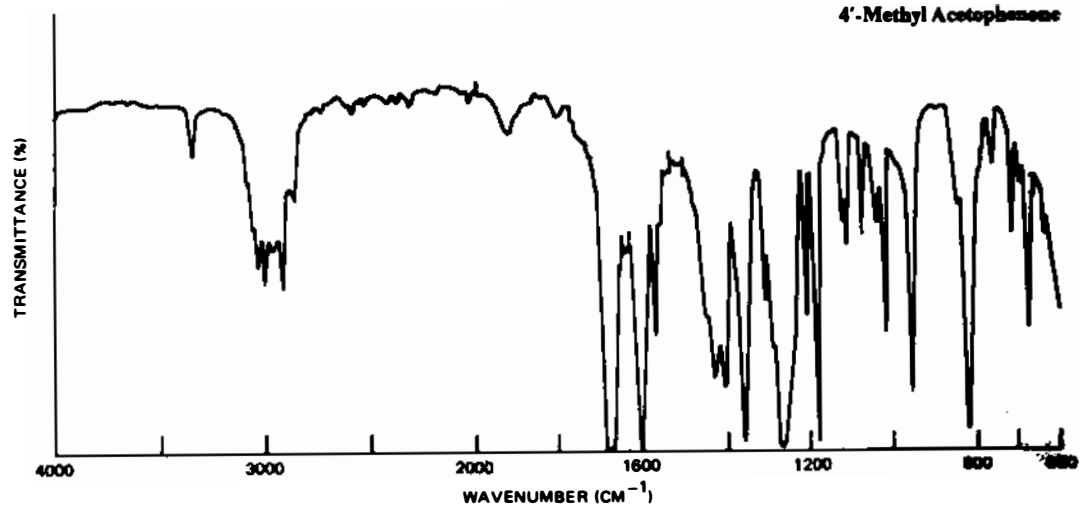


Linalyl Acetate, Synthetic

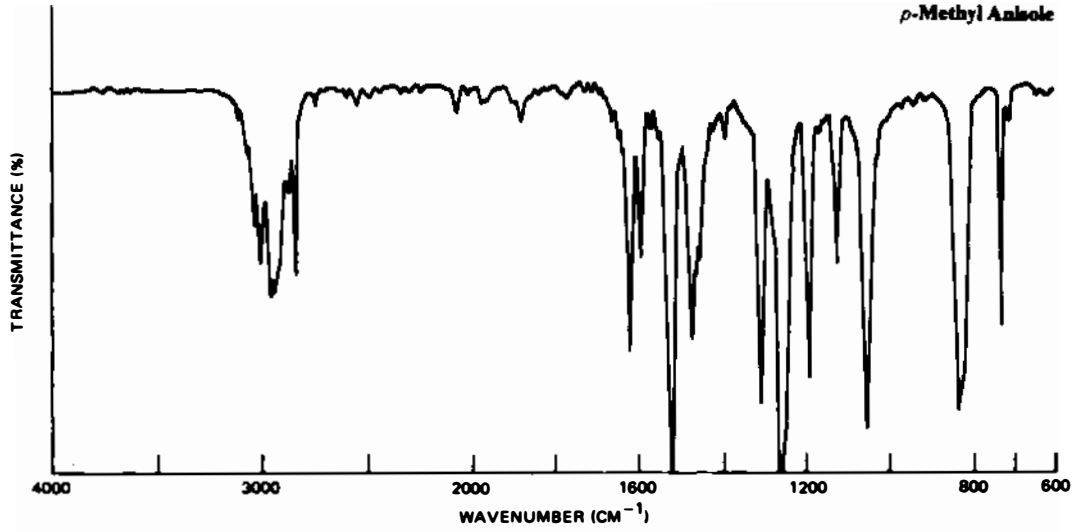



Menthol (*l*-form)
TRANSMITTANCE (%)
4000
3000
2000
1800
1400
1000
800
600
WAVENUMBER (CM^{-1})

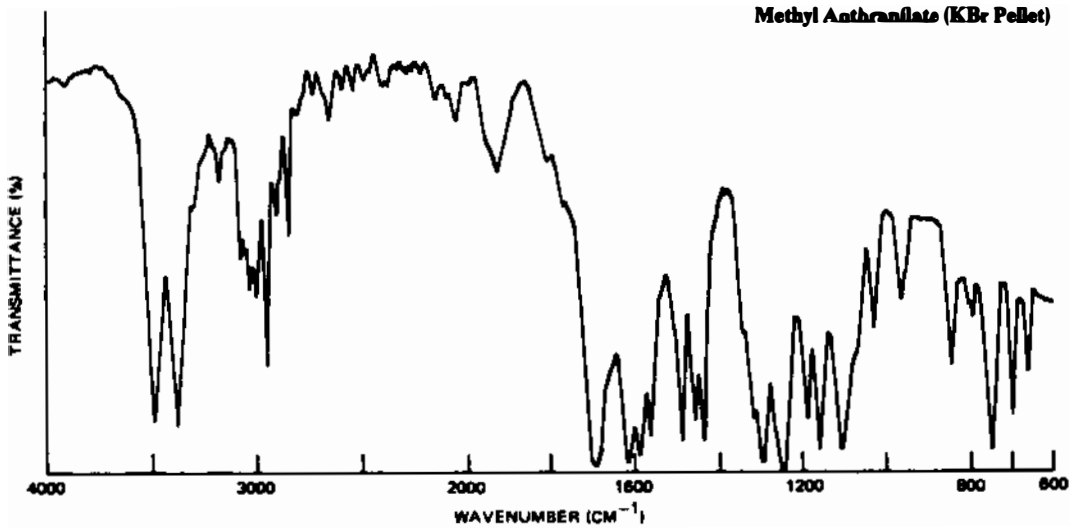

2-Methoxy-3(5)-methylpyrazine
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM^{-1})


4'-Methyl Acetophenone
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
WAVENUMBER (CM^{-1})

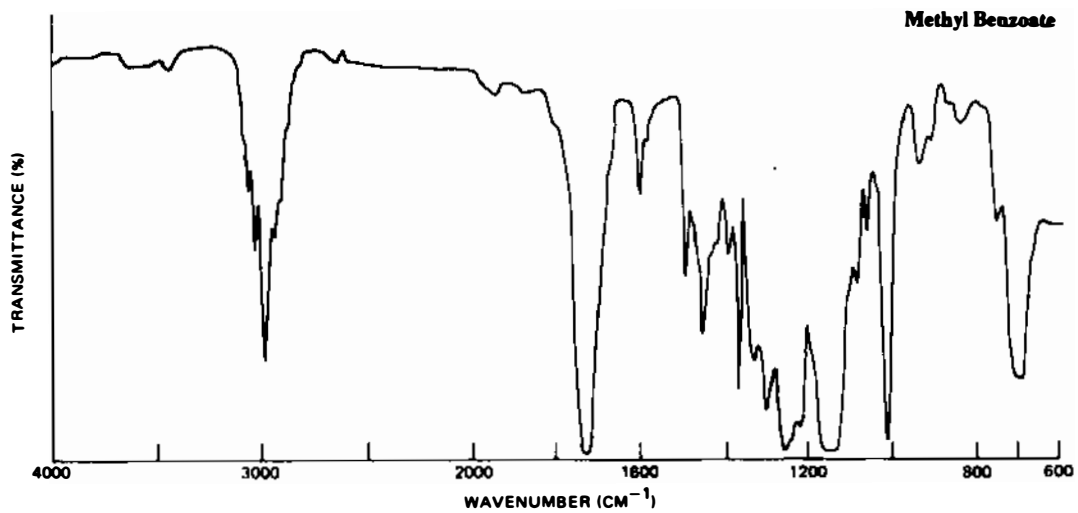
p-Methyl Anisole



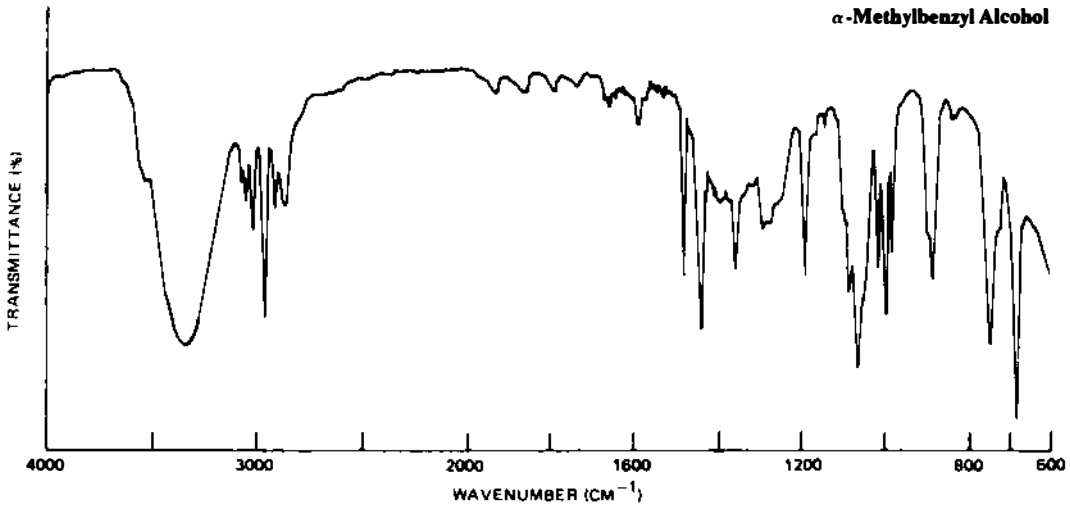
Methyl Anthranilate (KBr Pellet)



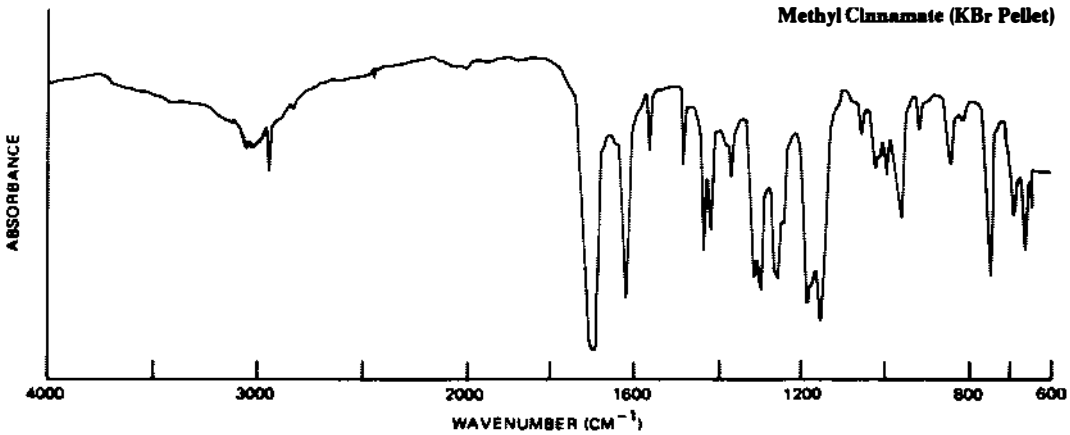
Methyl Benzoate



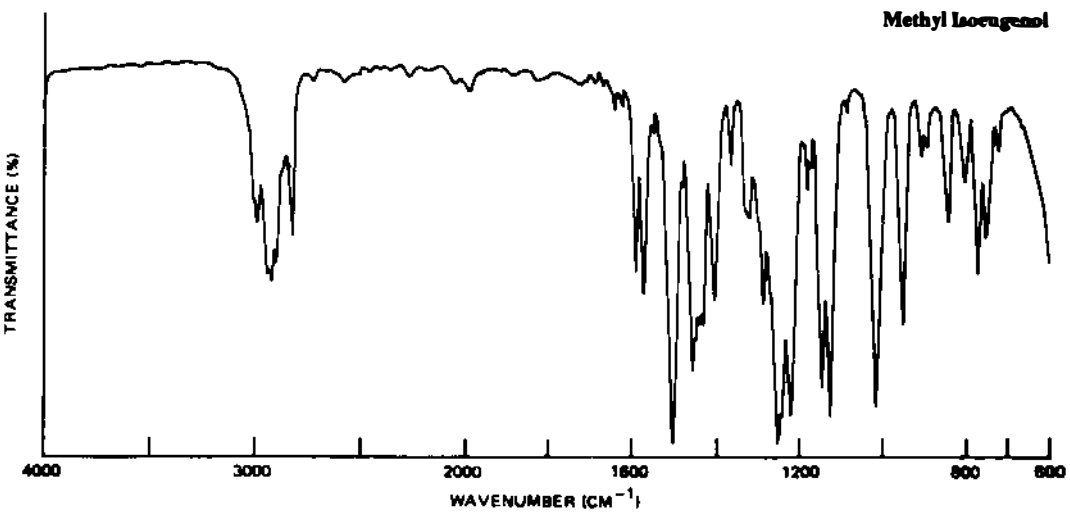
α-Methylbenzyl Alcohol



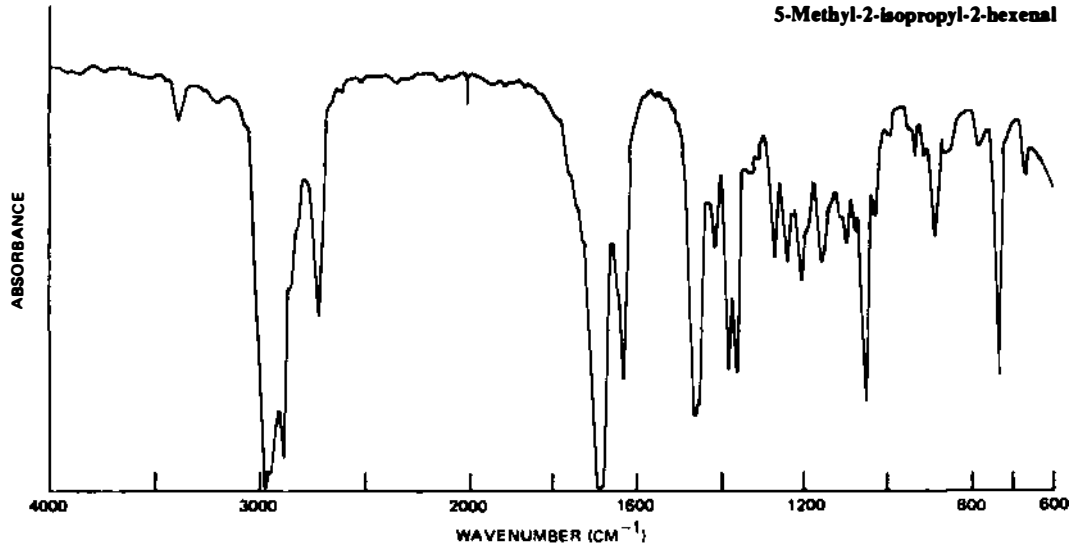
Methyl Cinnamate (KBr Pellet)



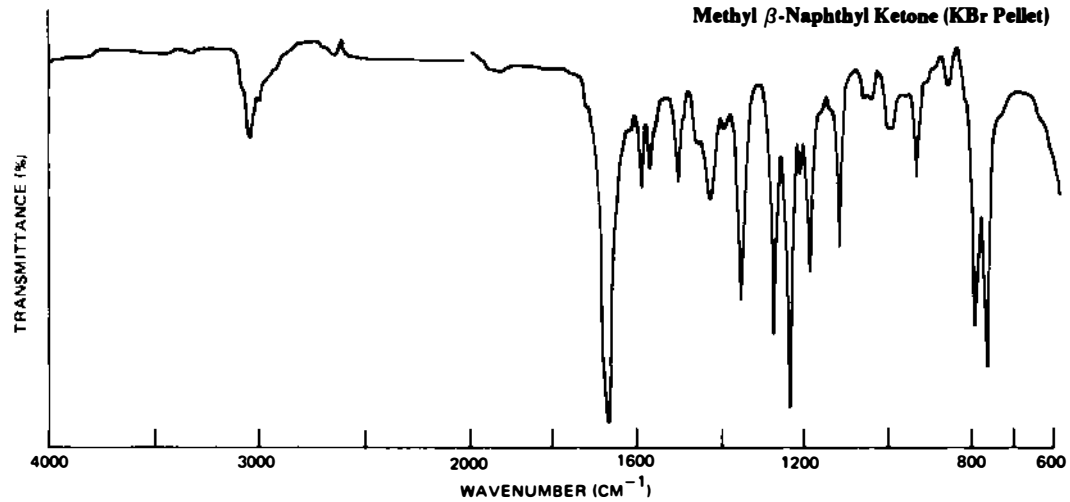
Methyl Isoeugenol



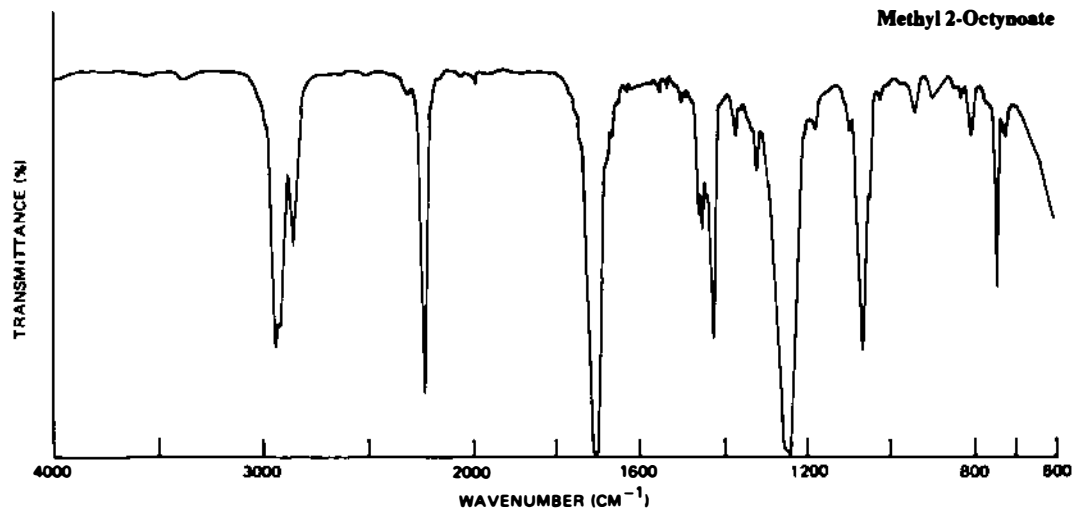
5-Methyl-2-isopropyl-2-hexenal



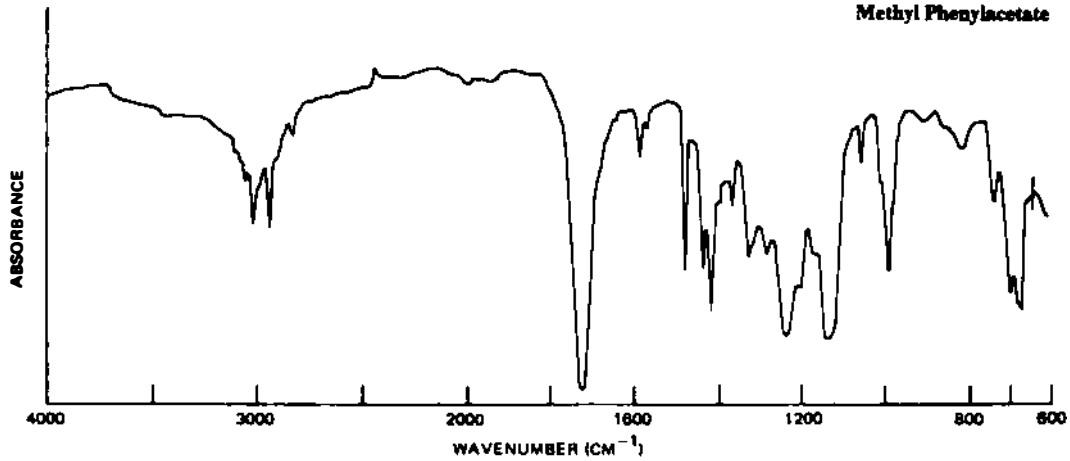
Methyl β-Naphthyl Ketone (KBr Pellet)



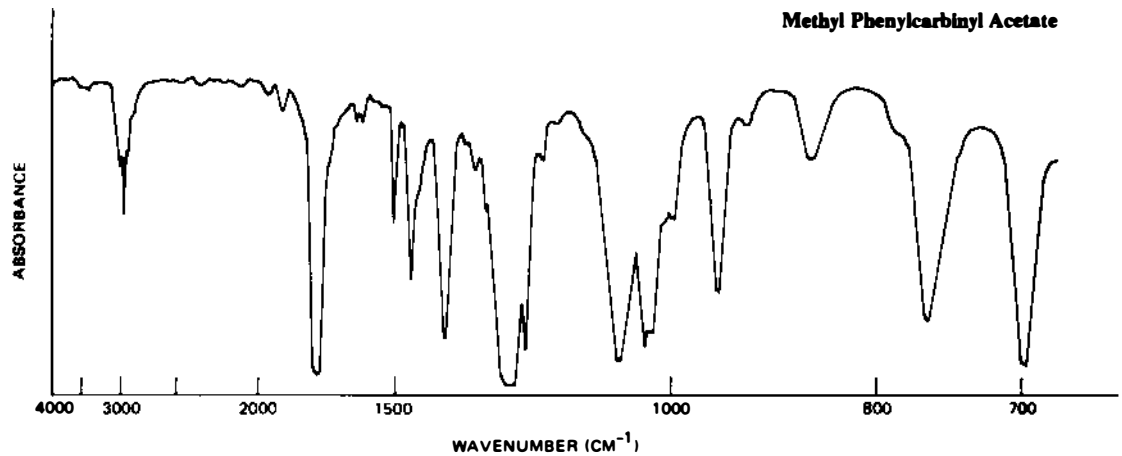
Methyl 2-Octynoate



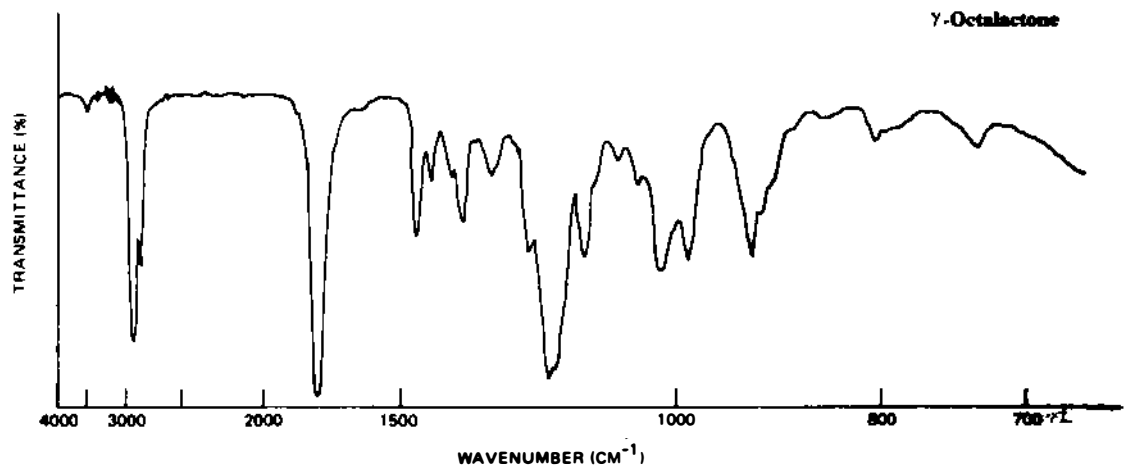
Methyl Phenylacetate



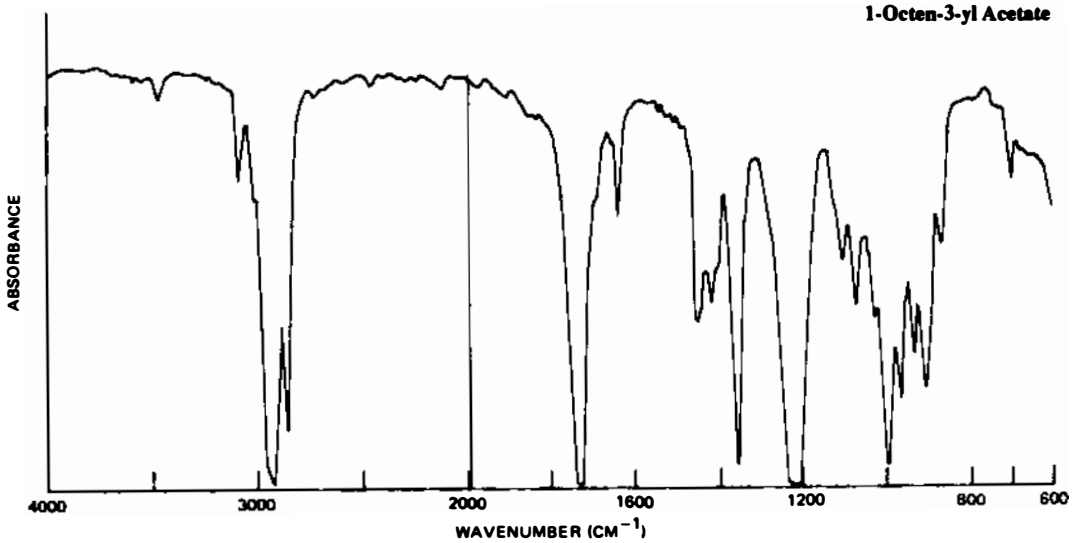
Methyl Phenylcarbinyl Acetate



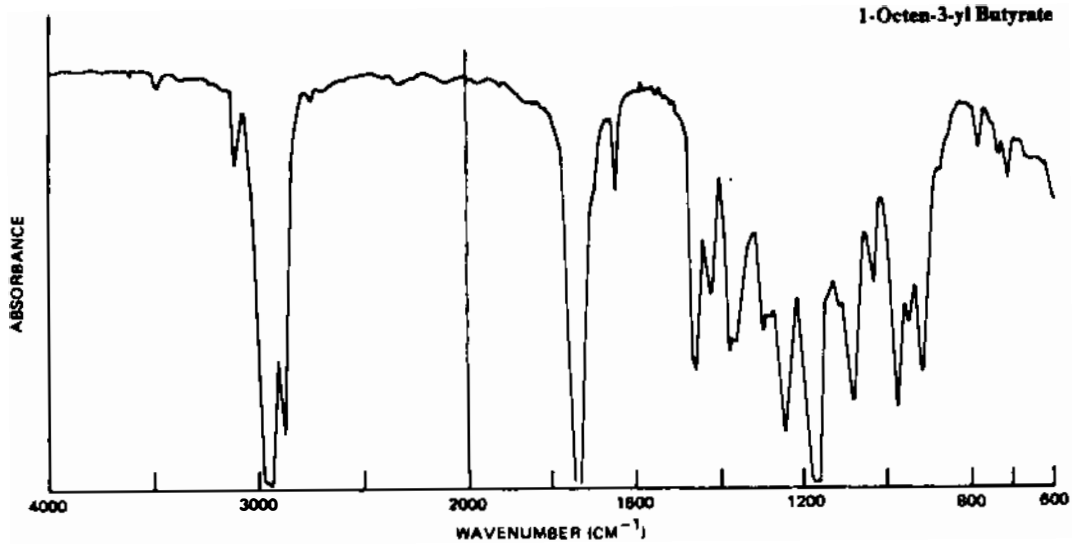
γ-Octalactone



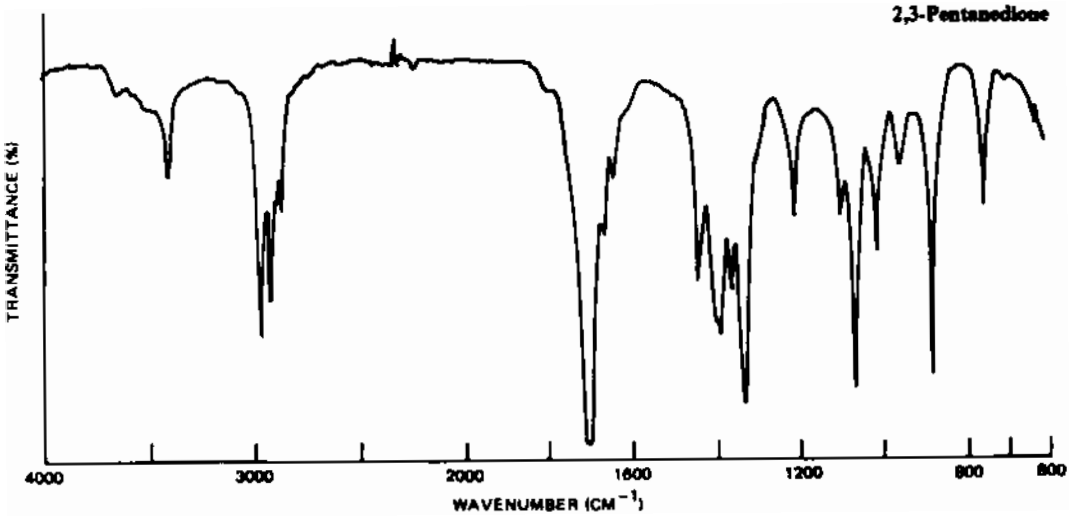
1-Octen-3-yl Acetate

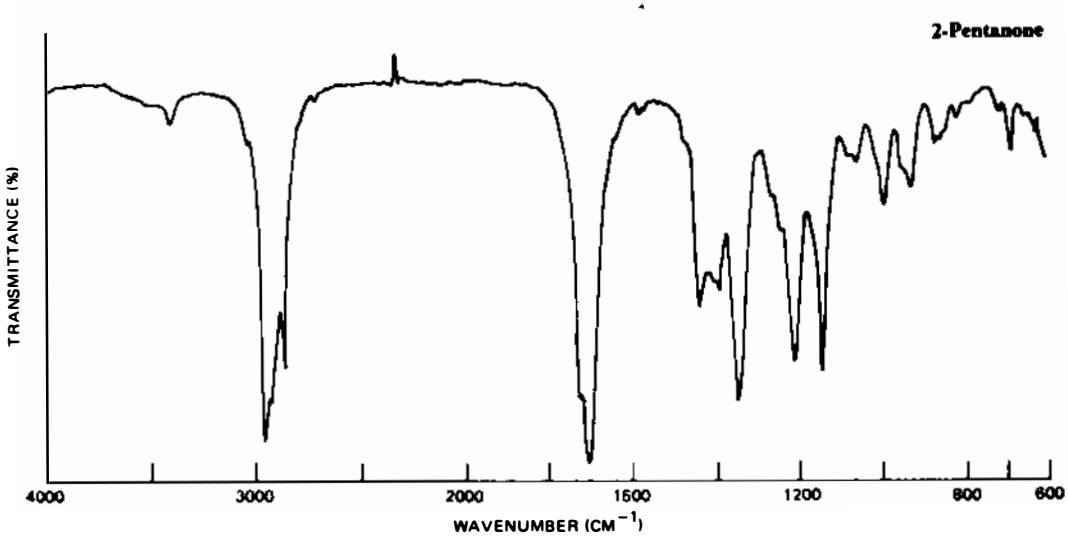


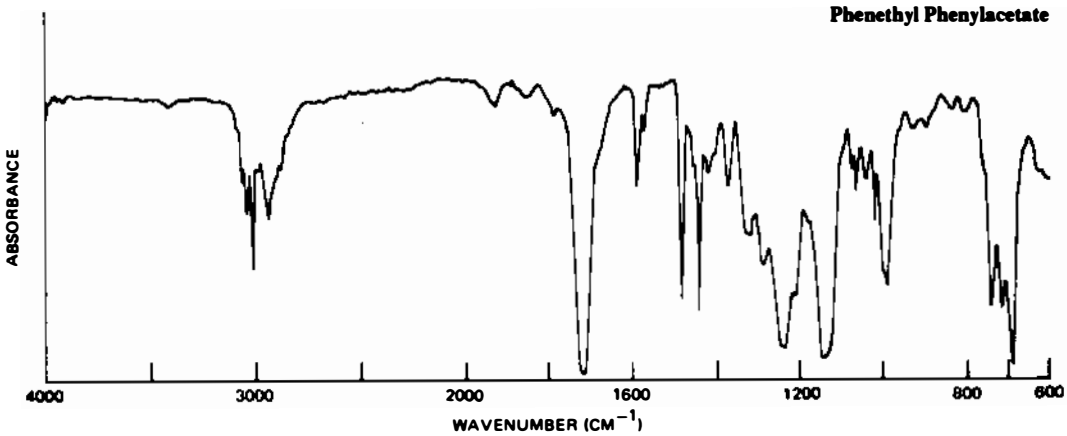
1-Octen-3-yl Butyrate

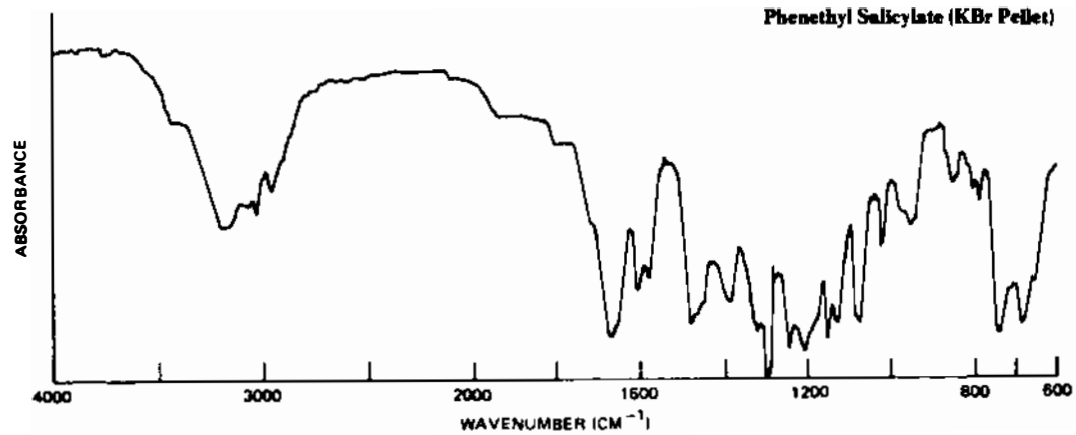


2,3-Pentanedione

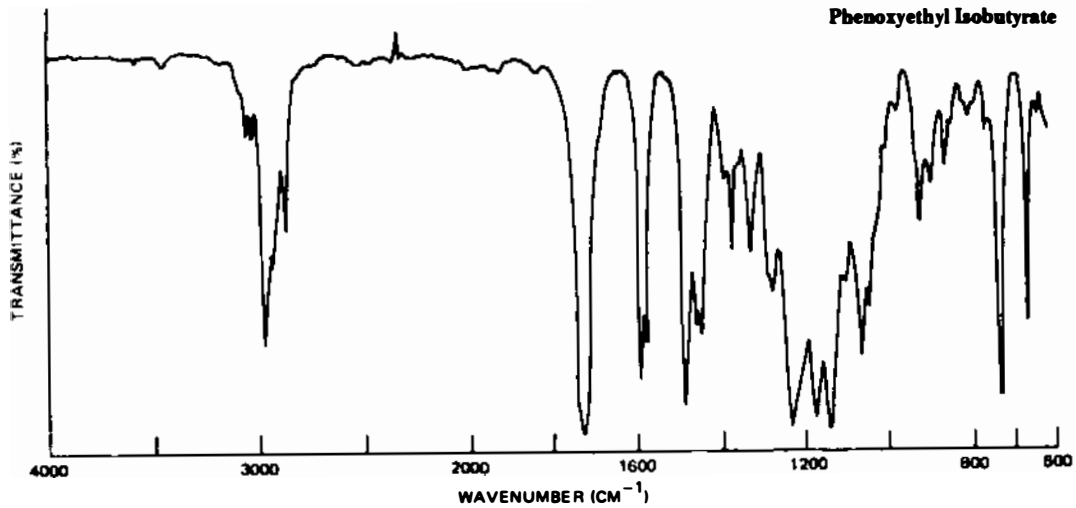



2-Pentanone
TRANSMITTANCE (%)
4000
3000
2000
1500
1200
800
600
WAVENUMBER (CM^{-1})

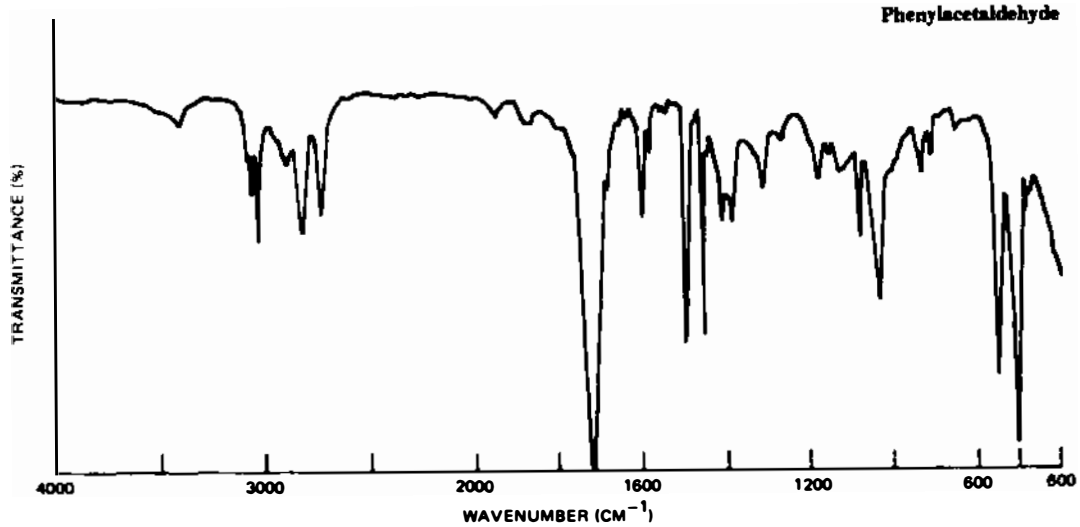

Phenethyl Phenylacetate
ABSORBANCE
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM^{-1})


Phenethyl Salicylate (KBr Pellet)
ABSORBANCE
4000
3000
2000
1500
1200
800
600
WAVENUMBER (CM^{-1})

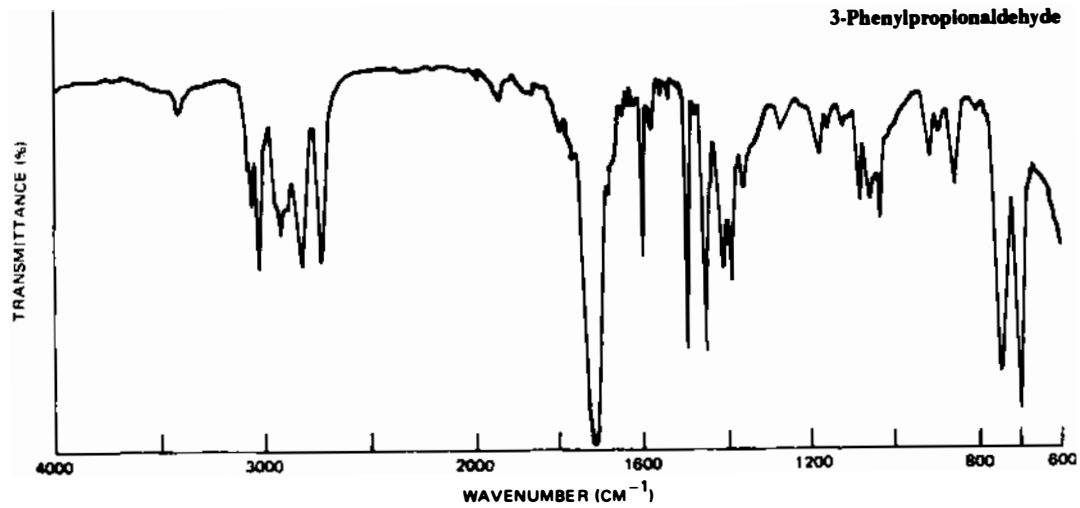
Phenoxyethyl Isobutyrate



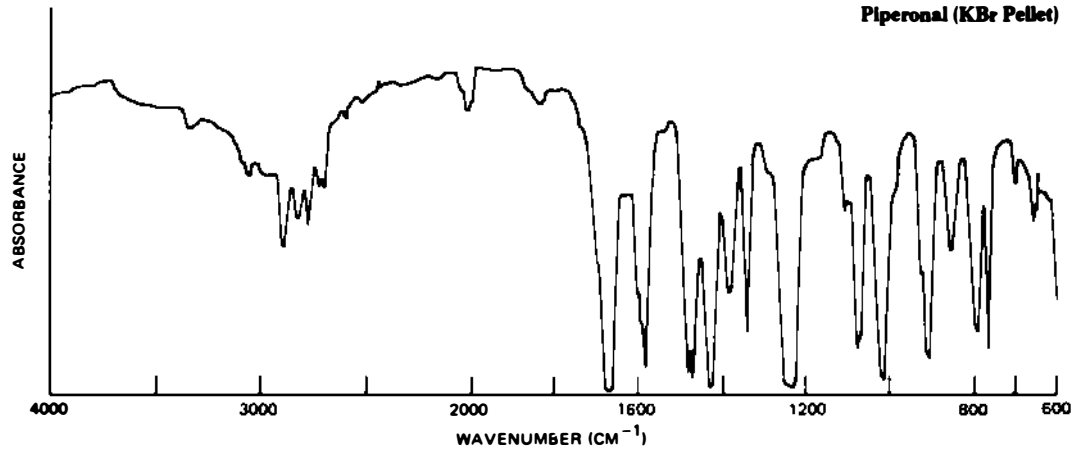
Phenylacetaldehyde



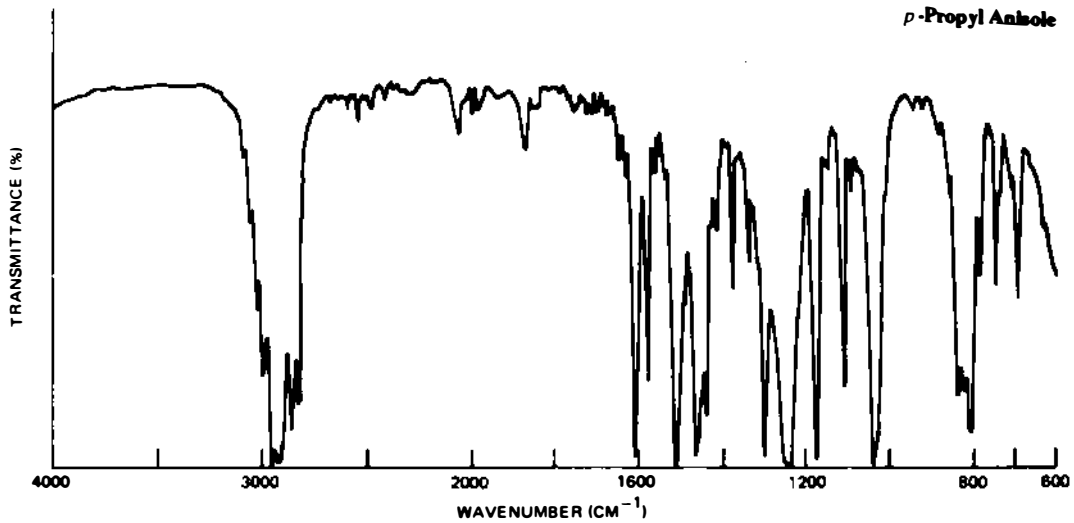
3-Phenylpropionaldehyde



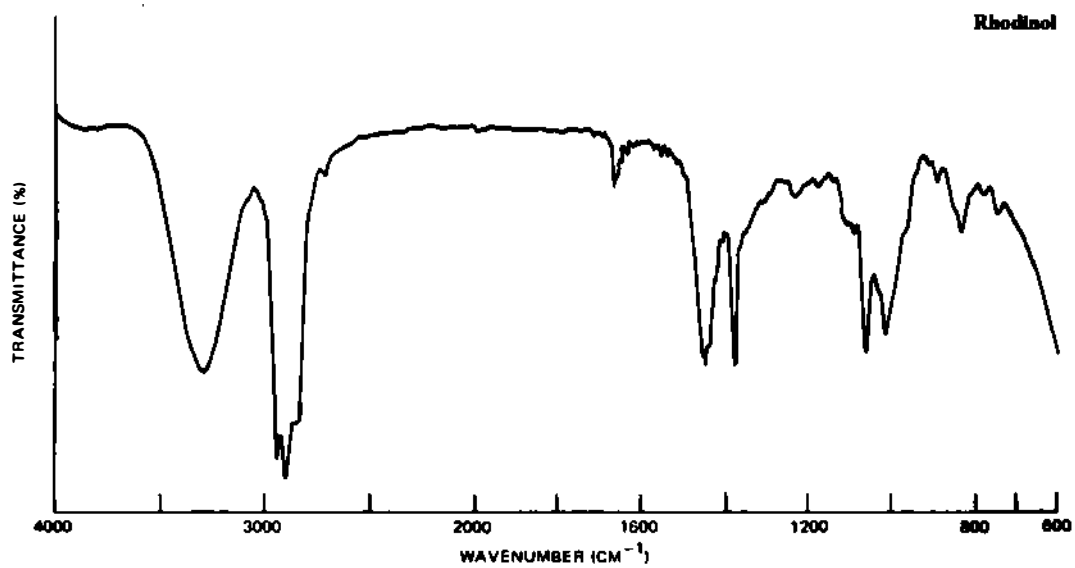
Piperonal (KBr Pellet)



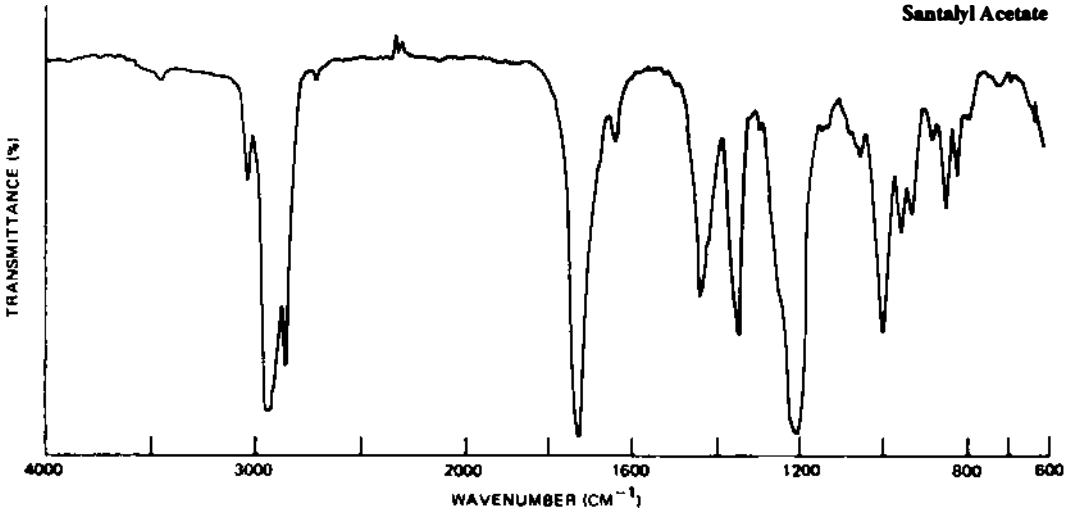
***p*-Propyl Anisole**



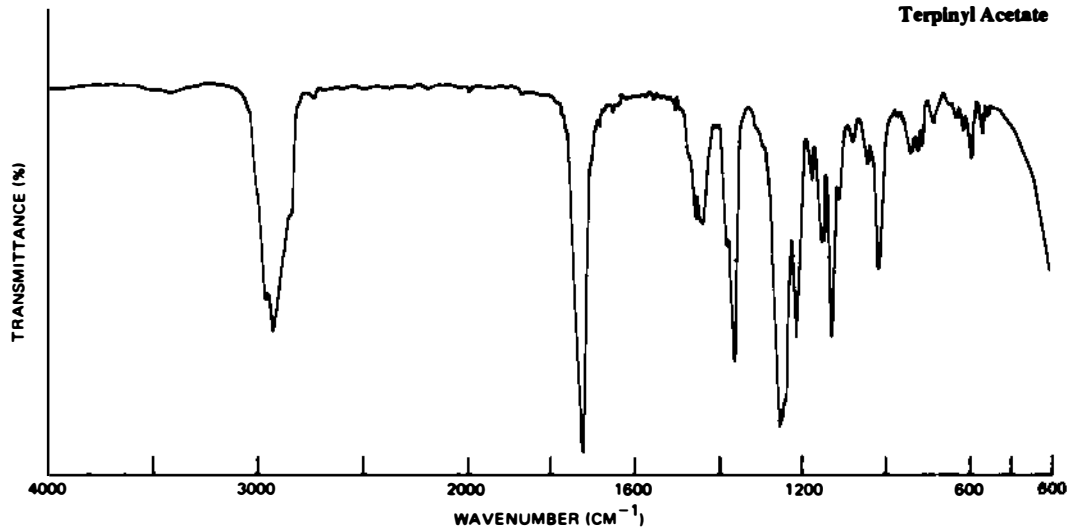
Rhodinol



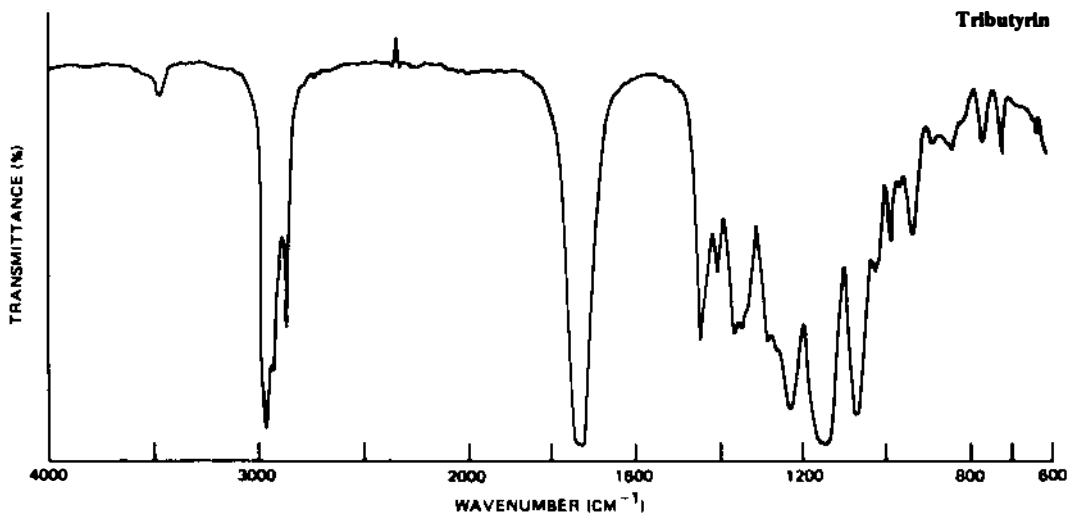
Santalyl Acetate



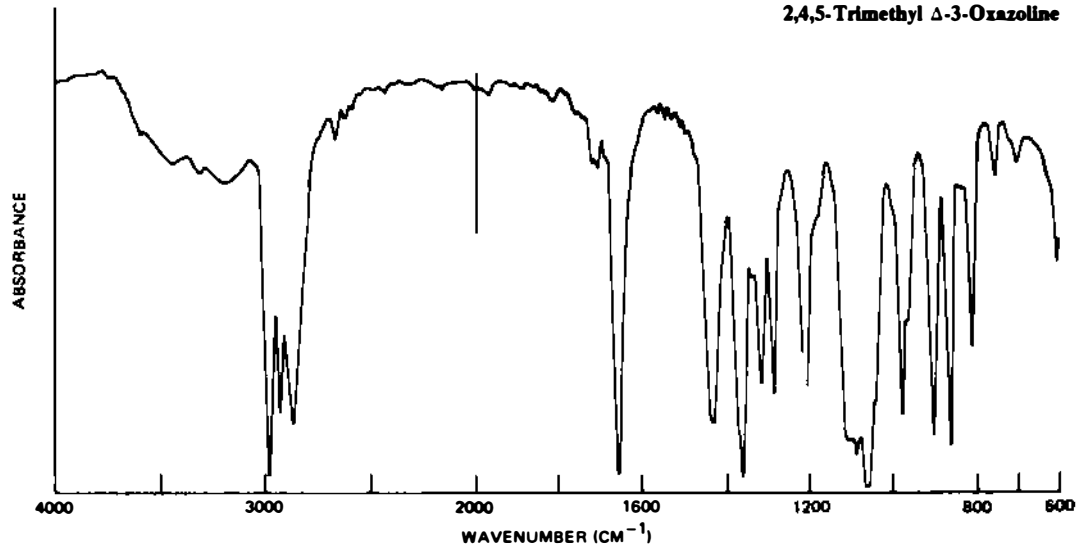
Terpinyl Acetate



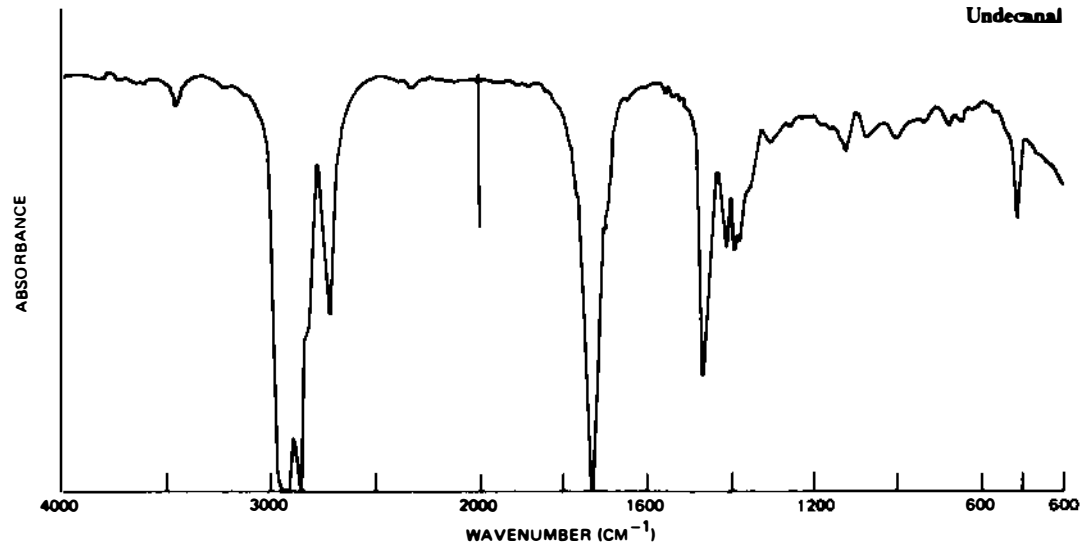
Tributyrin



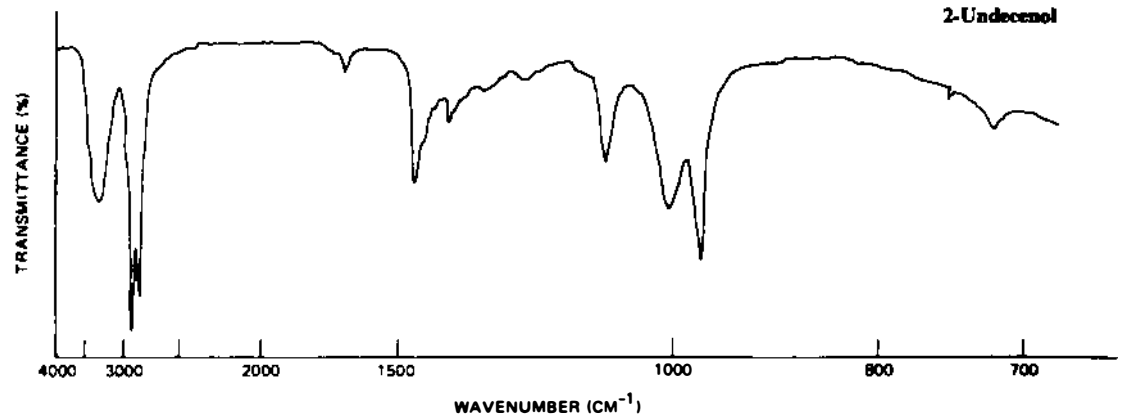
2,4,5-Trimethyl Δ-3-Oxazoline



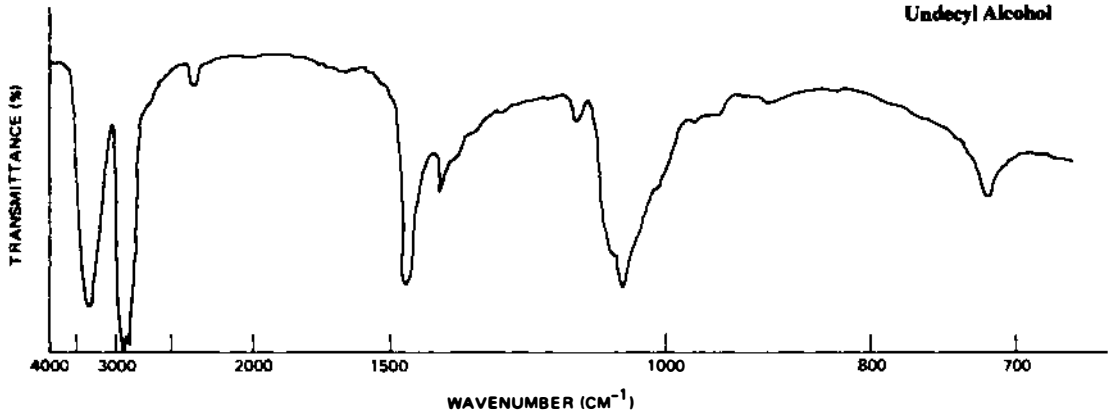
Undecanal



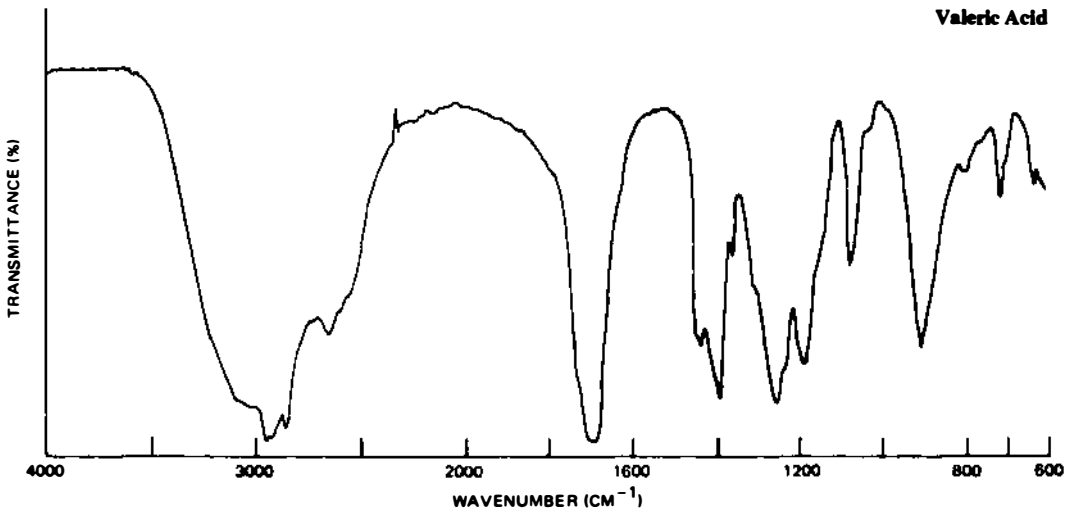
2-Undecenol



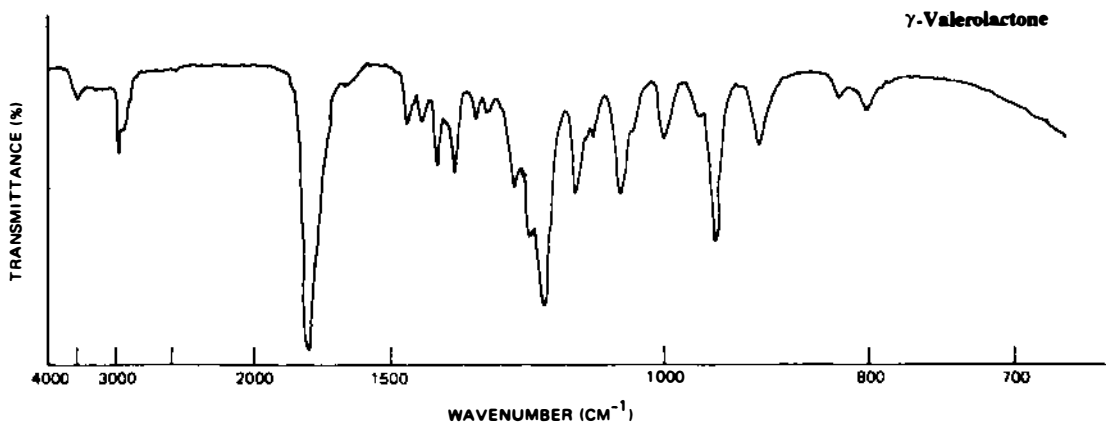
Undecyl Alcohol

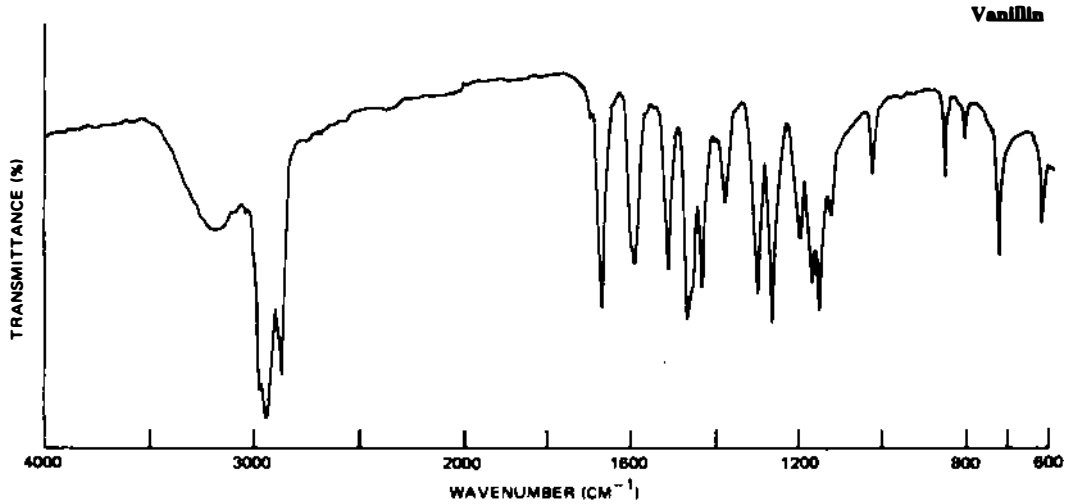


Valeric Acid

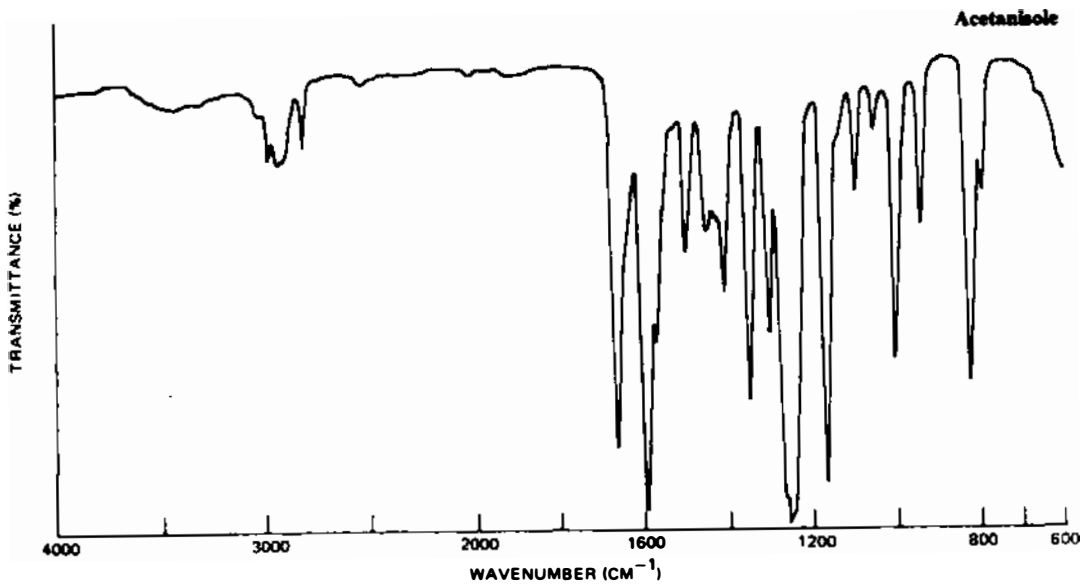


γ-Valerolactone

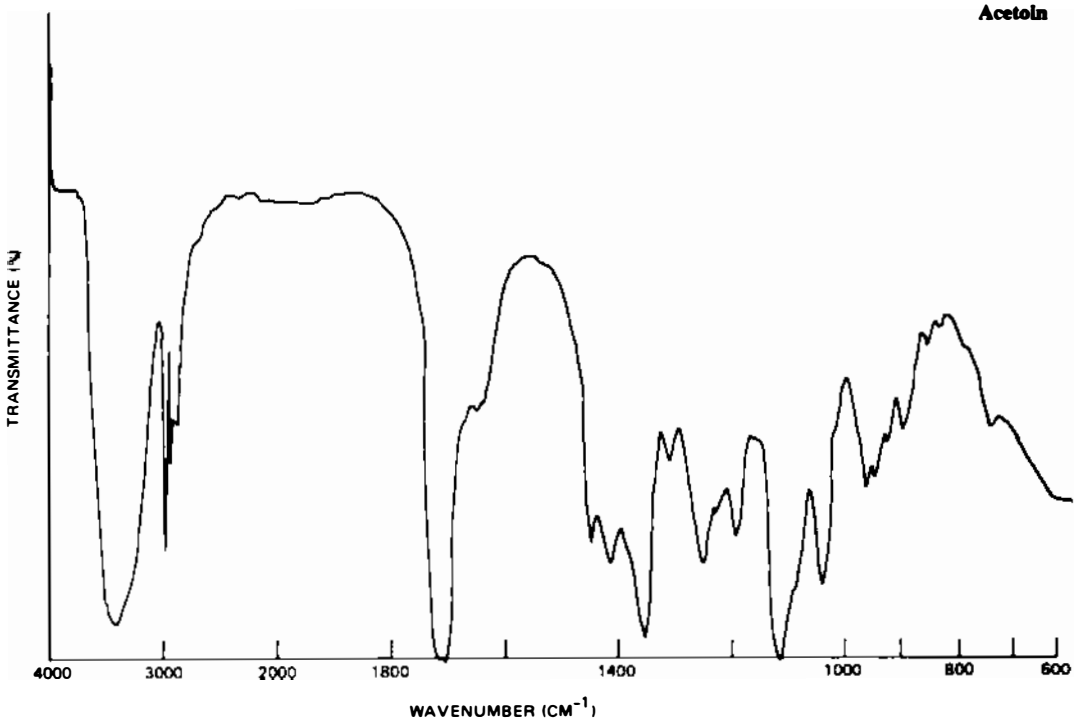




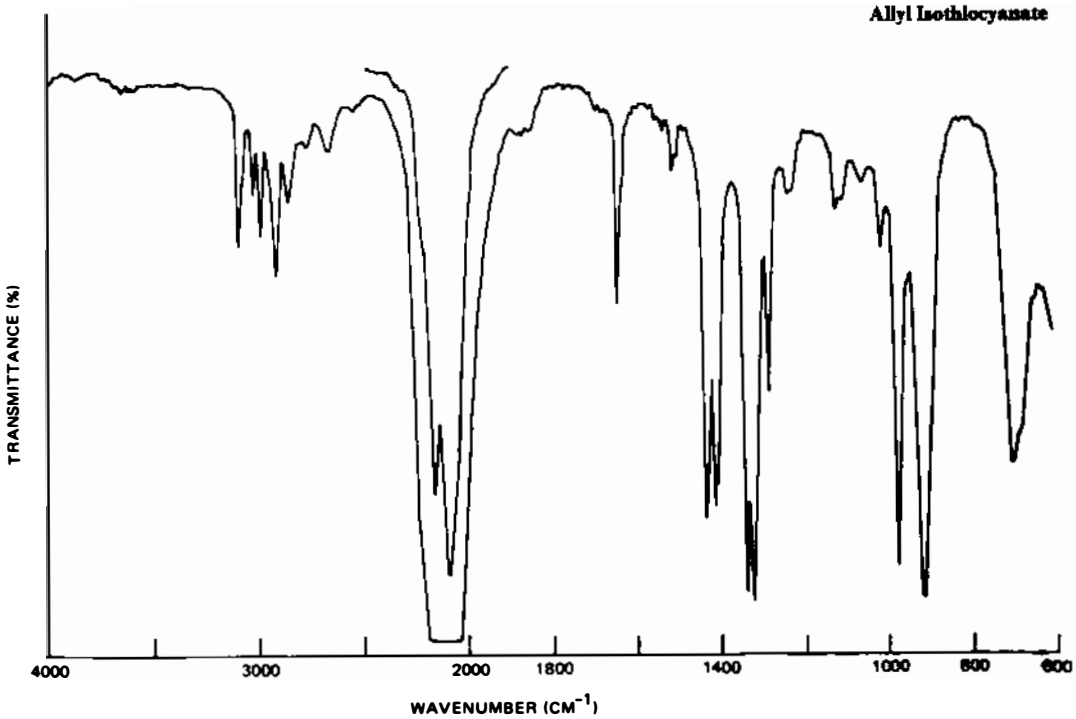
SERIES B-3



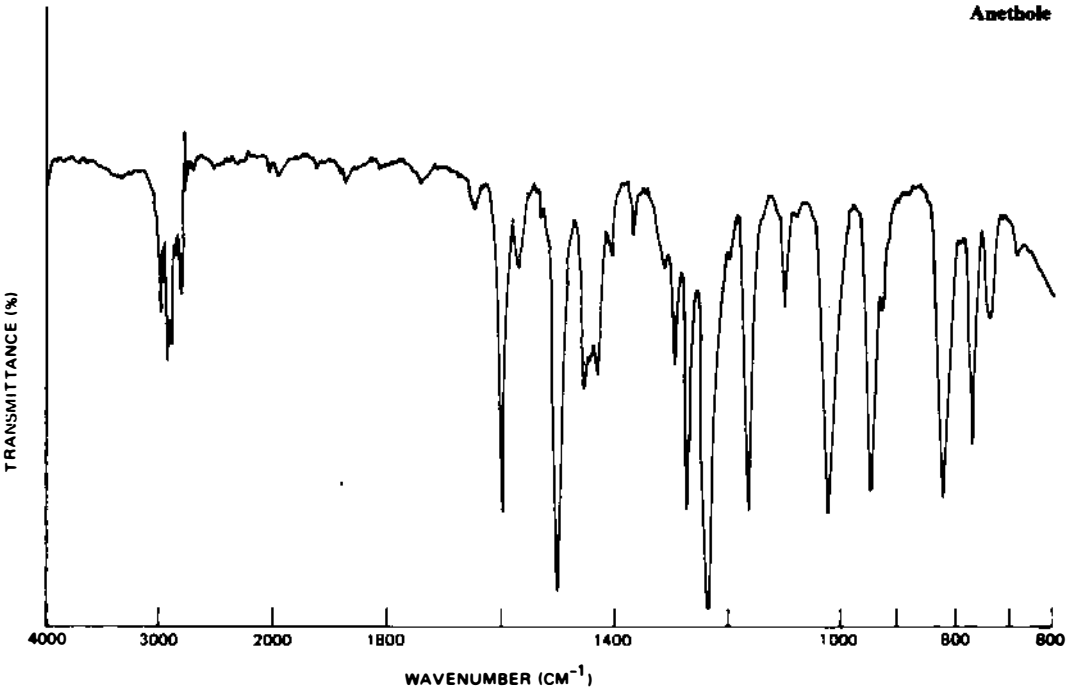
Acetoin



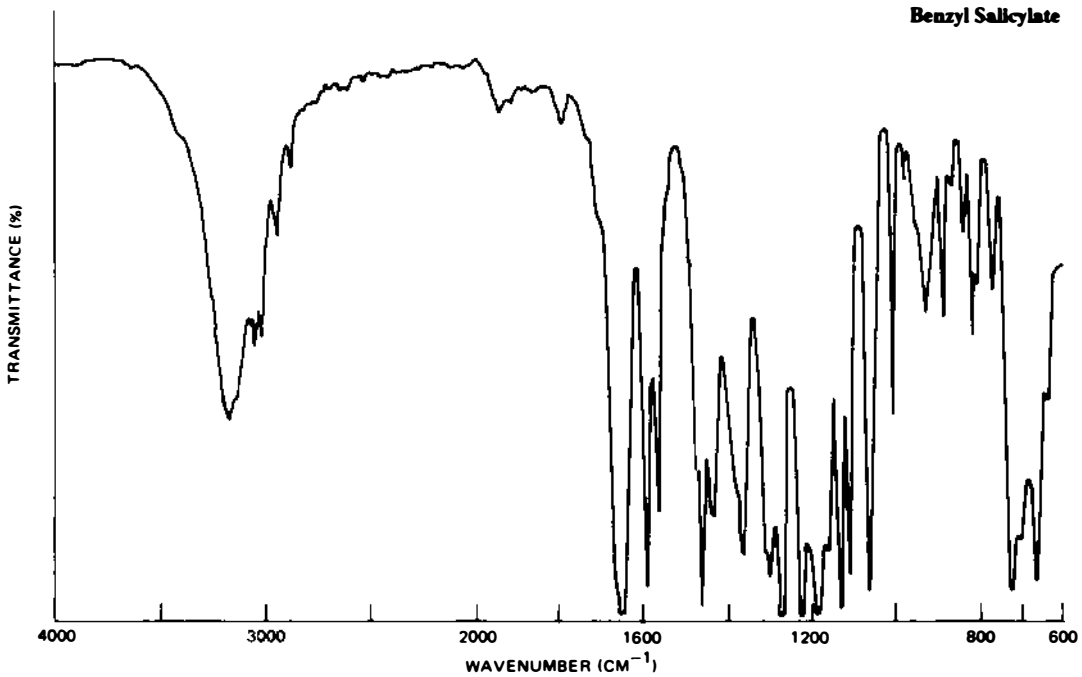
Allyl Isothiocyanate



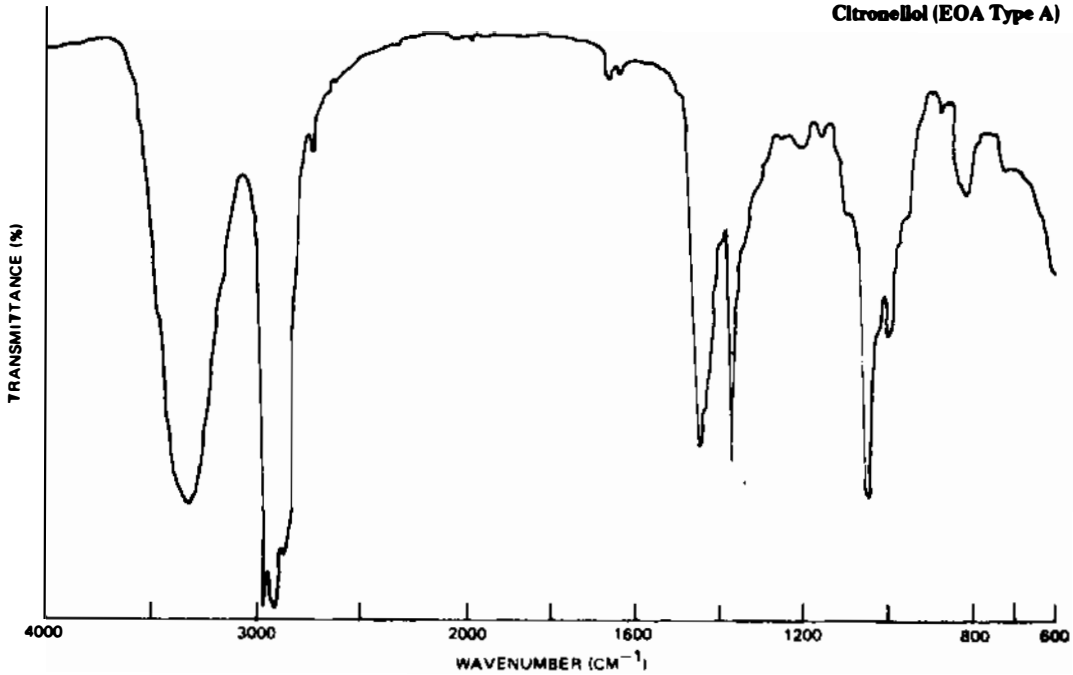
Anethole



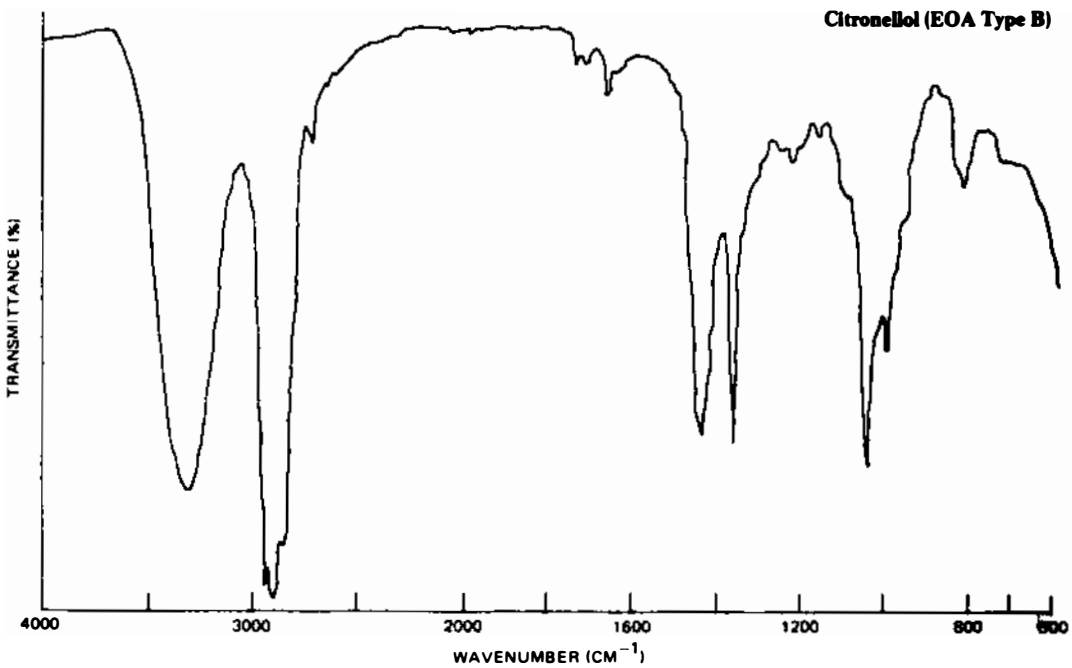
Benzyl Salicylate



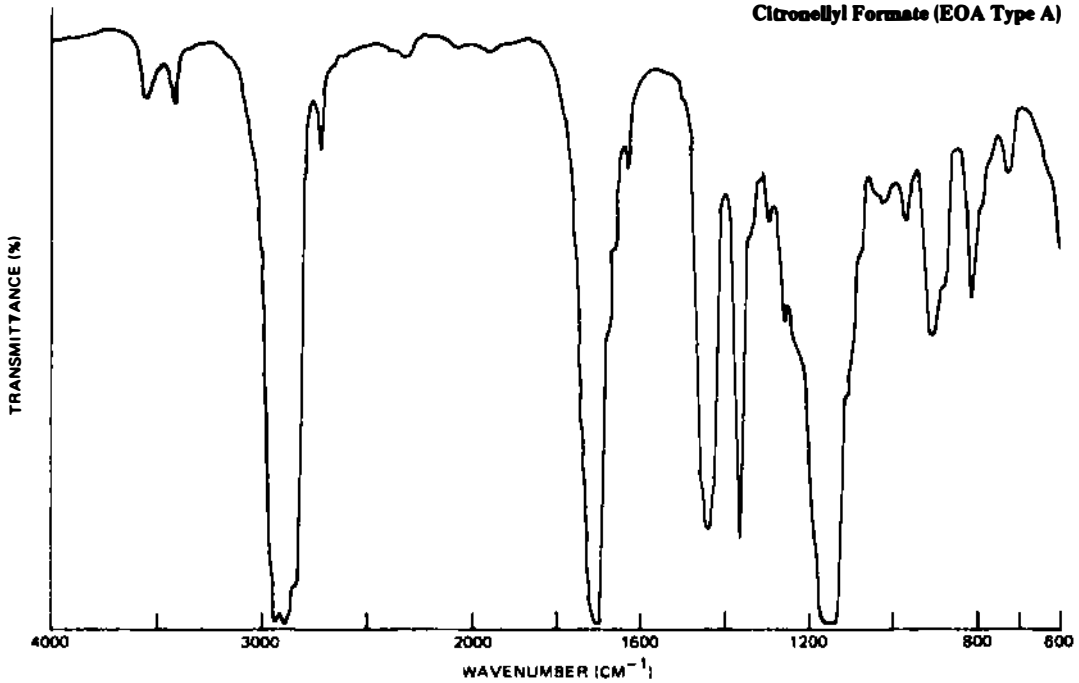
Citronellol (EOA Type A)



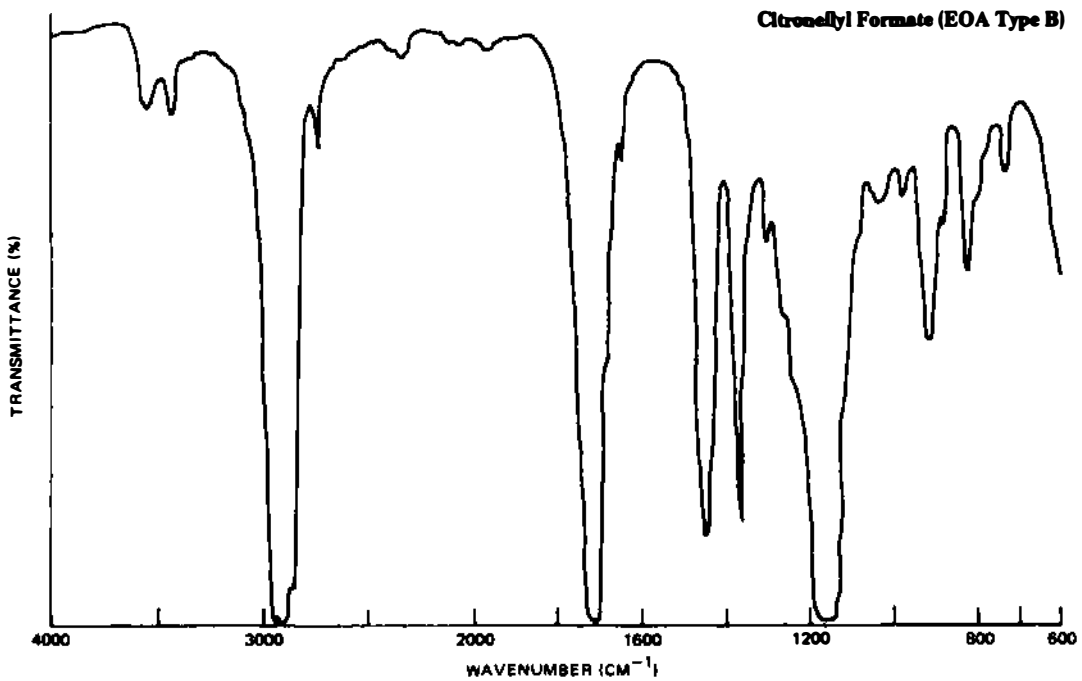
Citronellol (EOA Type B)



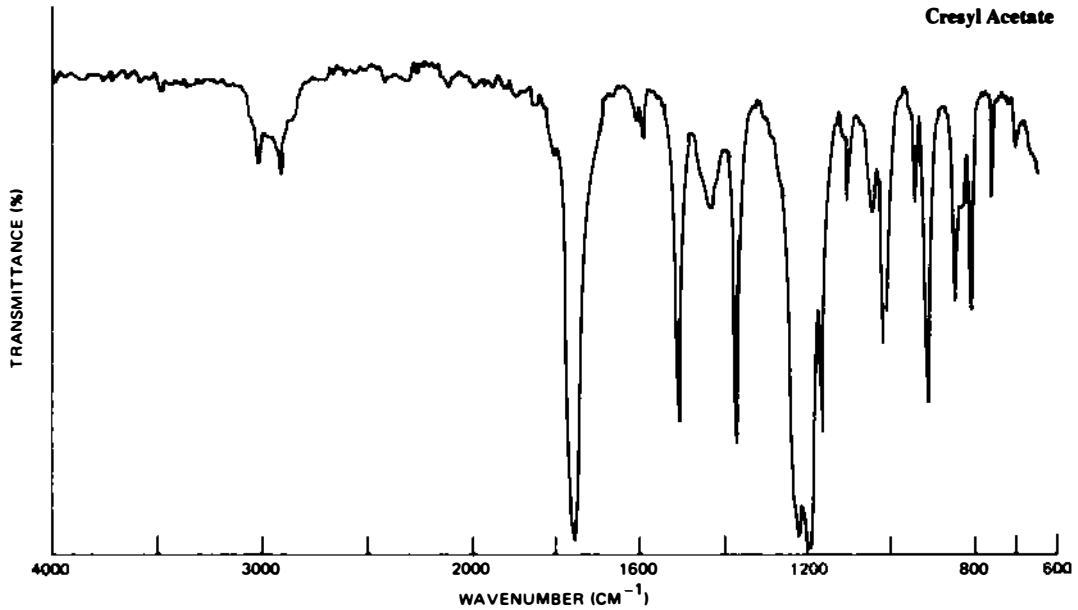
Citronellyl Formate (EOA Type A)



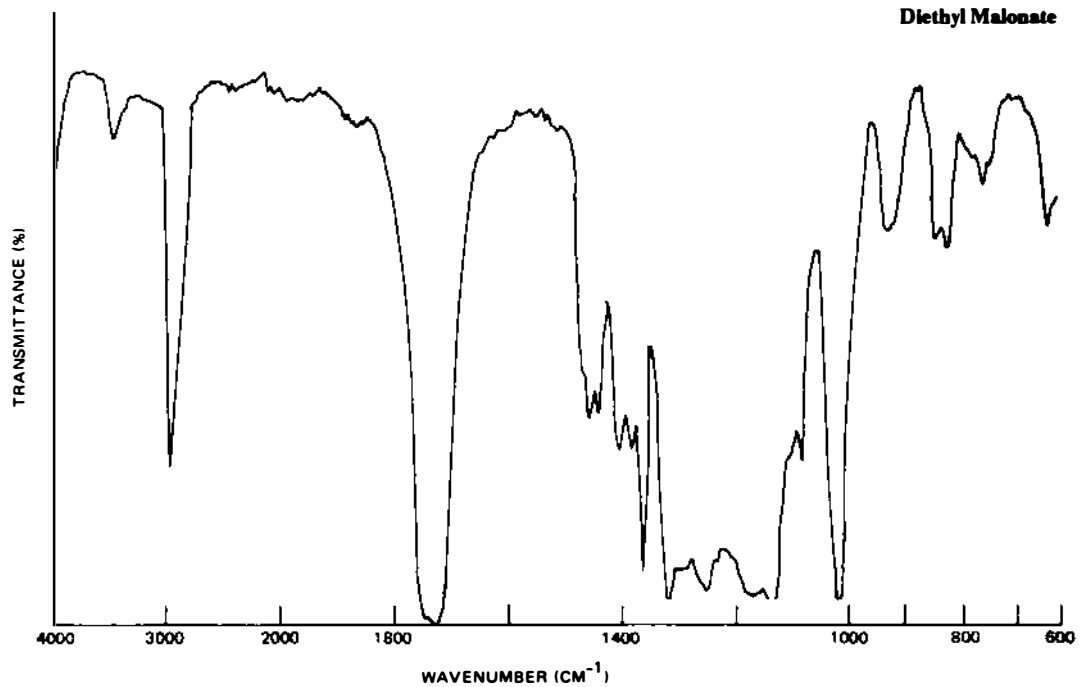
Citronellyl Formate (EOA Type B)



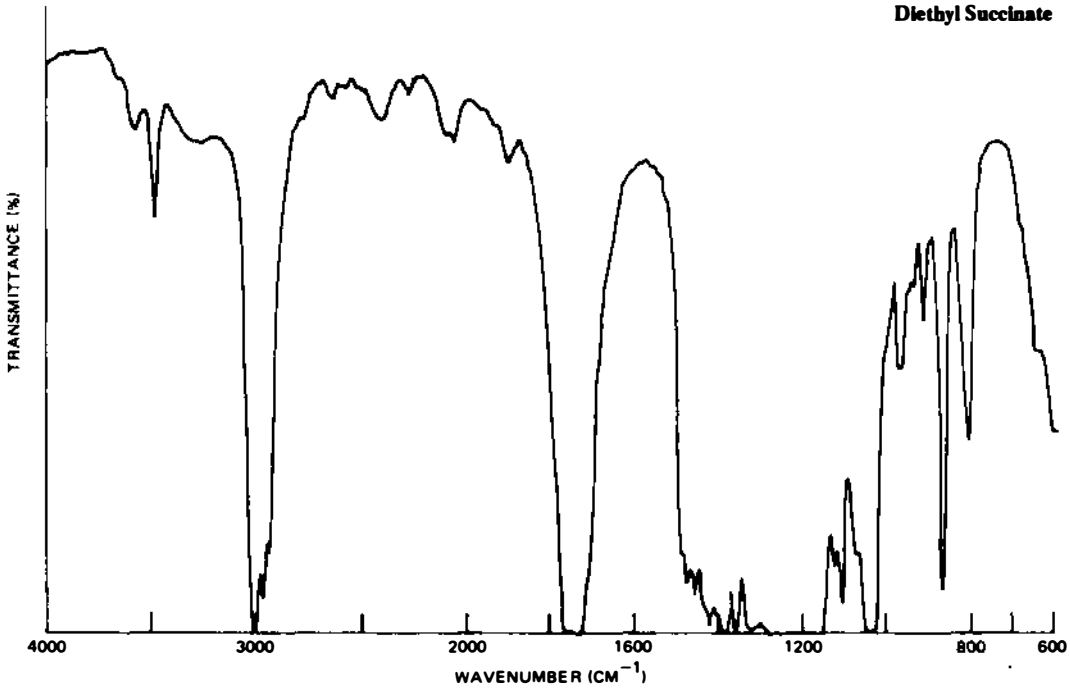
Cresyl Acetate



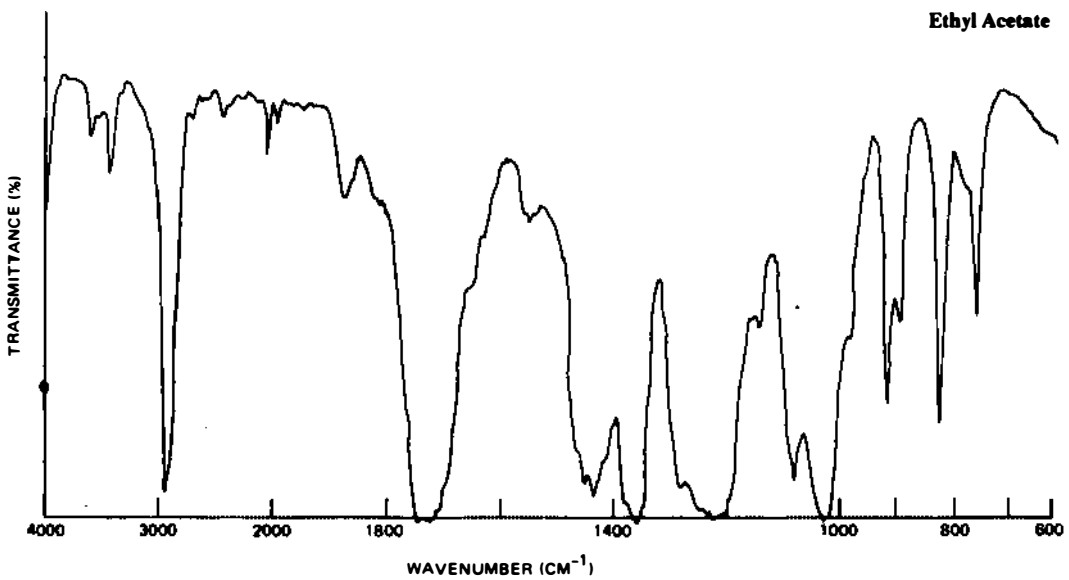
Diethyl Malonate



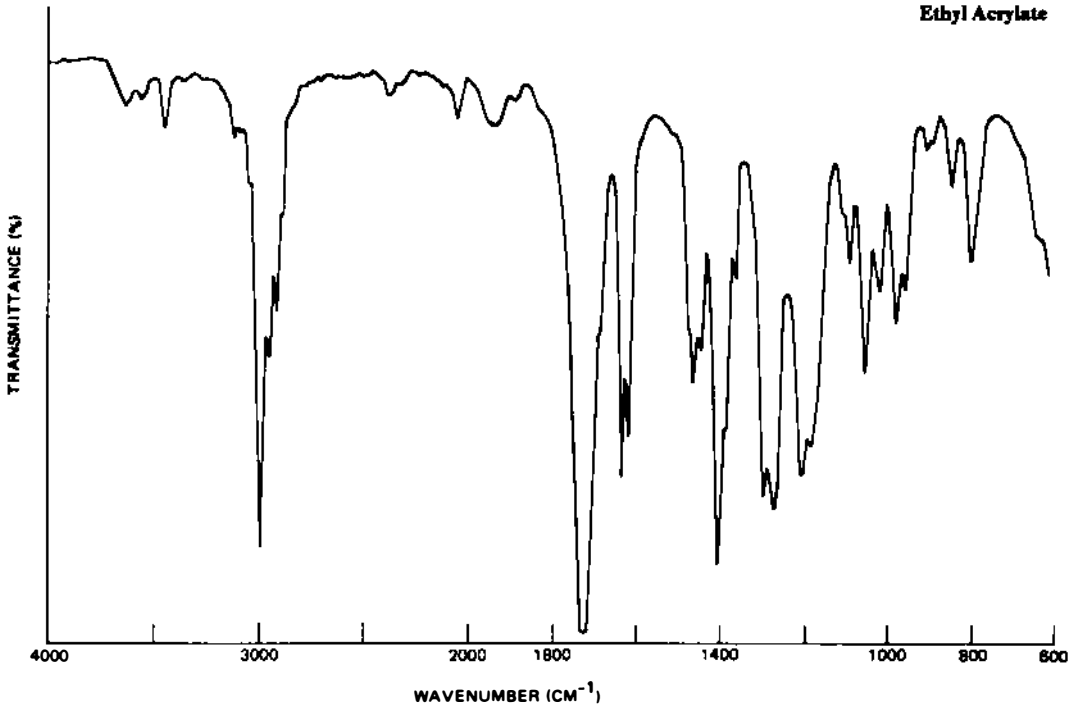
Diethyl Succinate



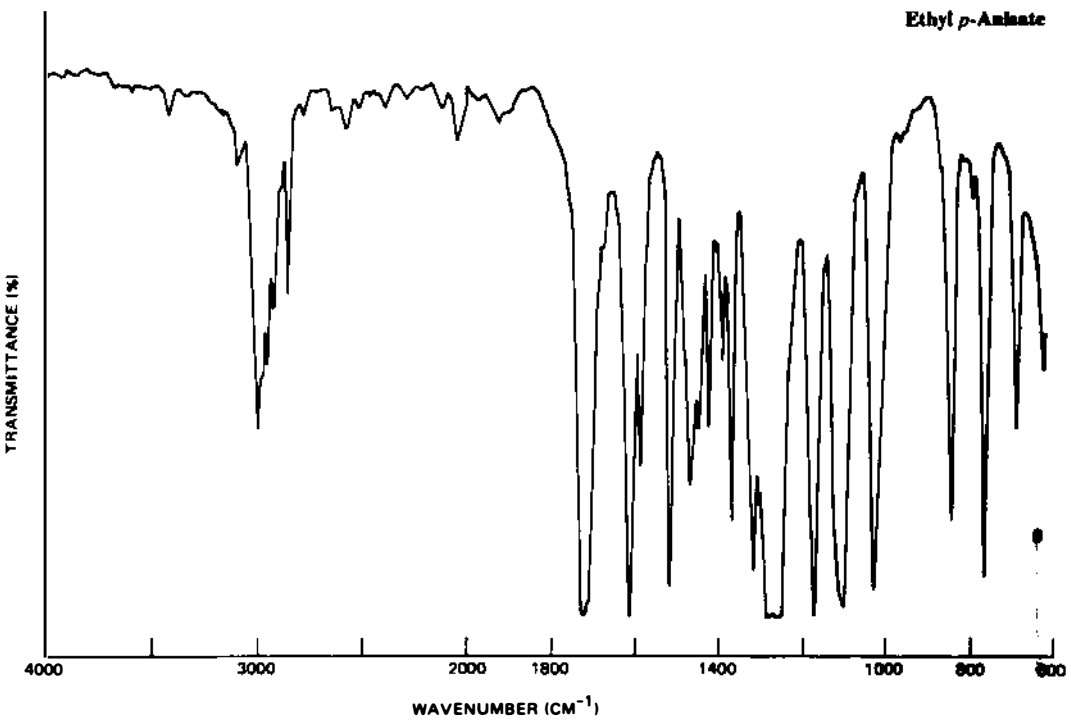
Ethyl Acetate



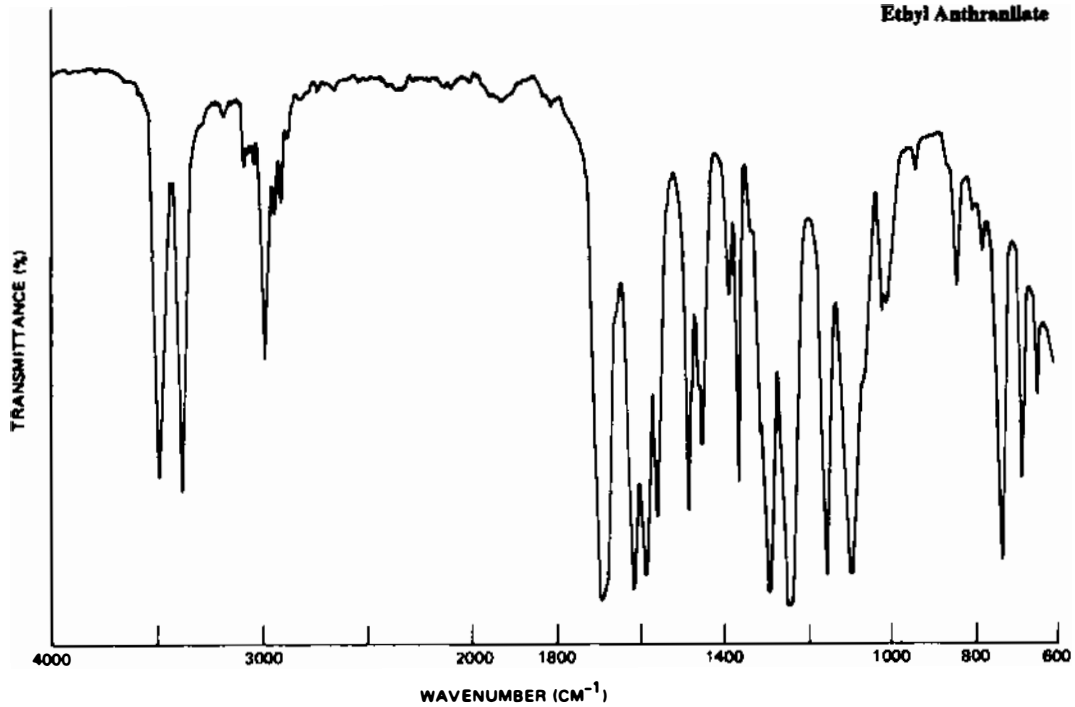
Ethyl Acrylate



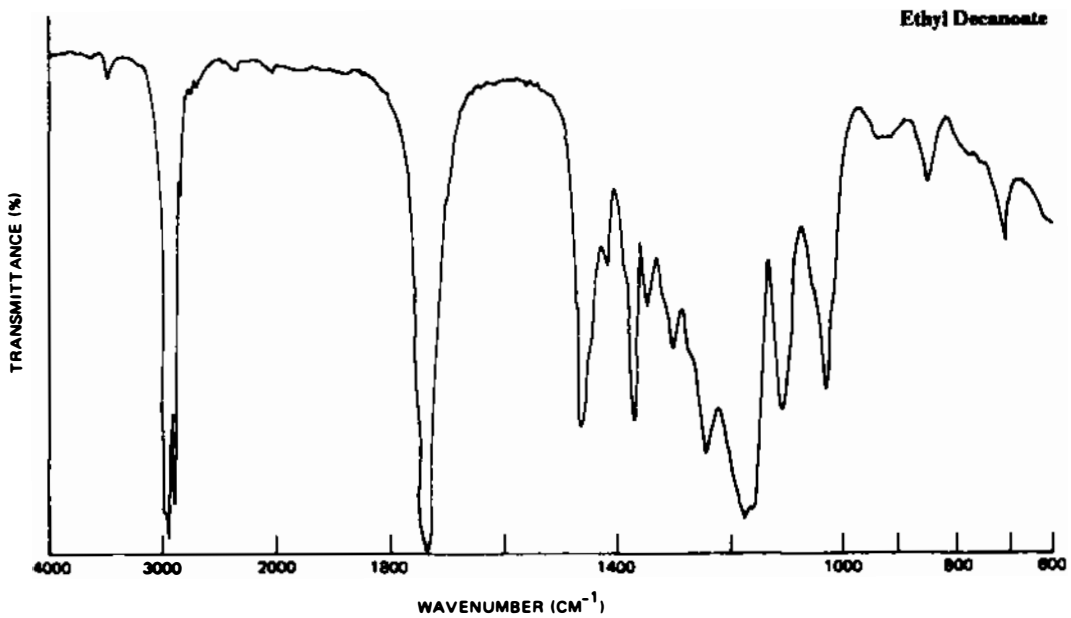
Ethyl *p*-Anisate



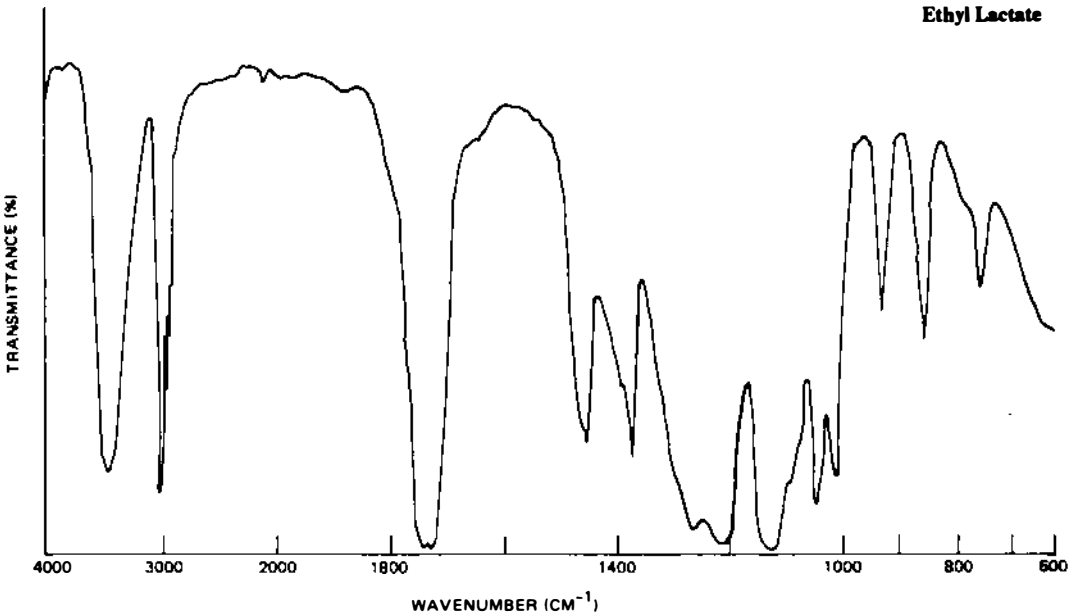
Ethyl Anthranilate



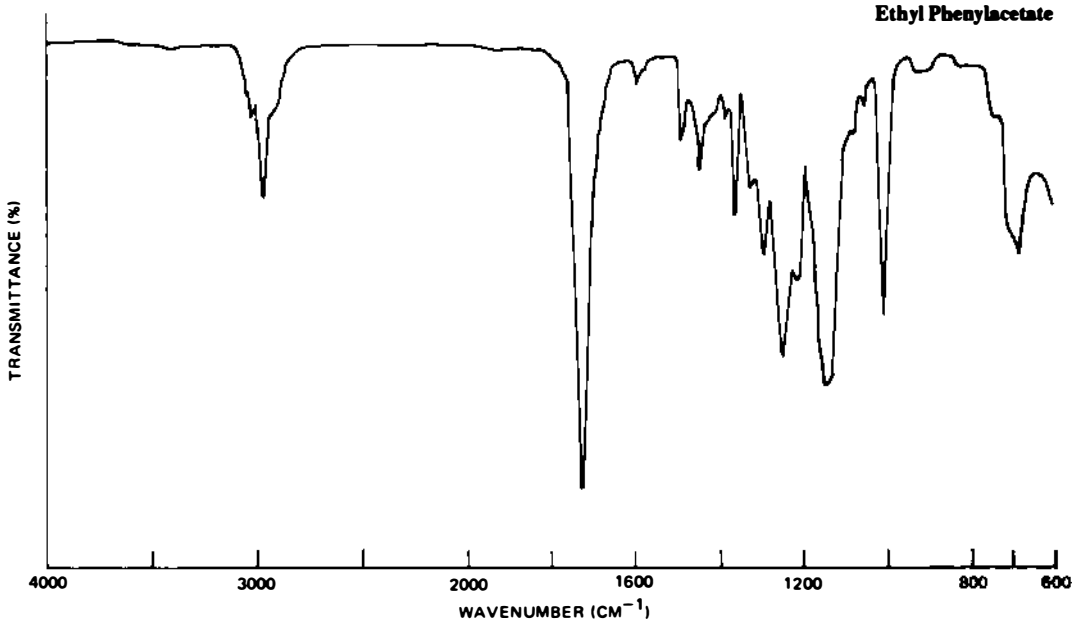
Ethyl Decanoate



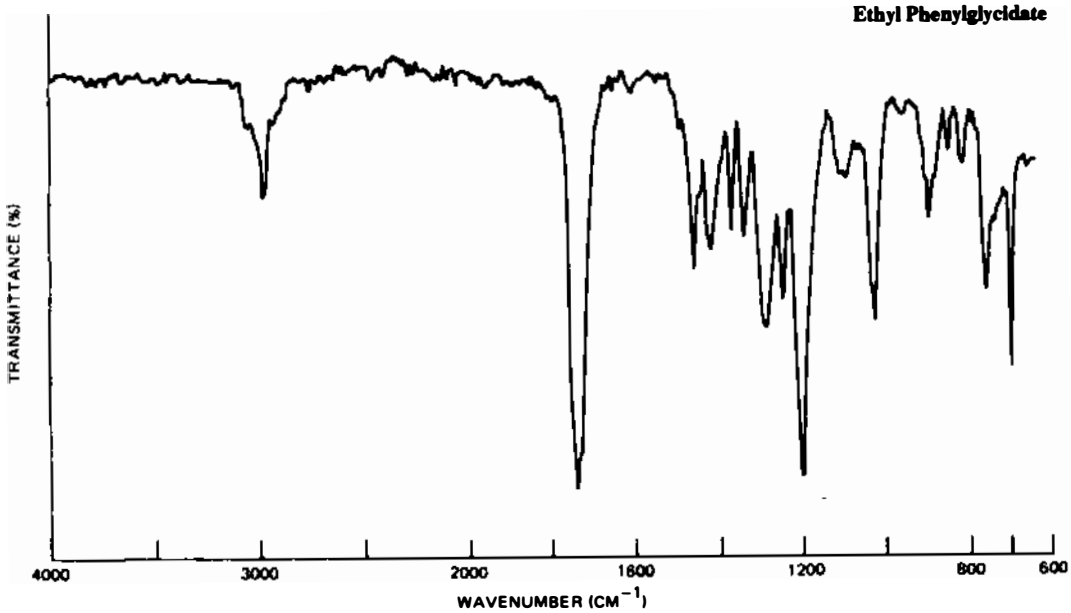
Ethyl Lactate



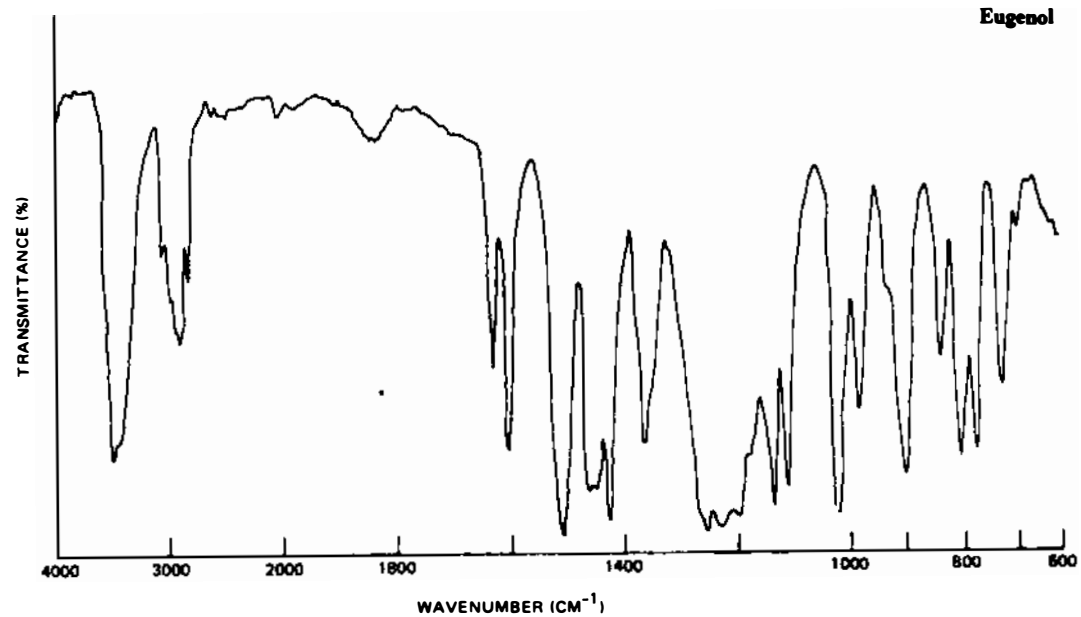
Ethyl Phenylacetate

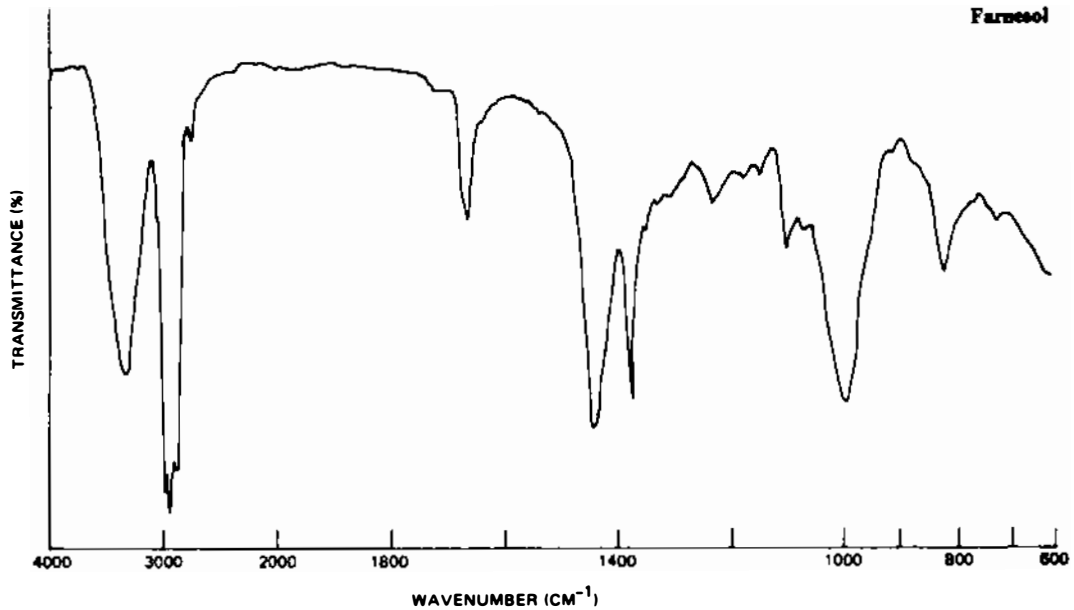


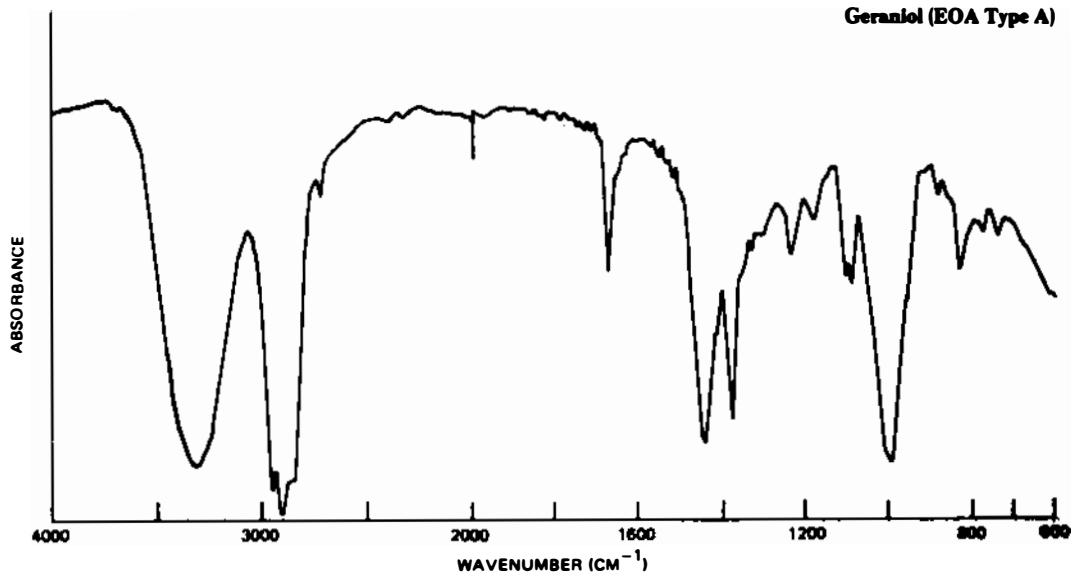
Ethyl Phenylglycidate



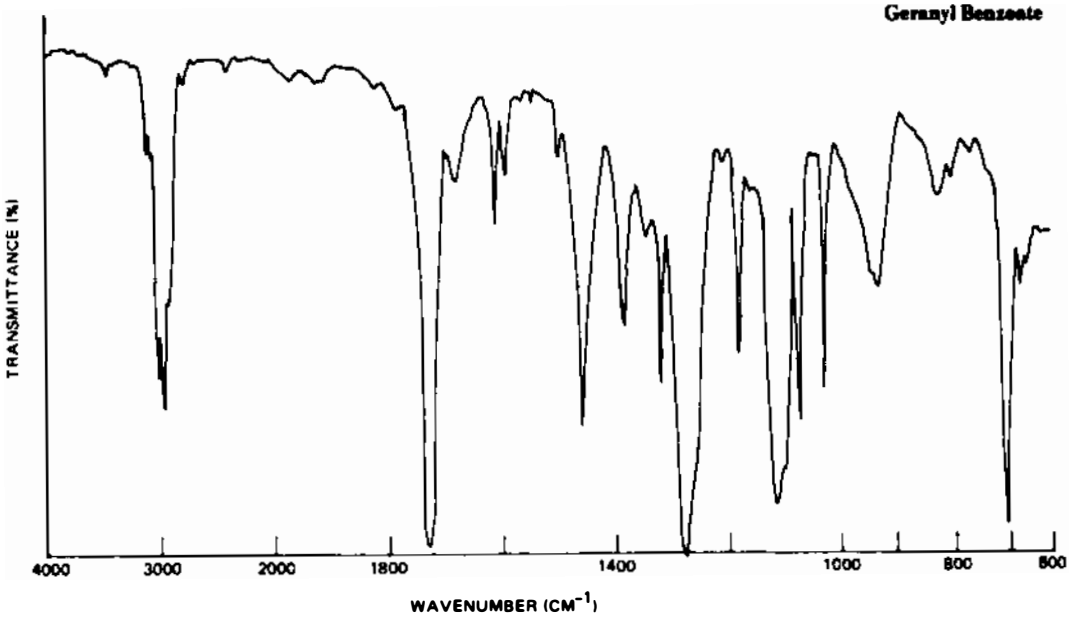
Eugenol



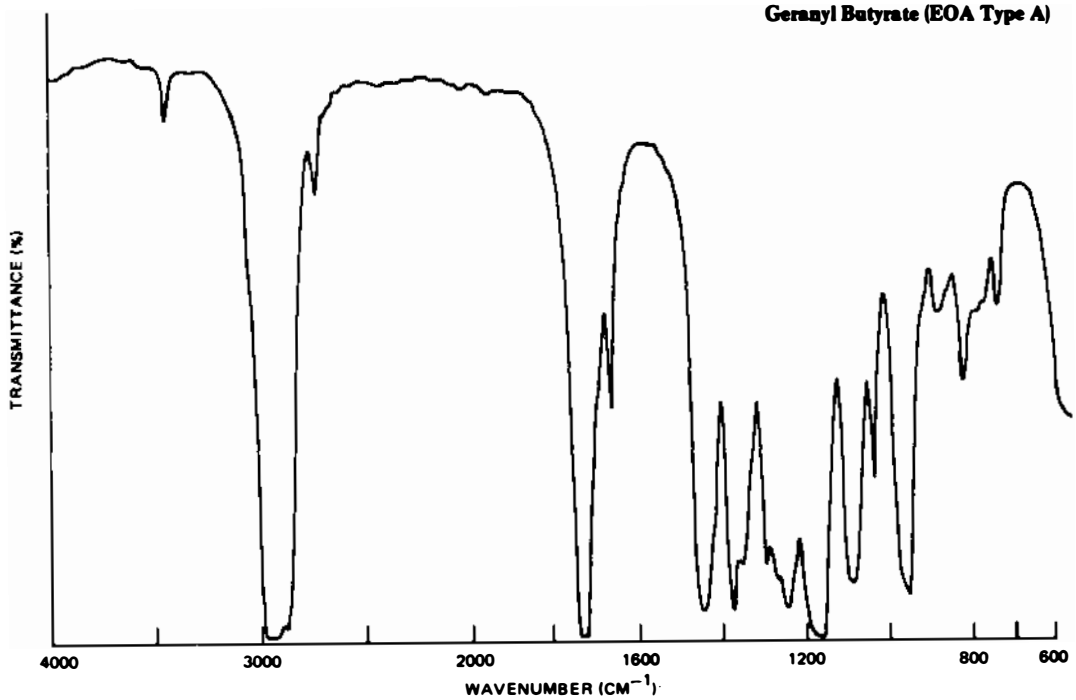




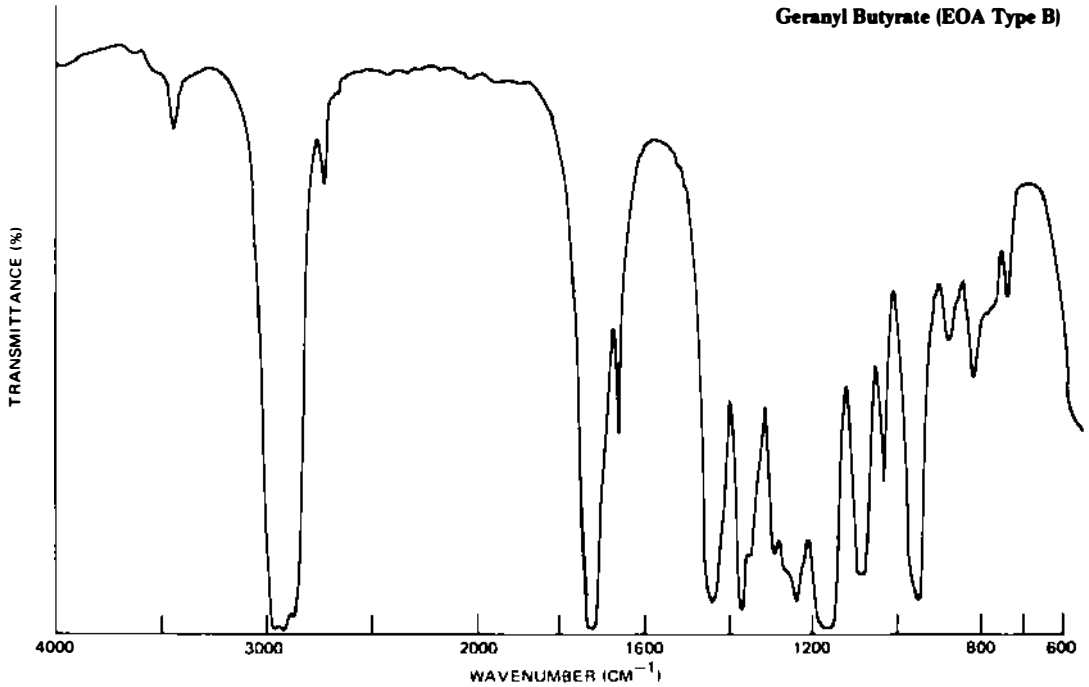
Geranyl Benzoate



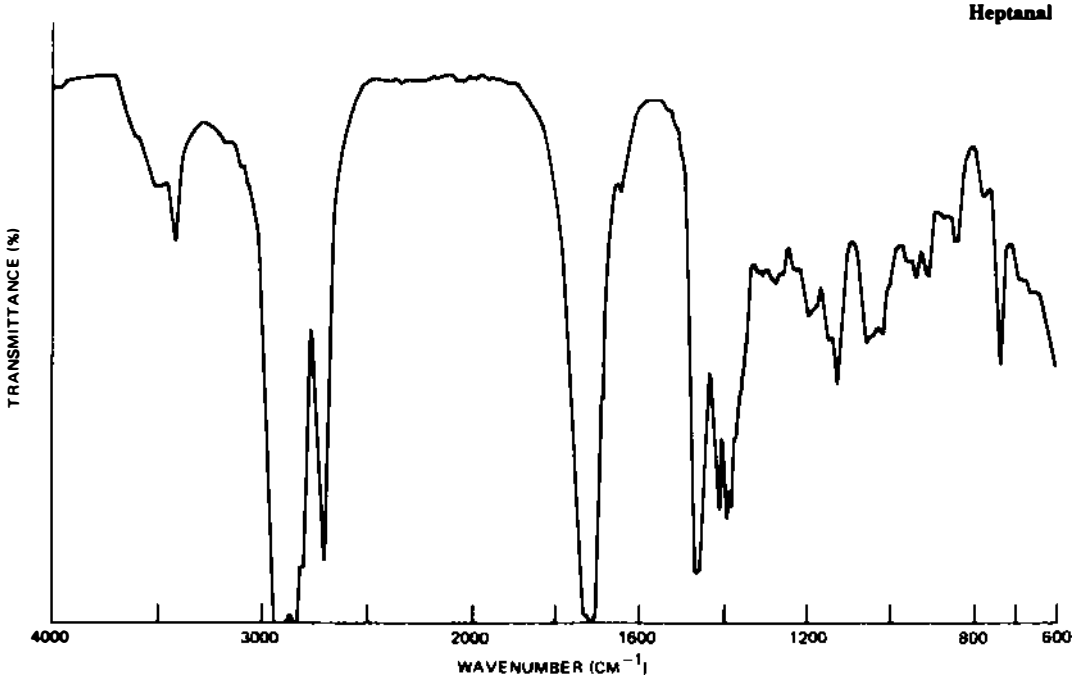
Geranyl Butyrate (EOA Type A)



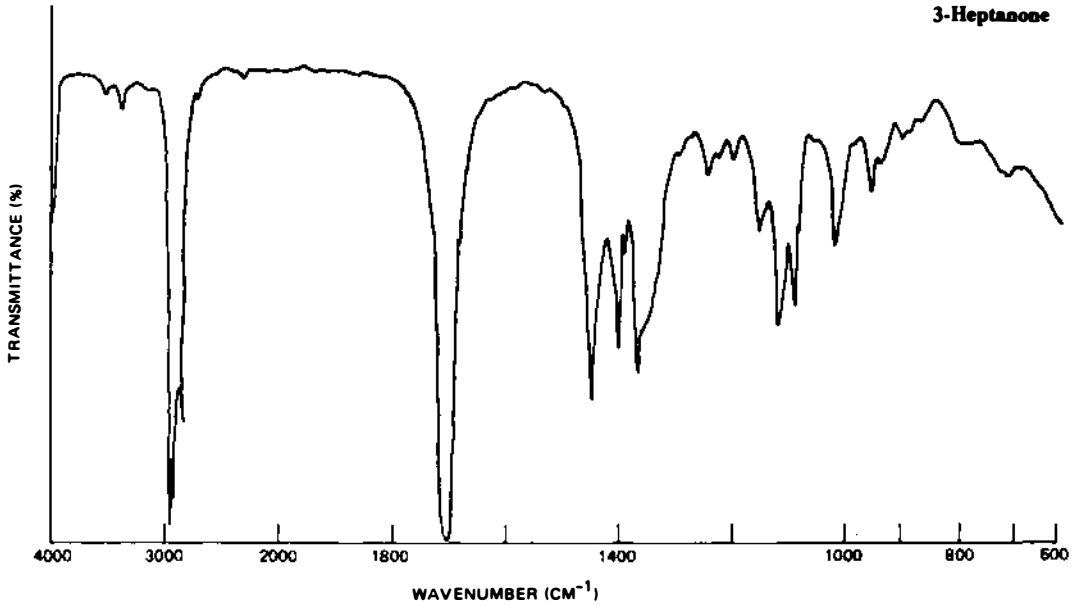
Geranyl Butyrate (EOA Type B)



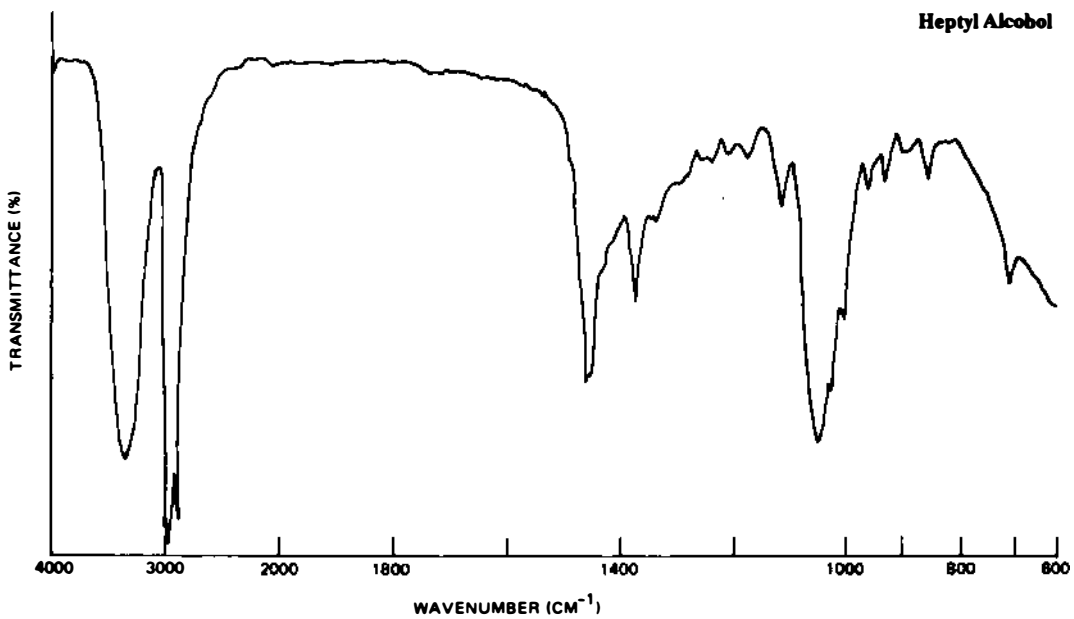
Heptanal

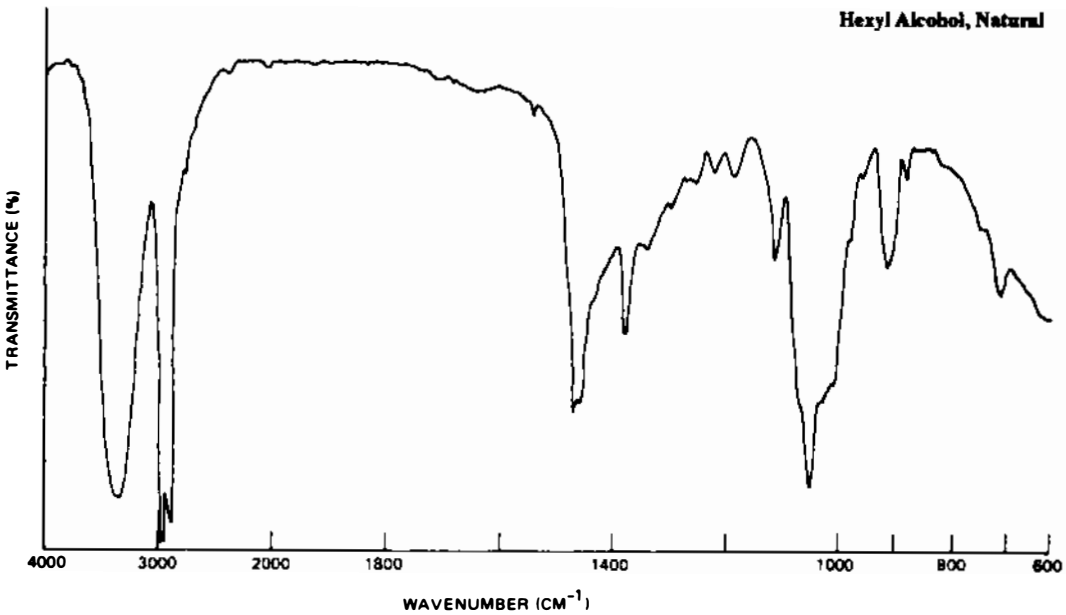


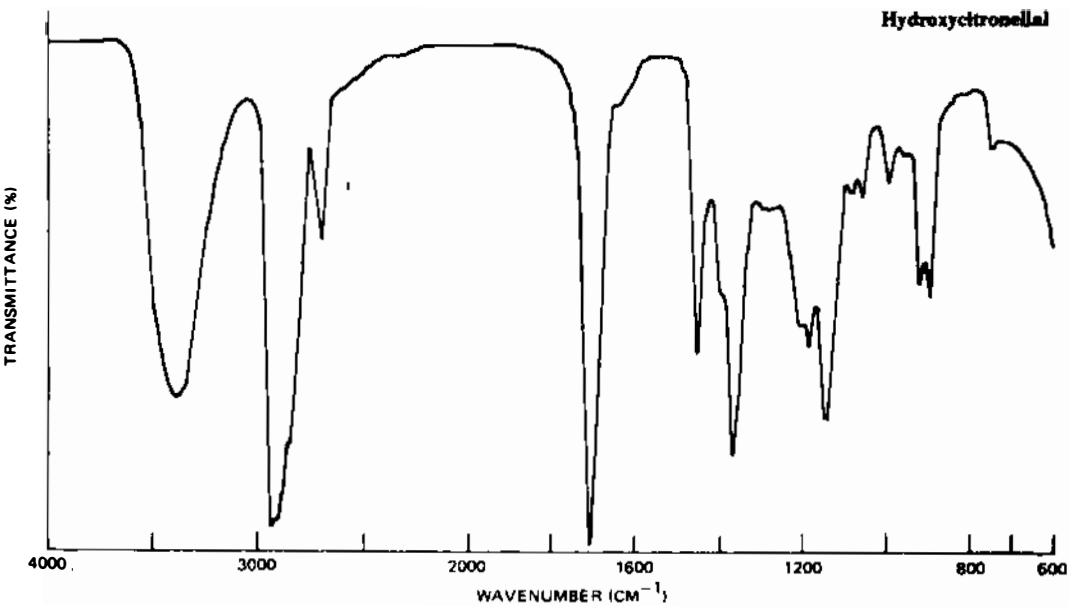
3-Heptanone

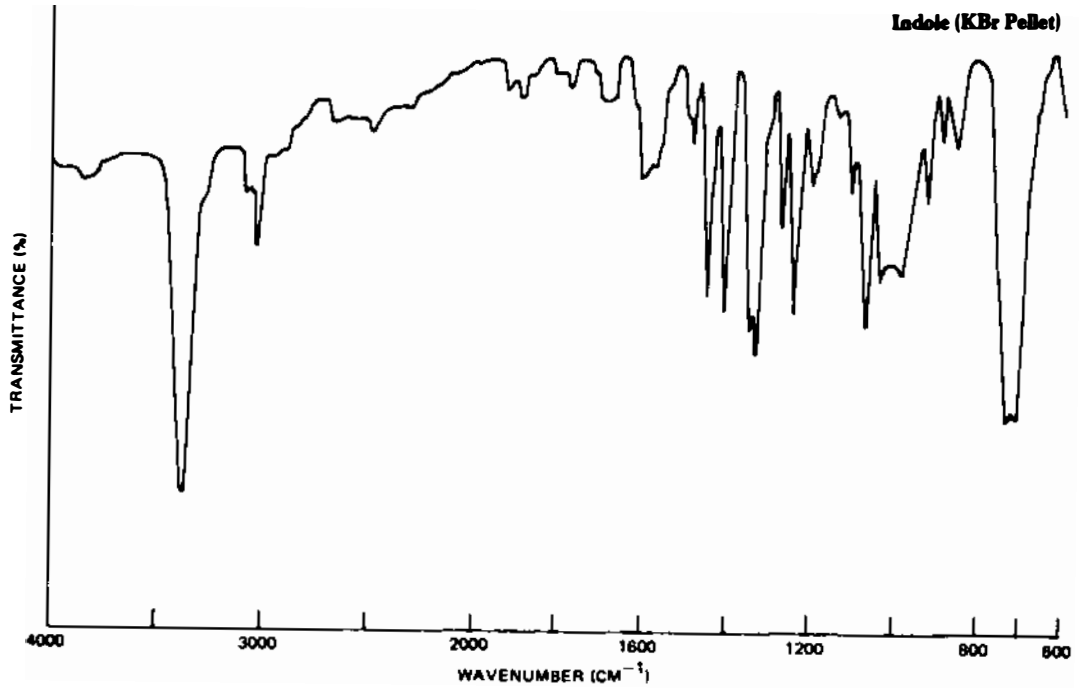


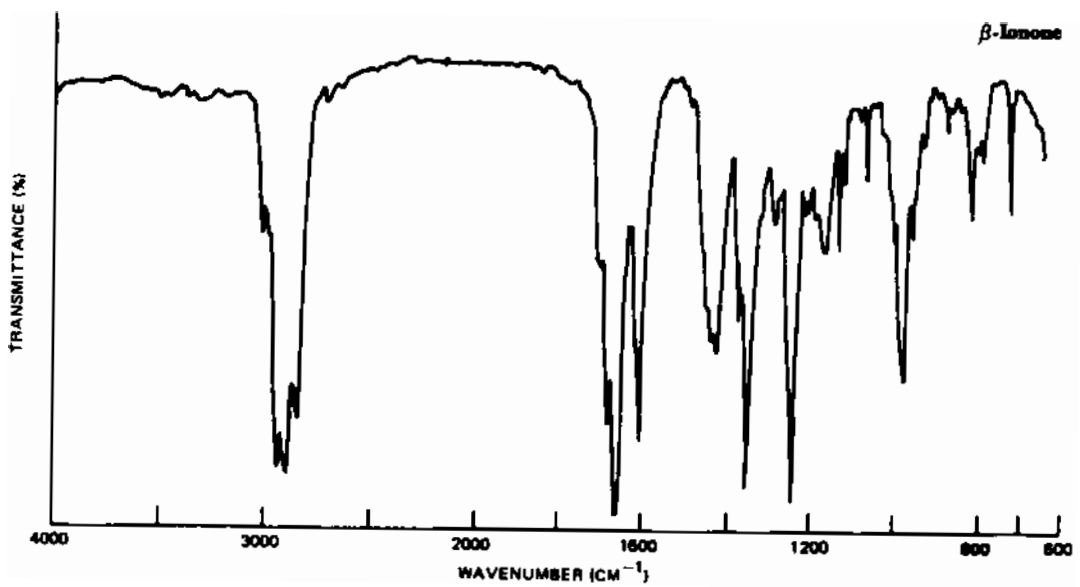
Heptyl Alcohol



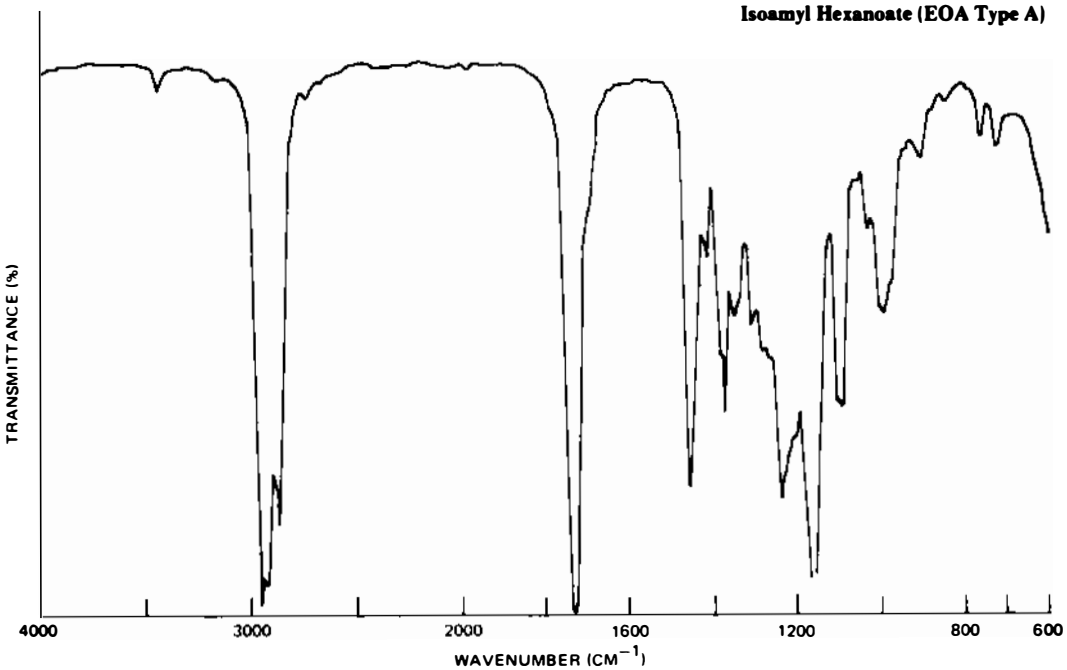




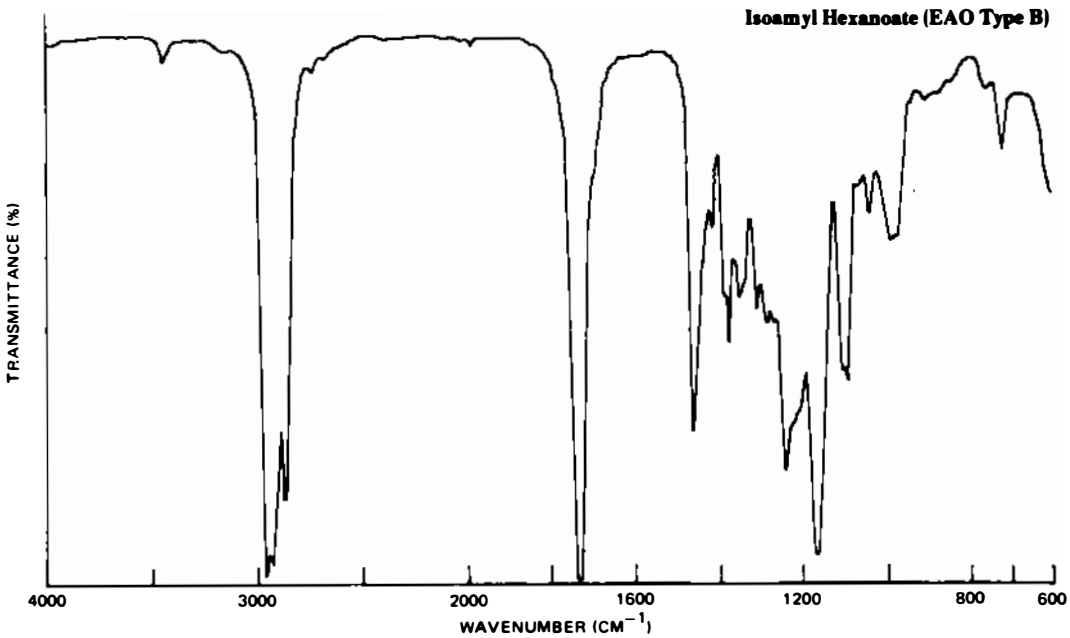




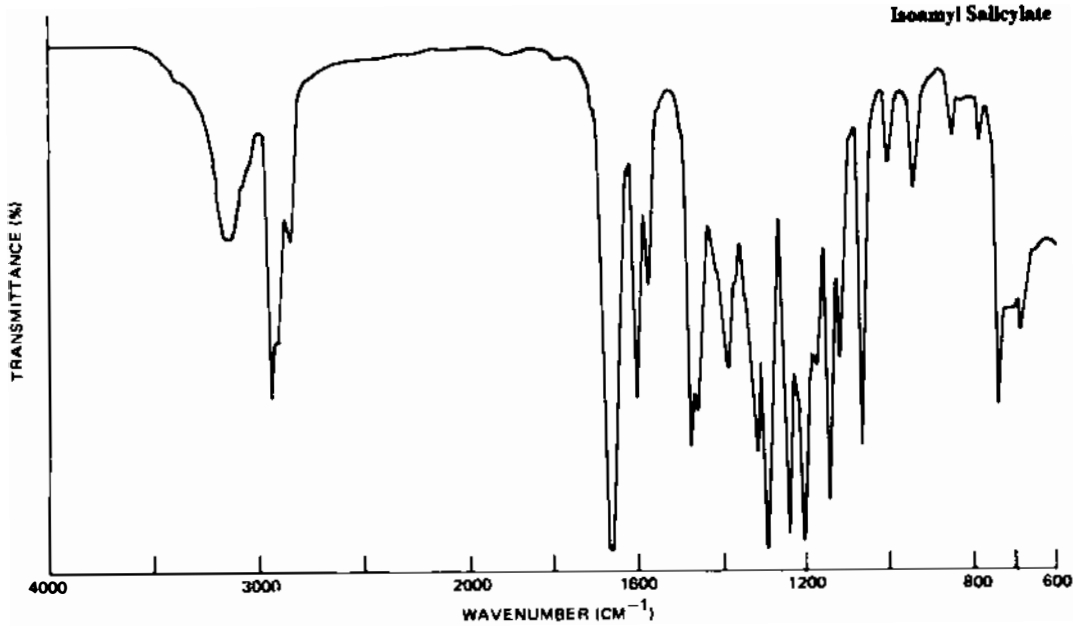
Isoamyl Hexanoate (EOA Type A)



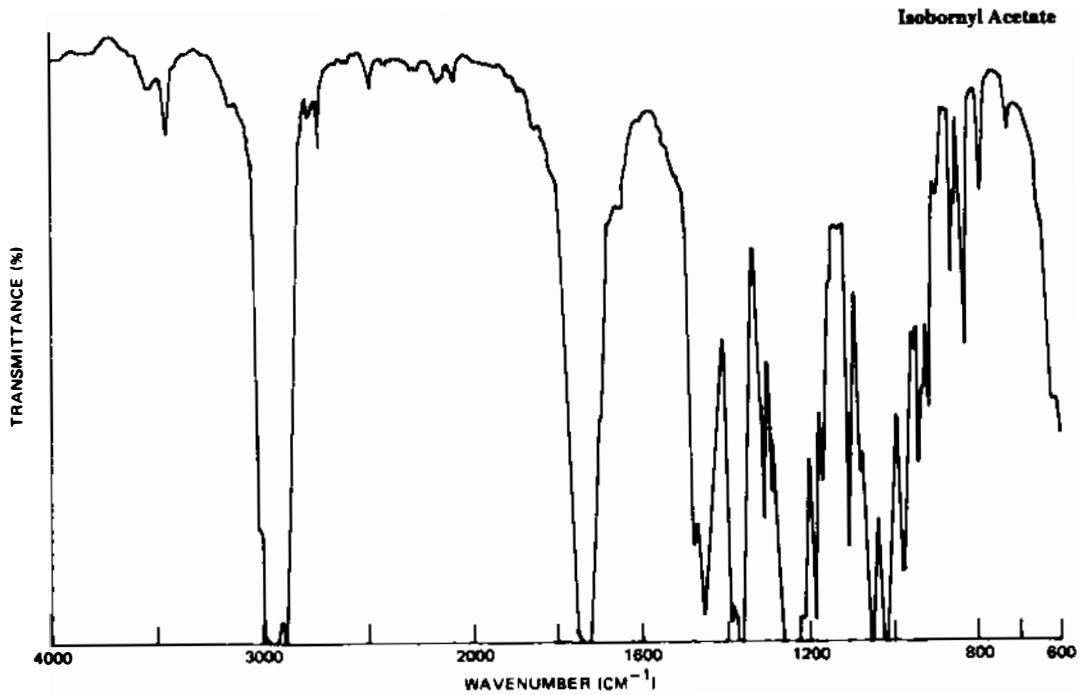
Isoamyl Hexanoate (EAO Type B)

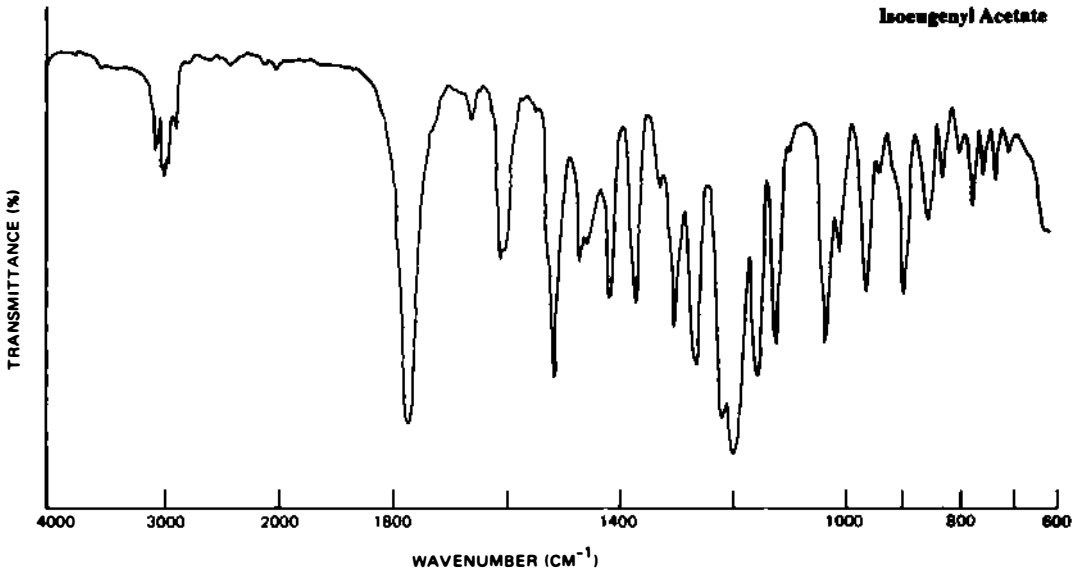


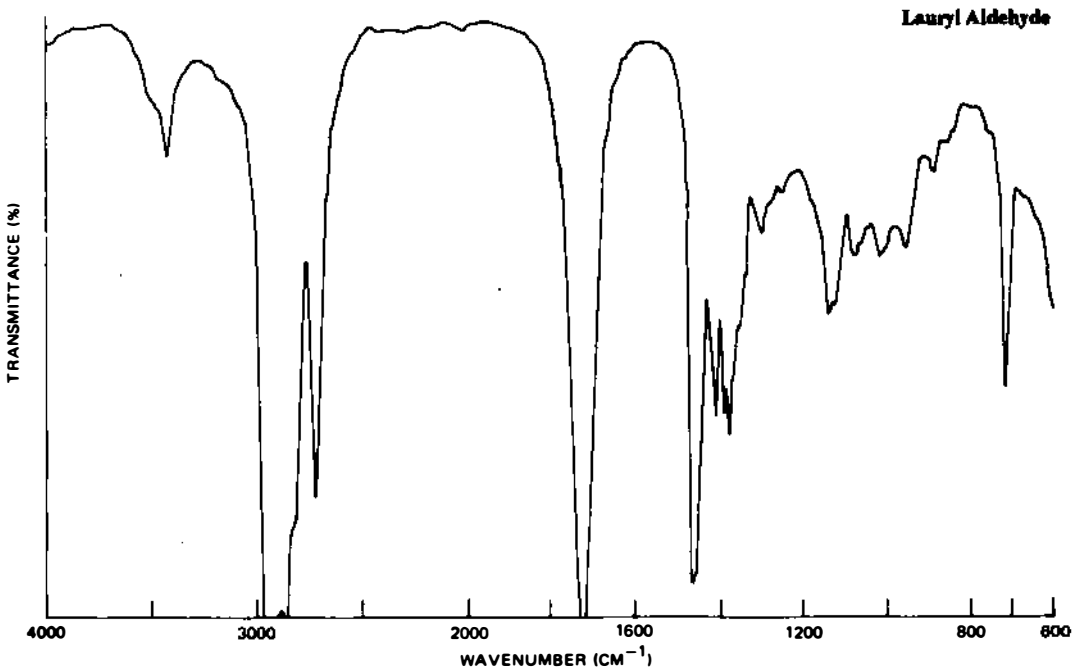
Isoamyl Salicylate

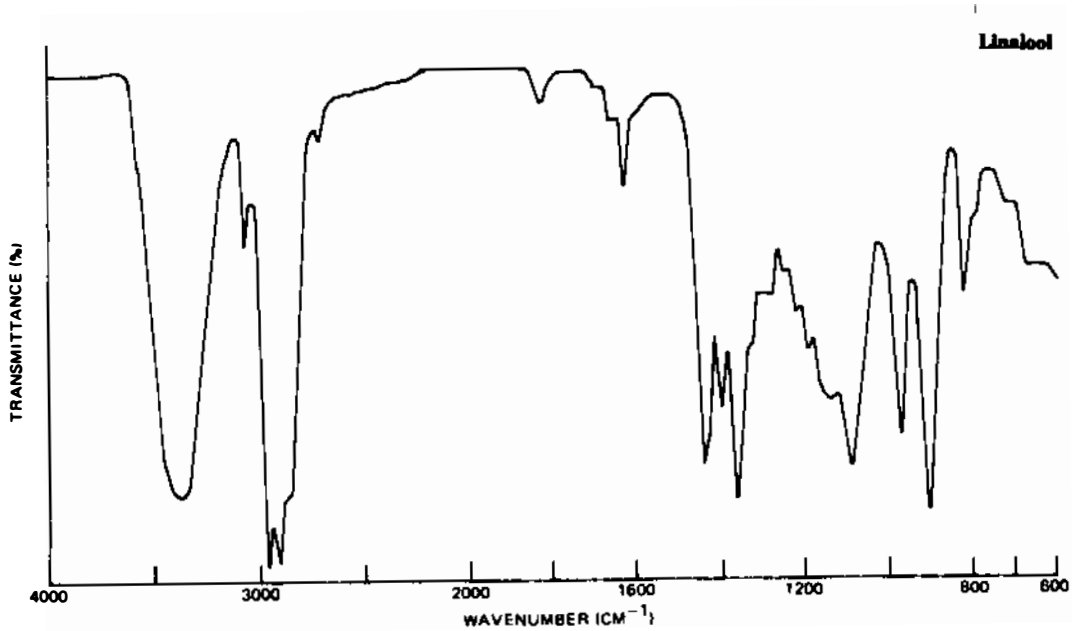


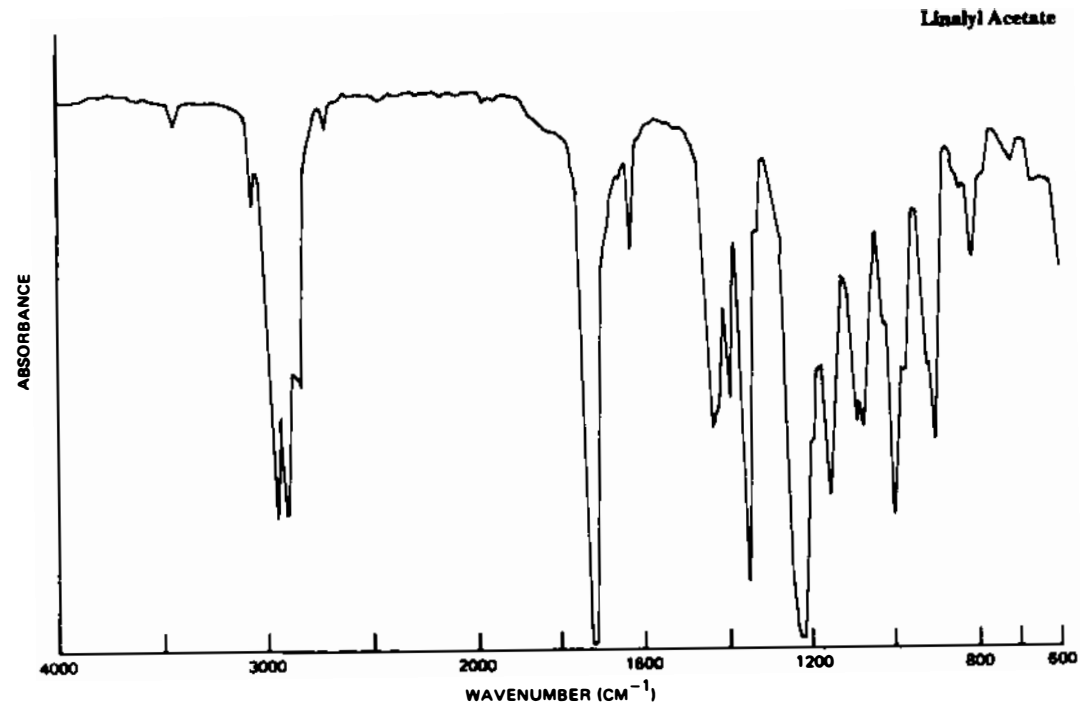
Isobornyl Acetate



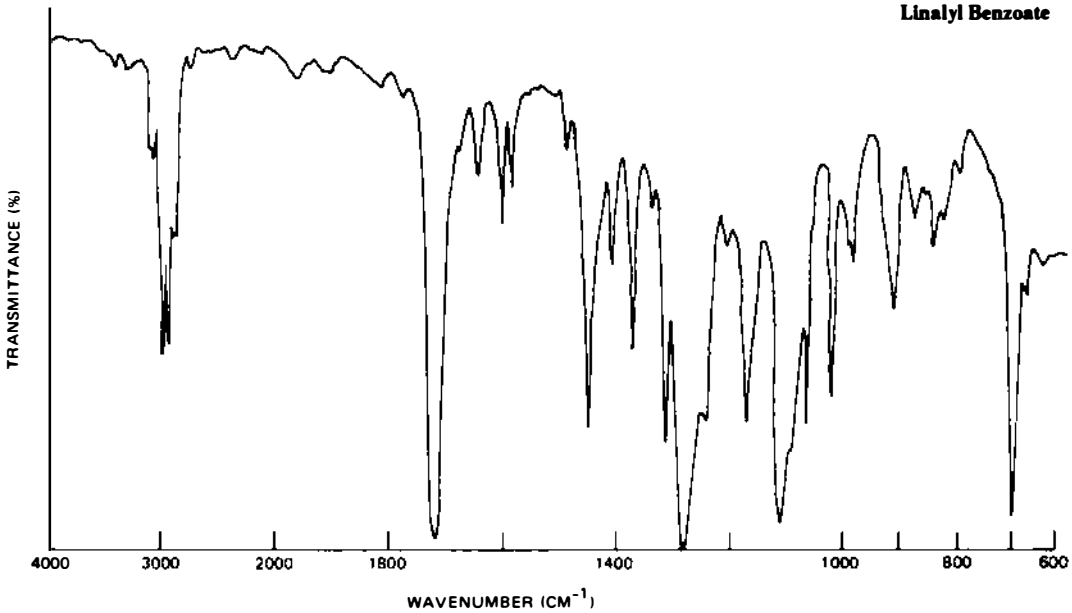

Isoeugenyl Acetate
TRANSMITTANCE (%)
4000
3000
2000
1800
1400
1000
800
600
WAVENUMBER (CM^{-1})


Lauryl Aldehyde
TRANSMITTANCE (%)
4000
3000
2000
1600
1200
800
600
WAVENUMBER (CM^{-1})

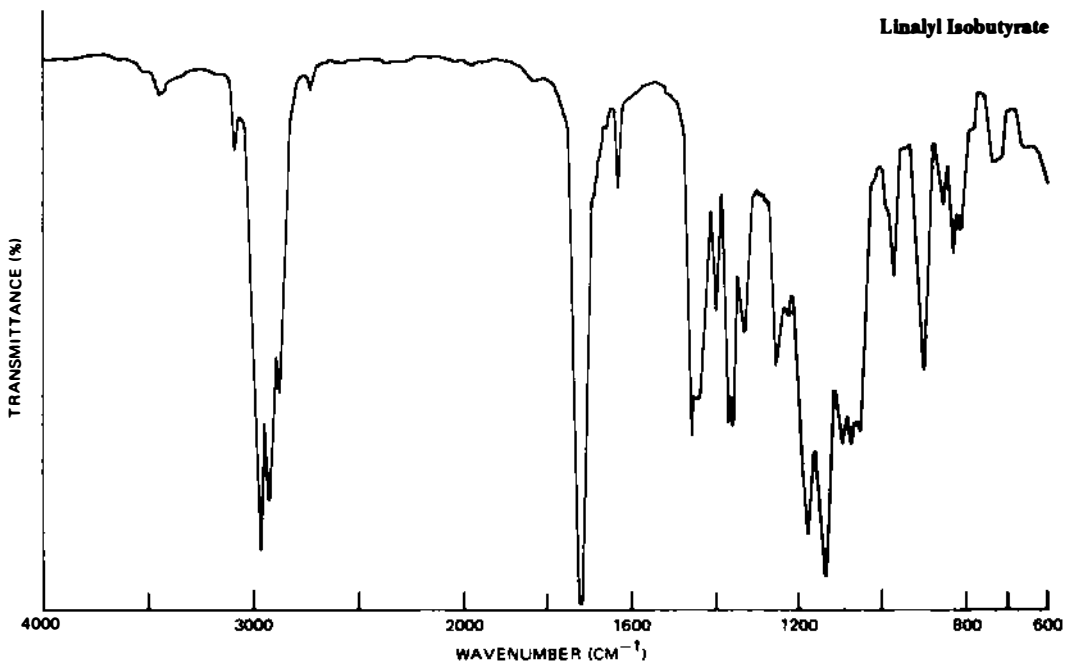




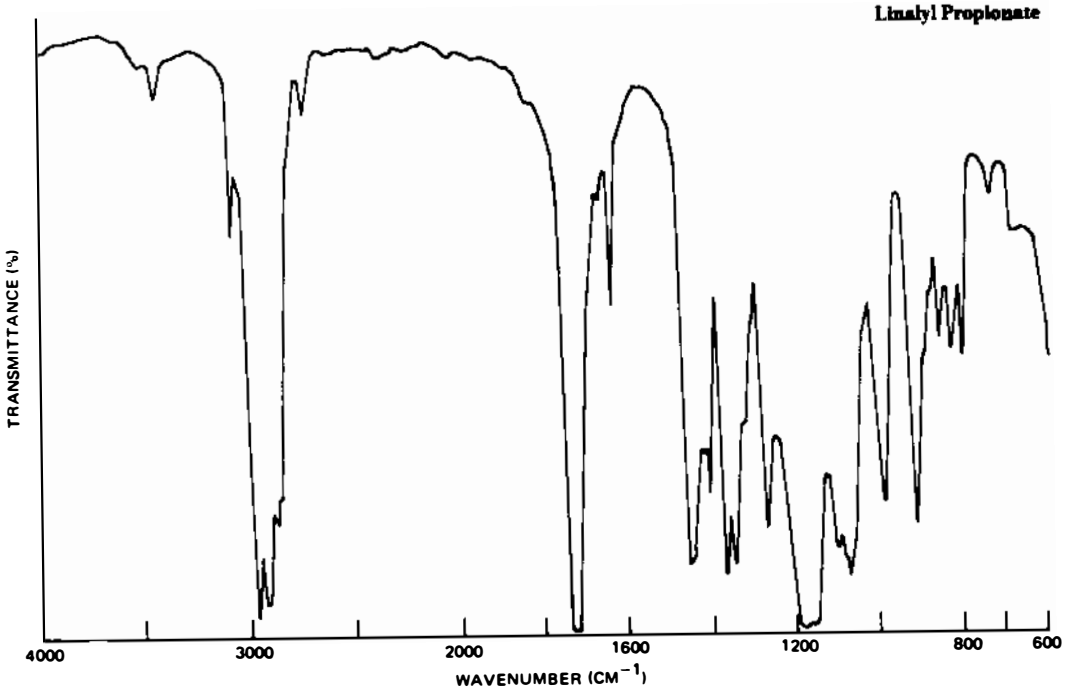
Linalyl Benzoate



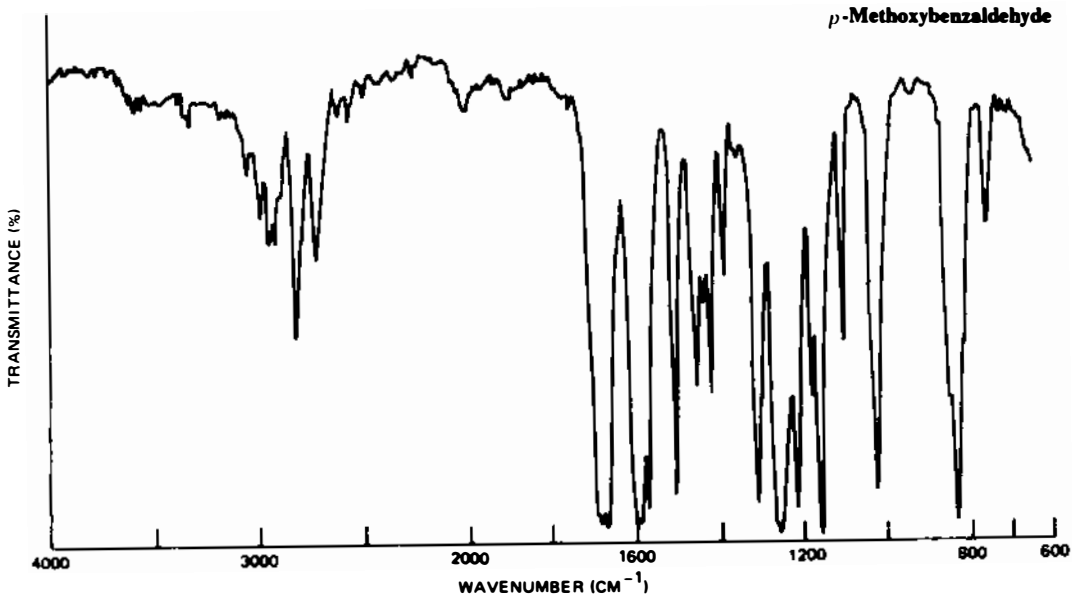
Linalyl Isobutyrate

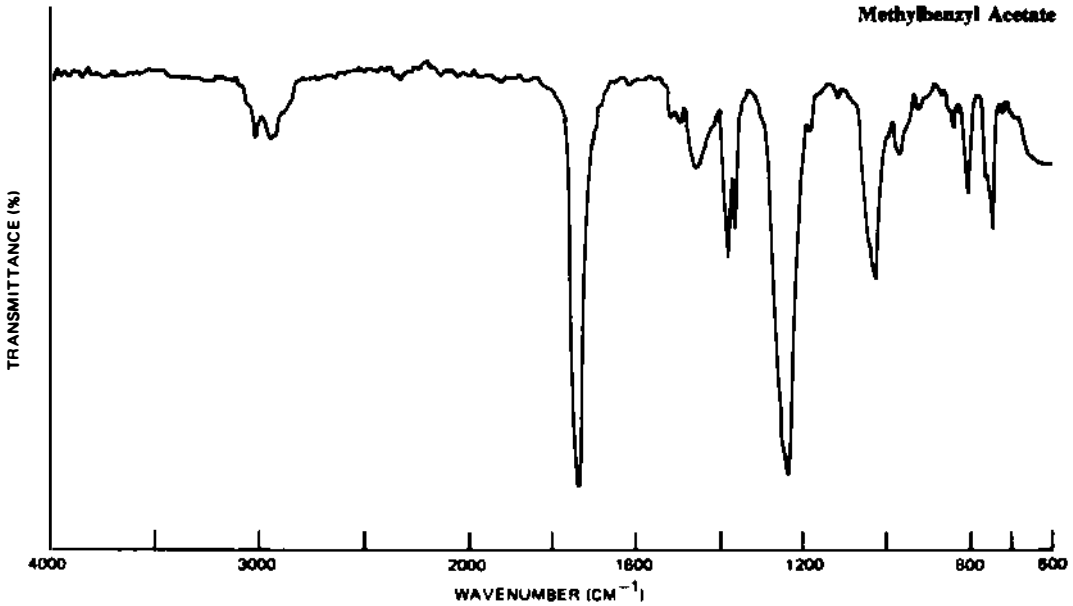


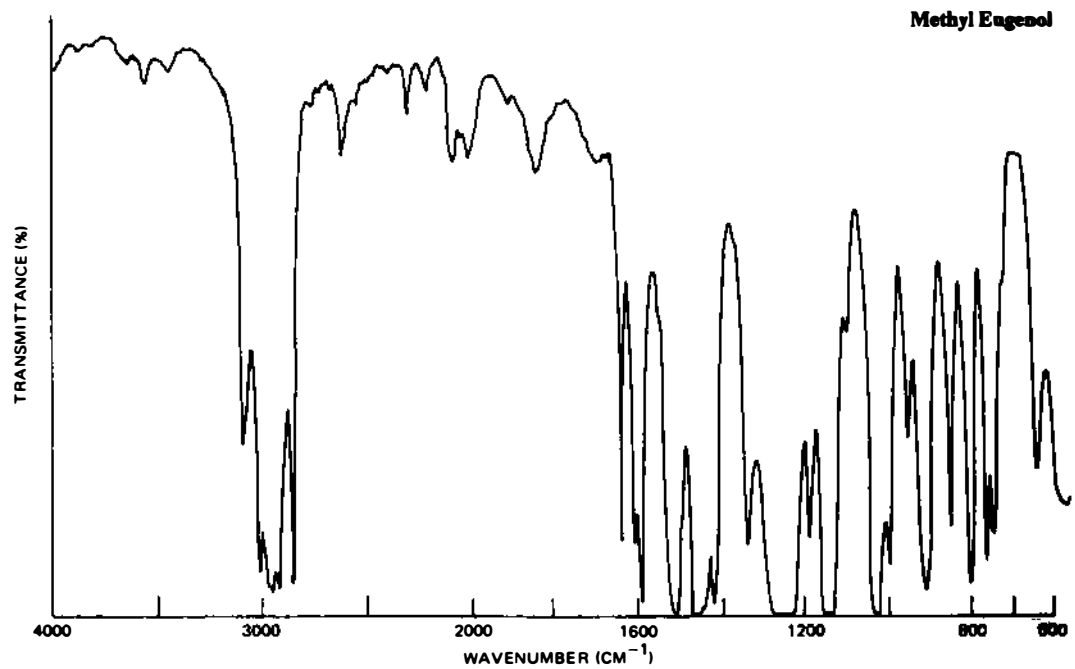
Linalyl Propionate

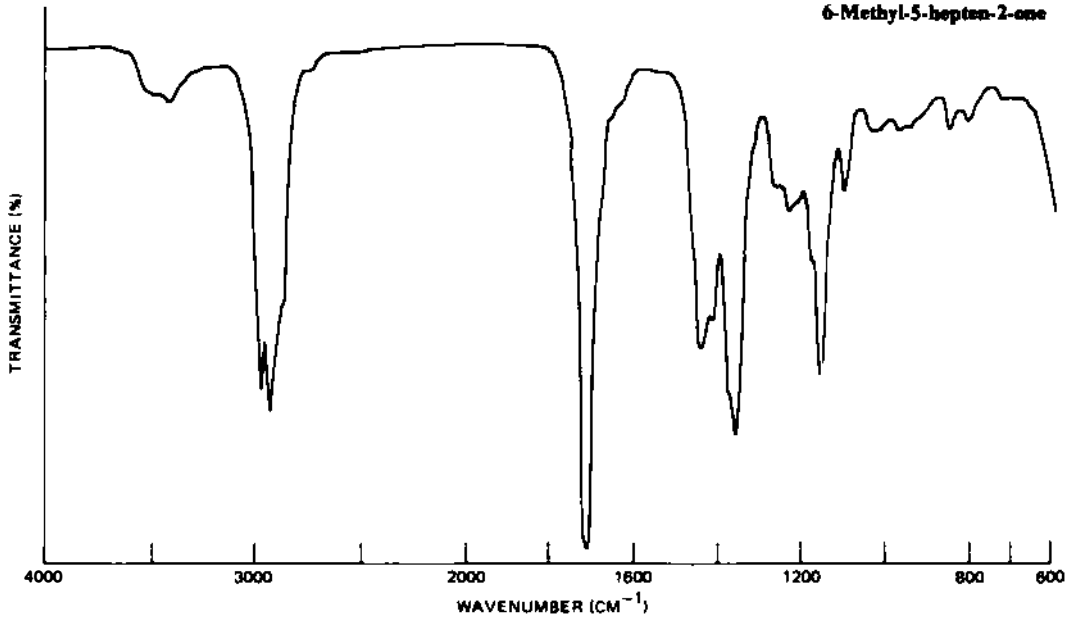


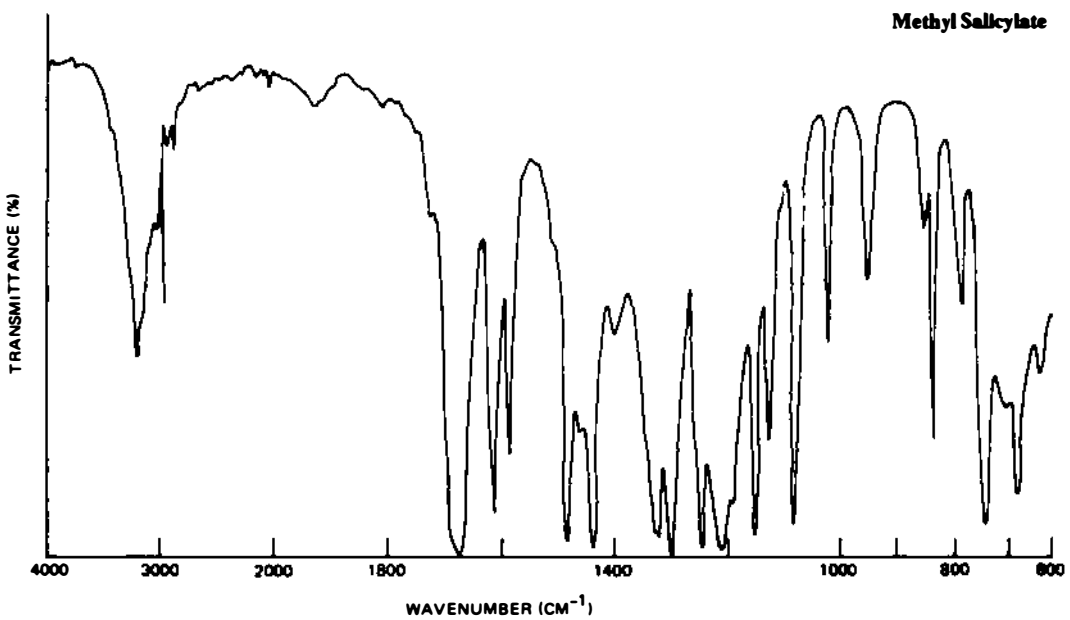
***p*-Methoxybenzaldehyde**

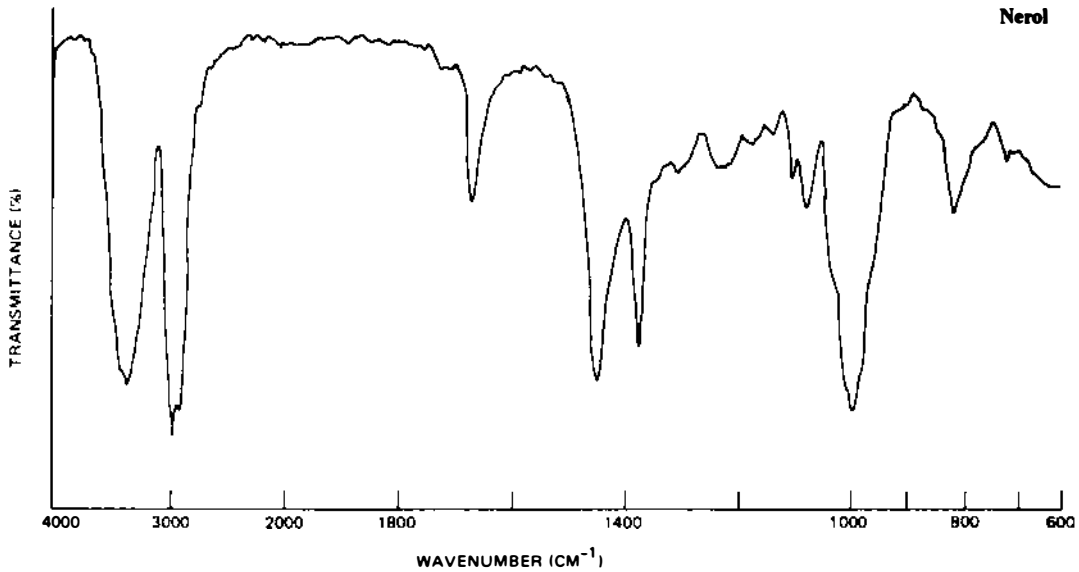


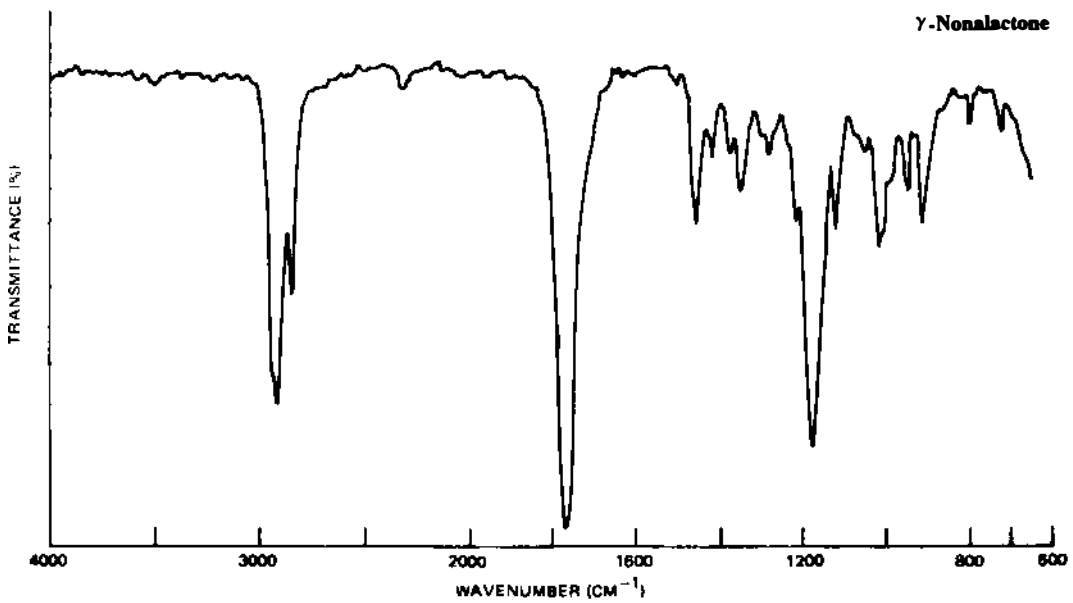




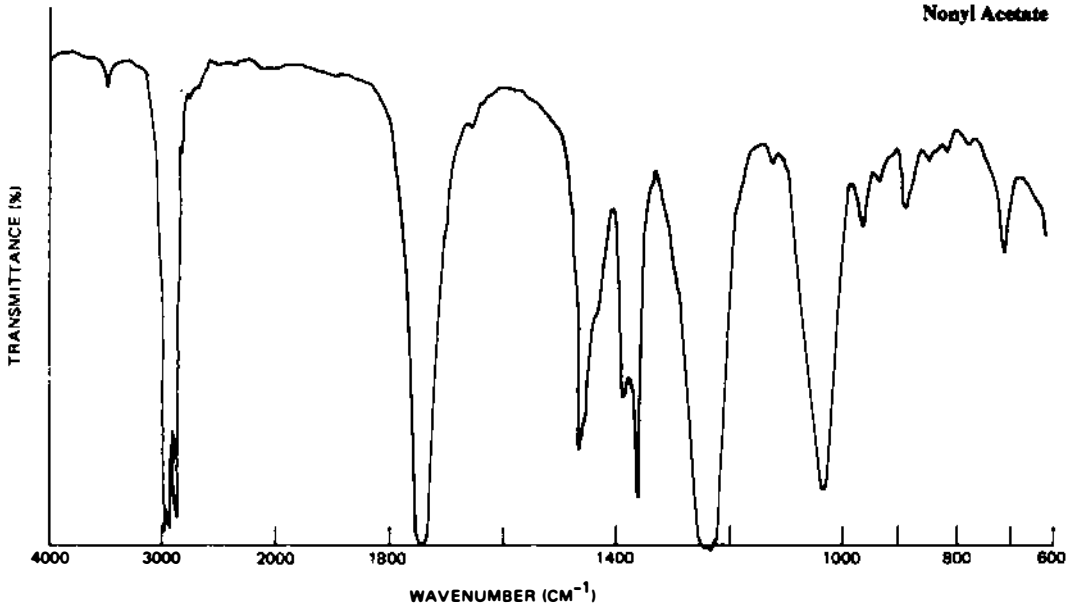




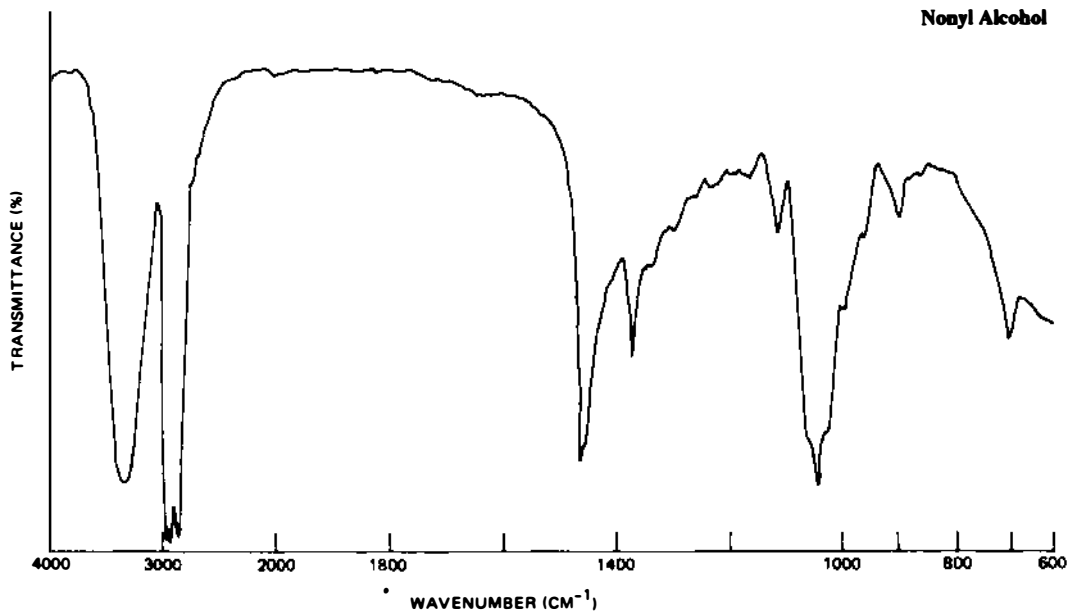


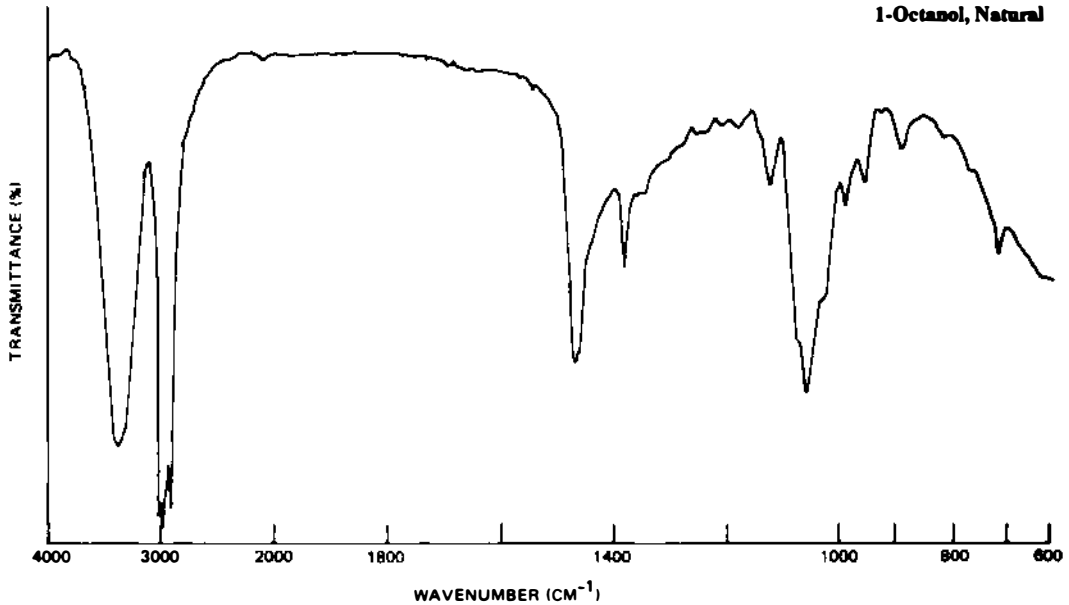


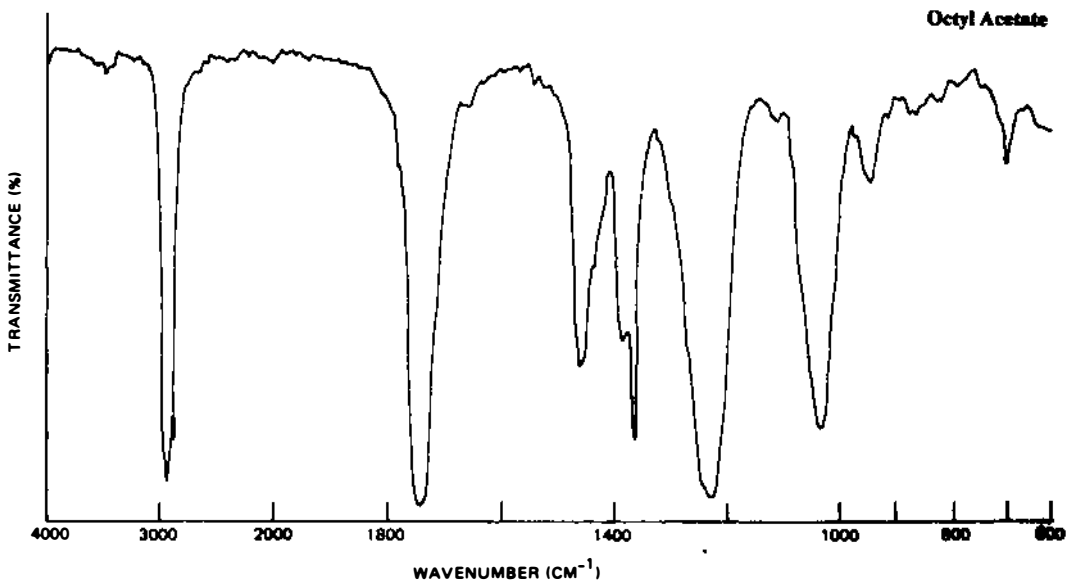
Nonyl Acetate



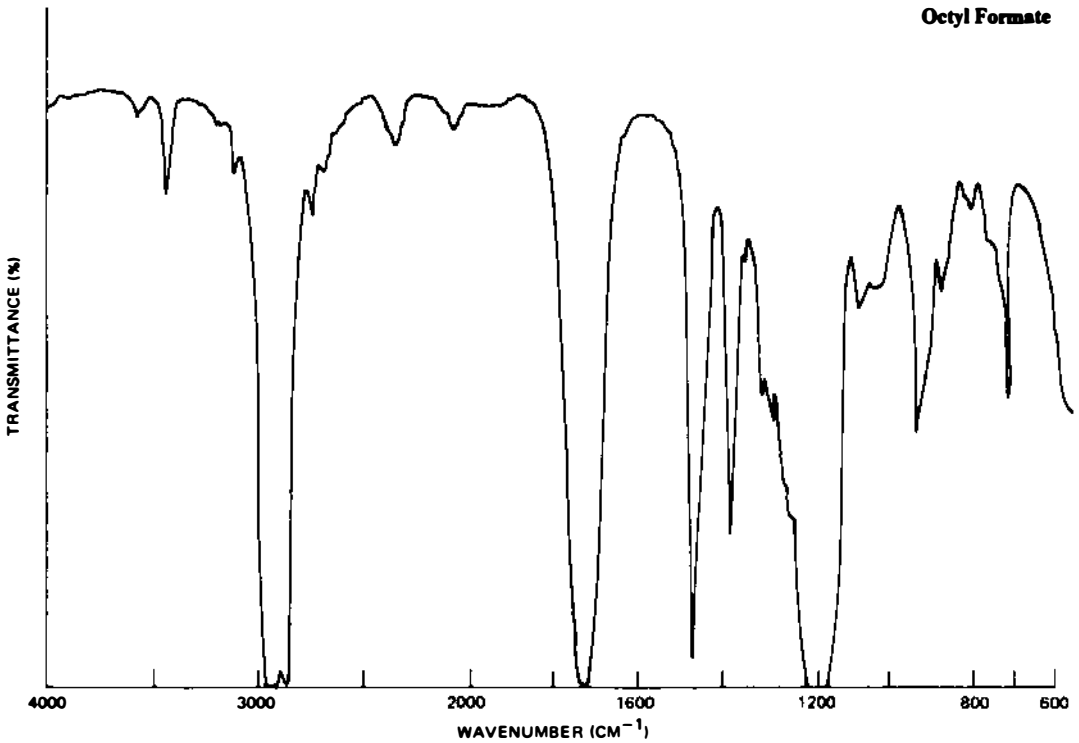
Nonyl Alcohol



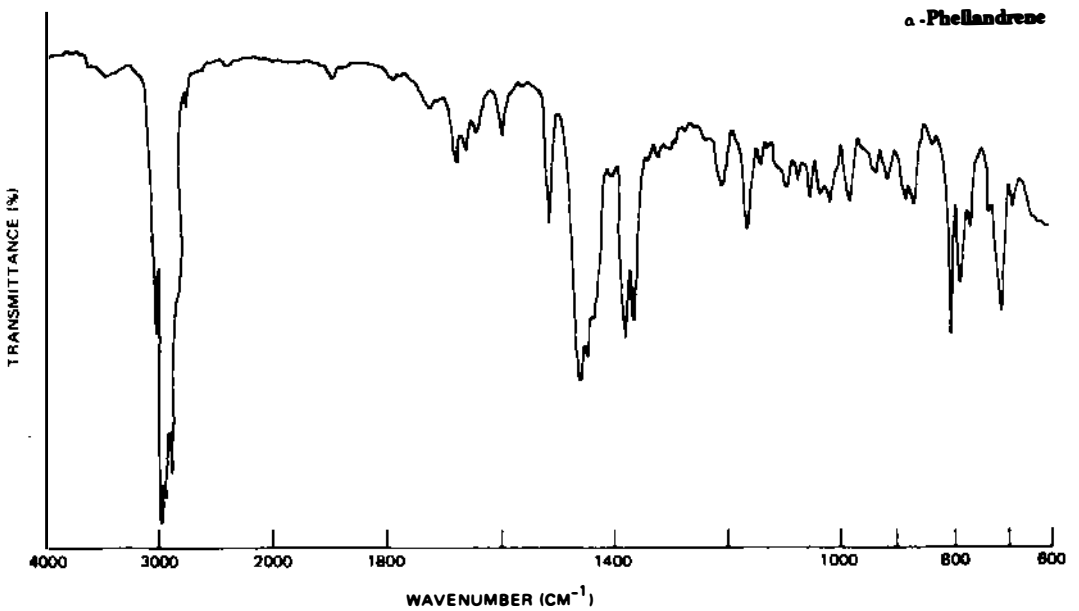


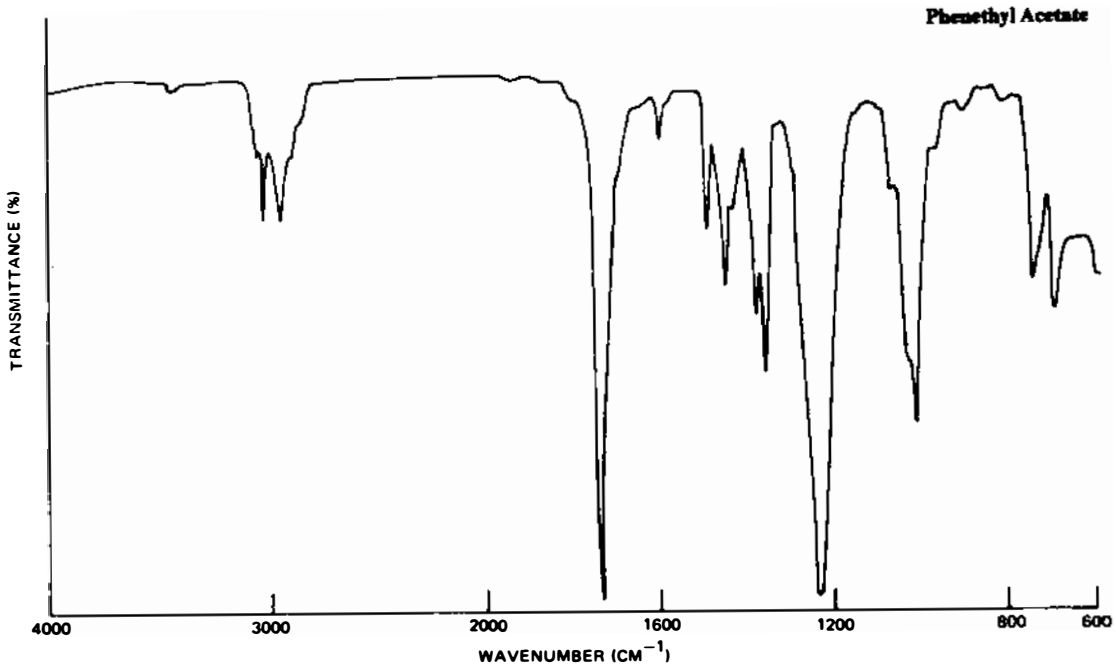


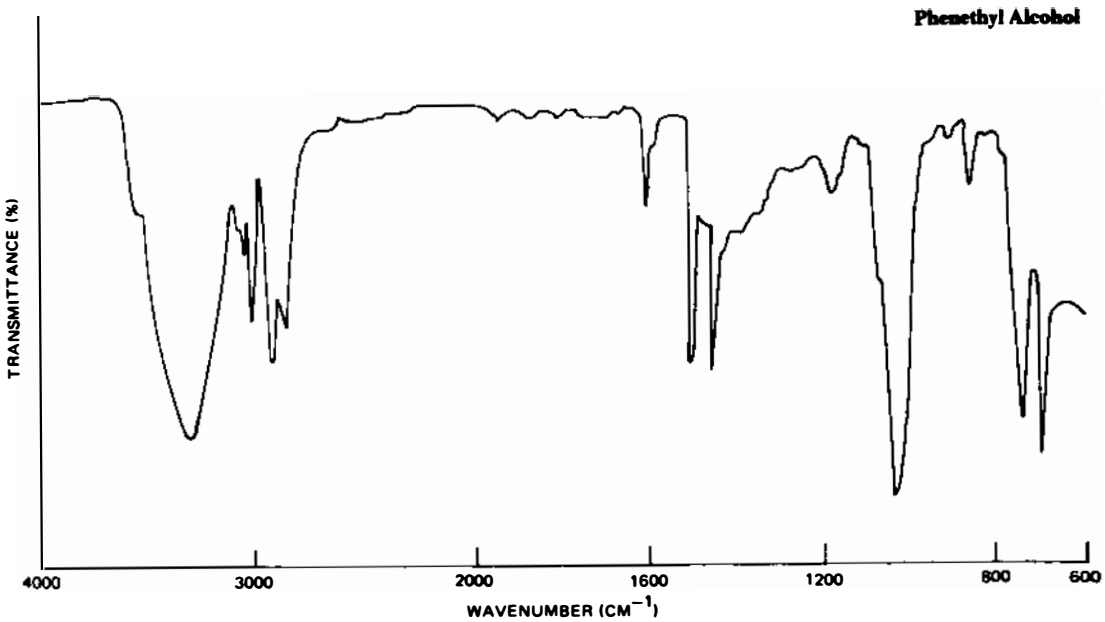
Octyl Formate

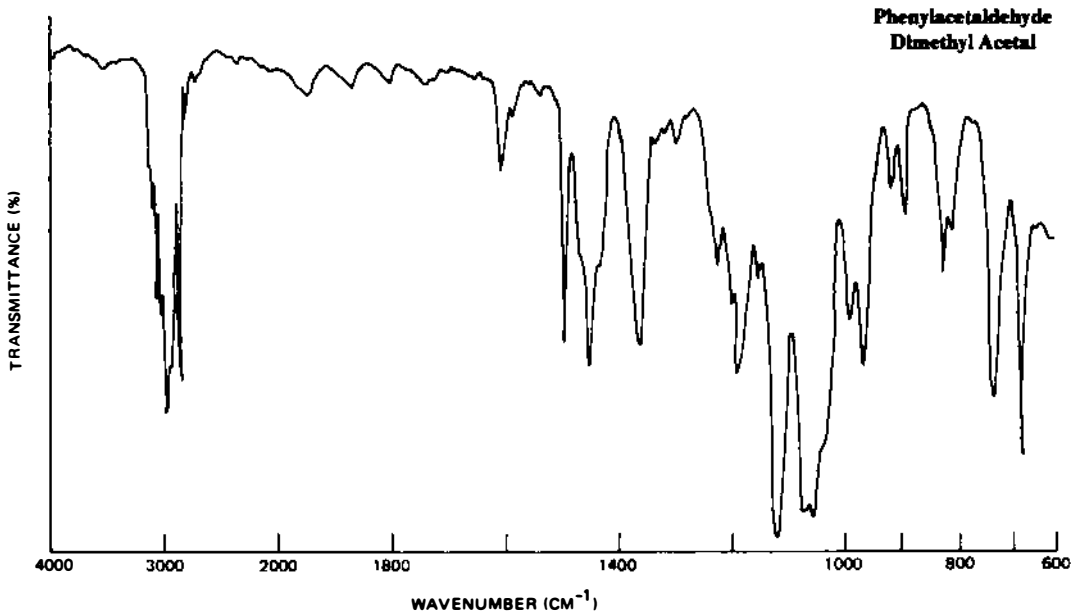


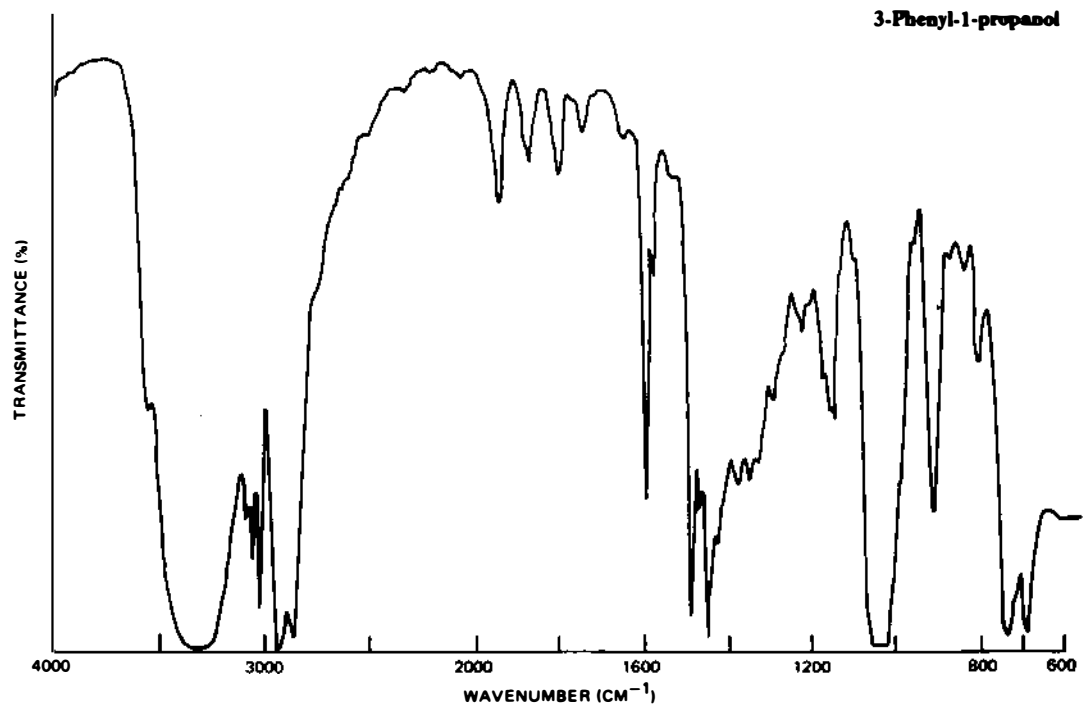
α-Phellandrene

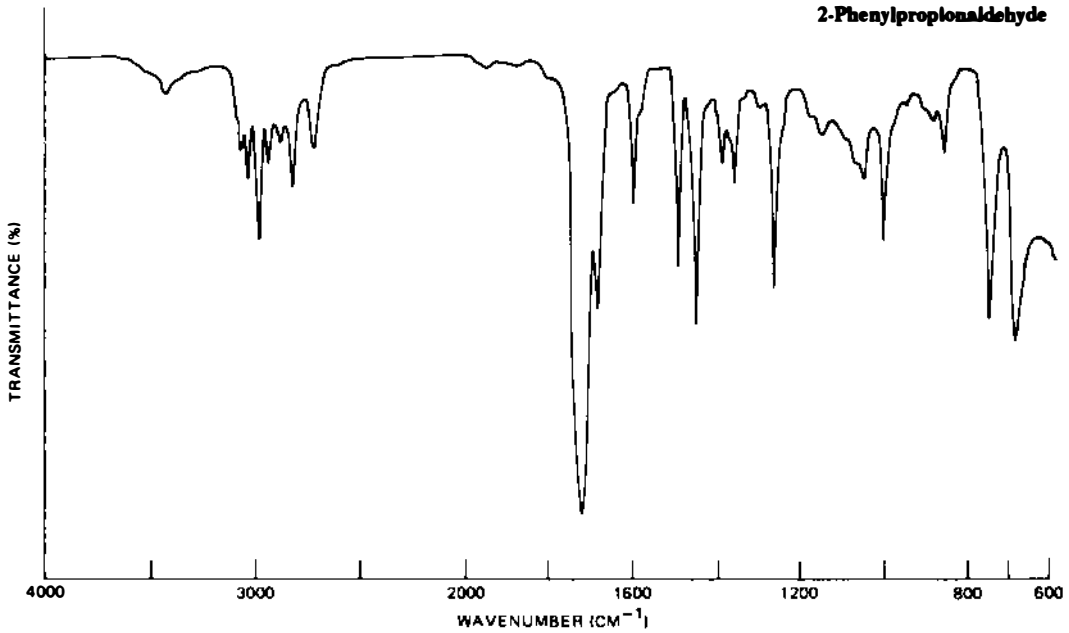


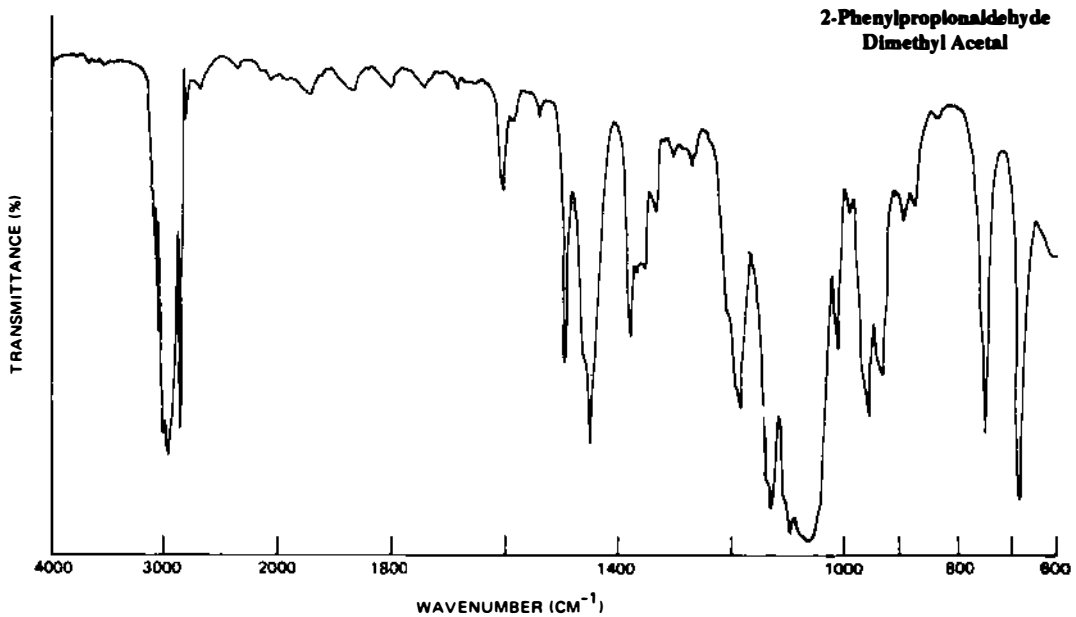


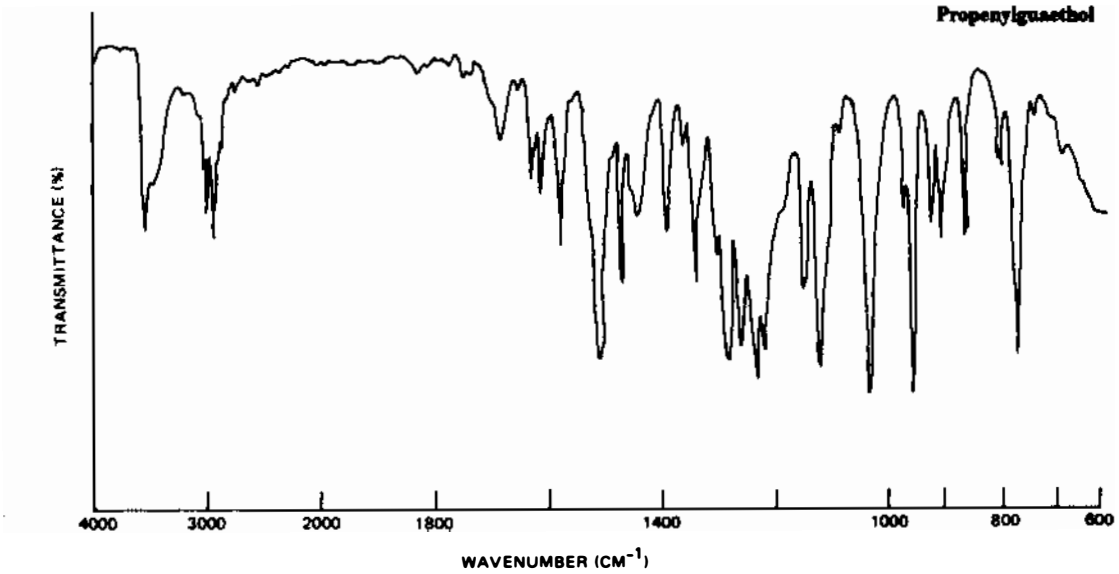


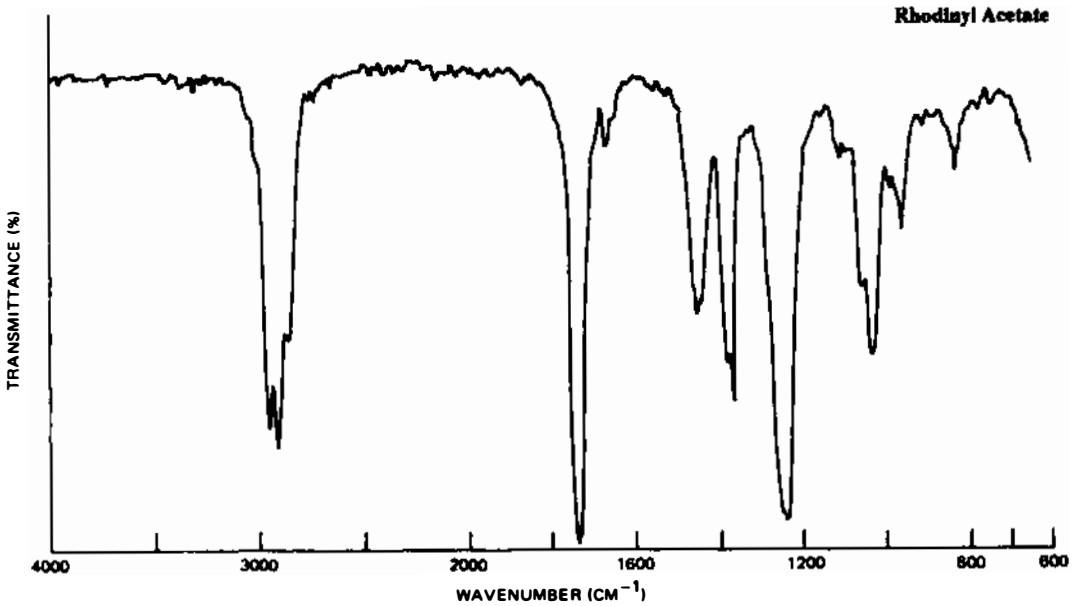












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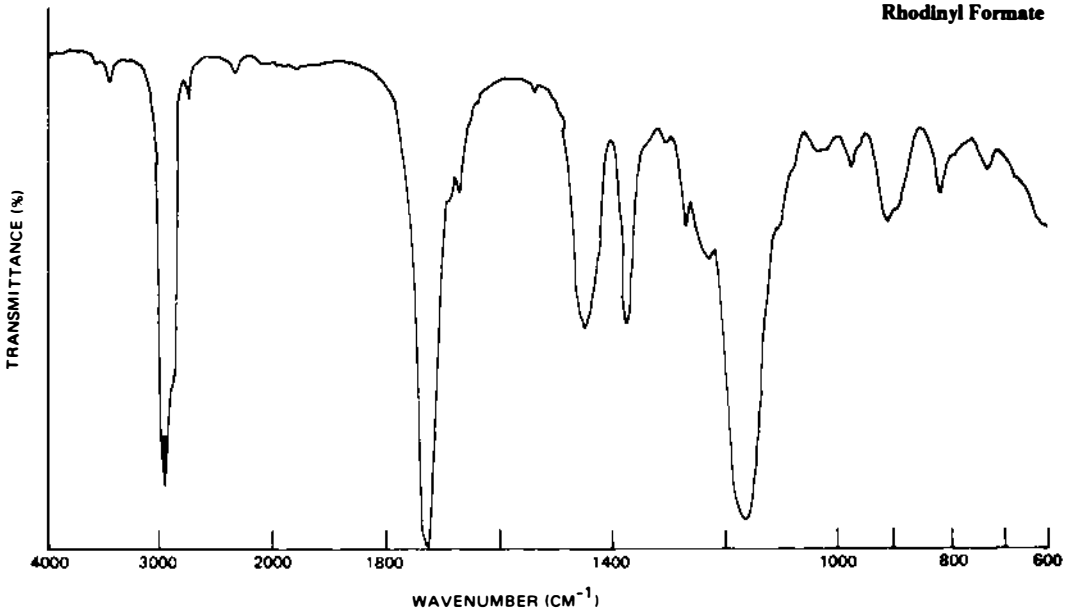
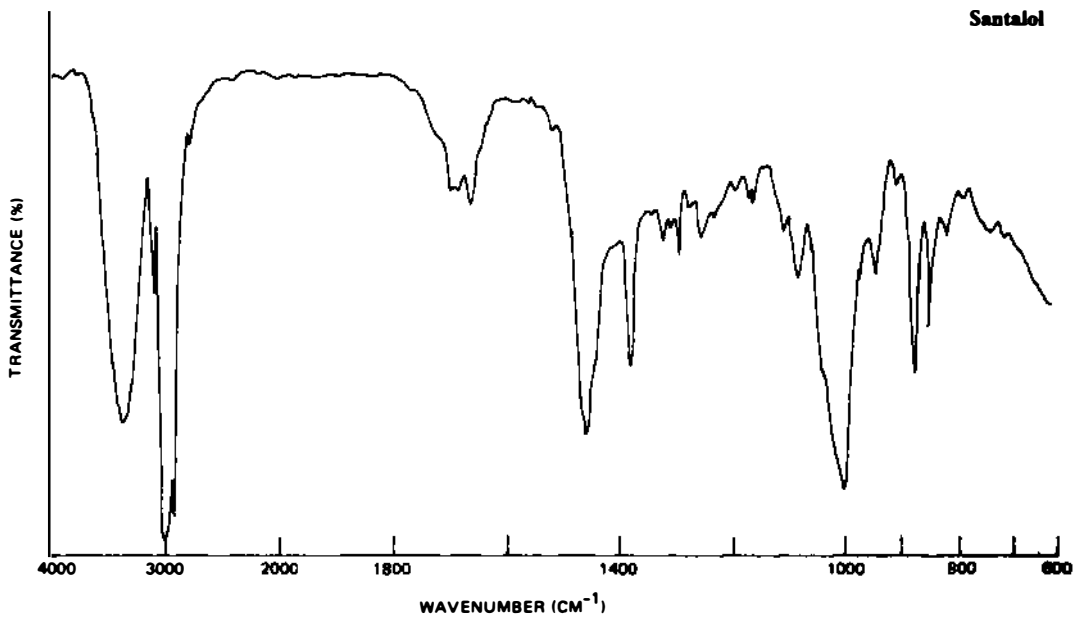
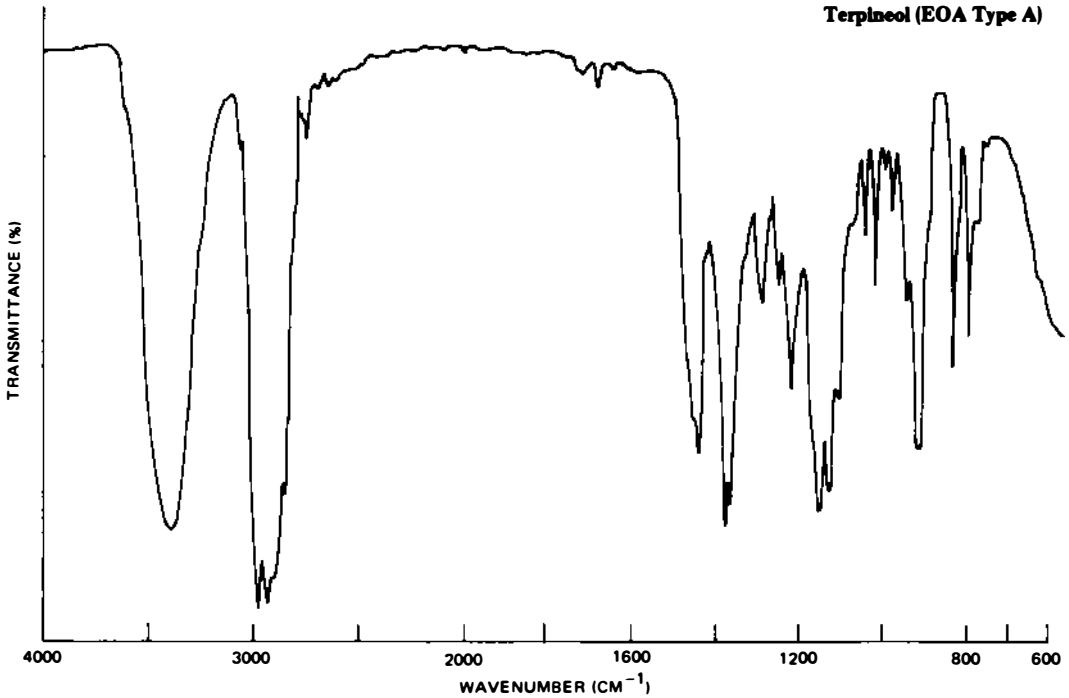


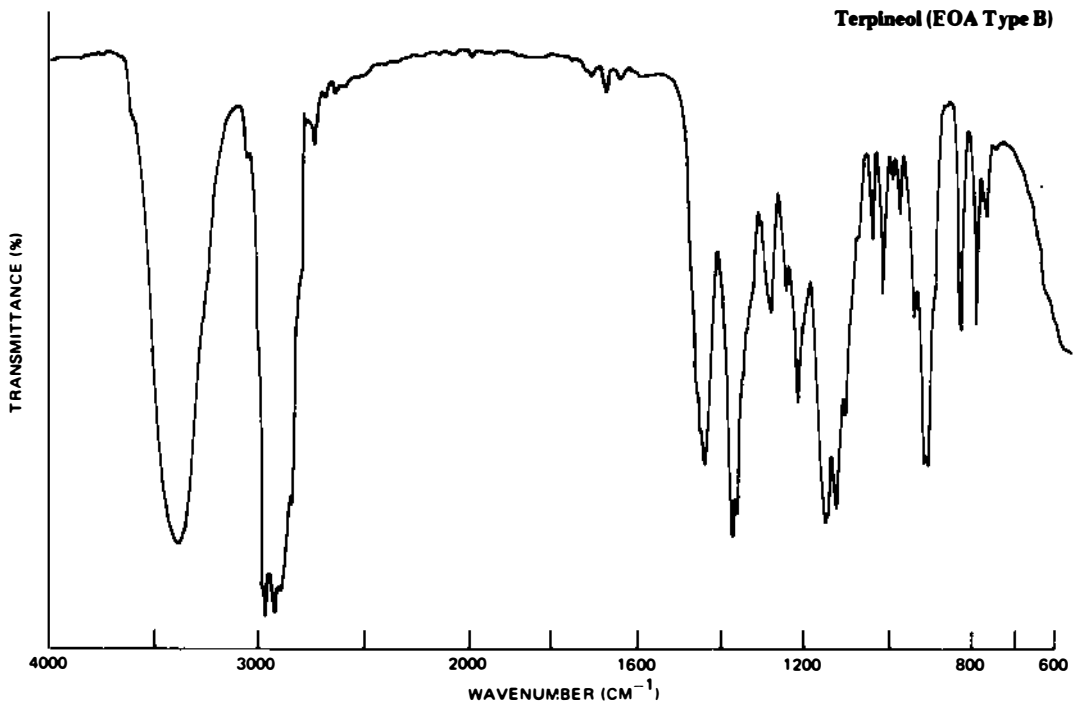
Santalol

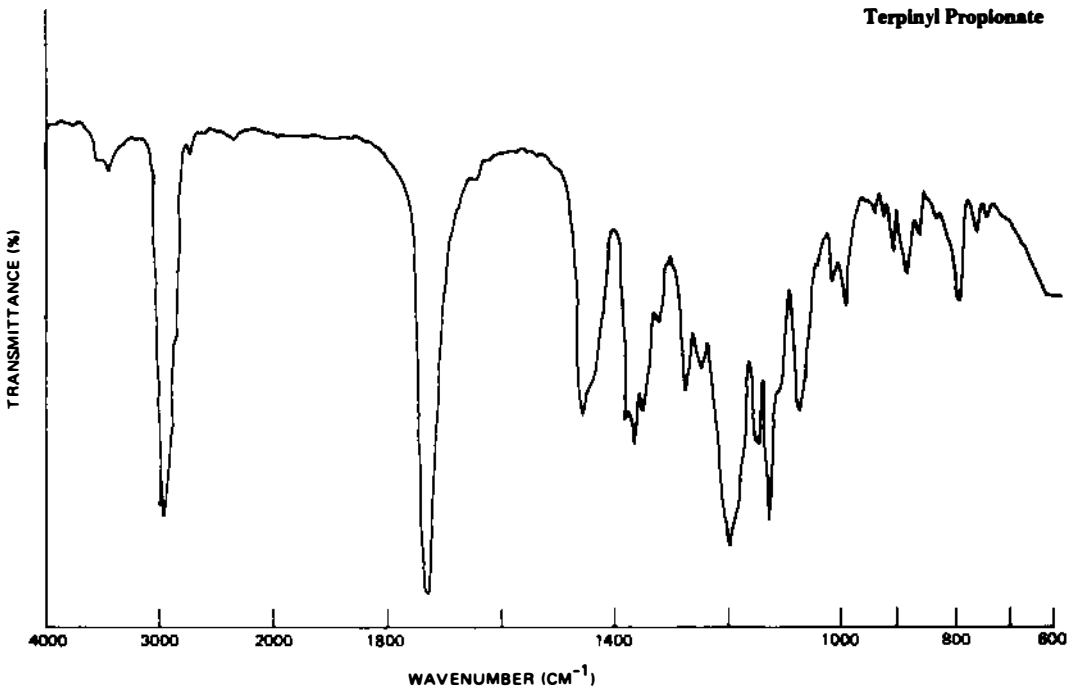


Terpineol (EOA Type A)



Terpineol (EOA Type B)

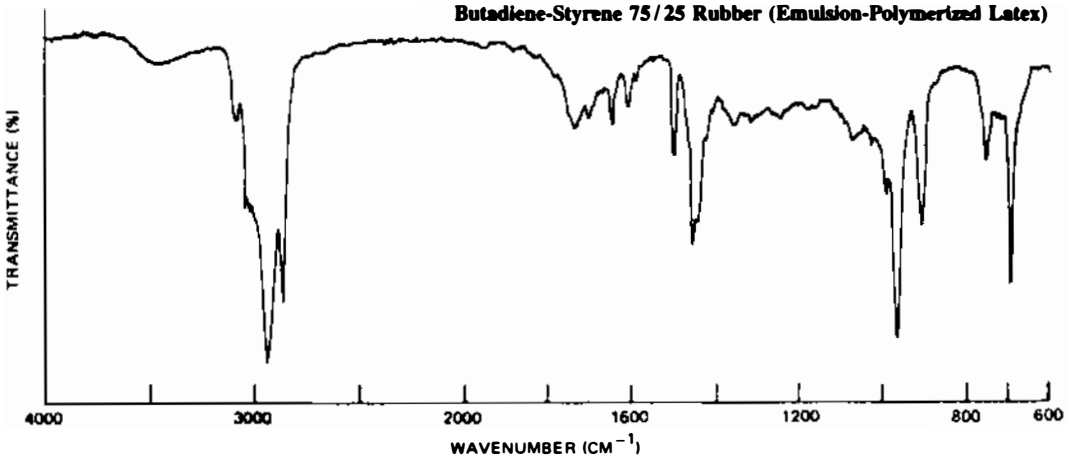




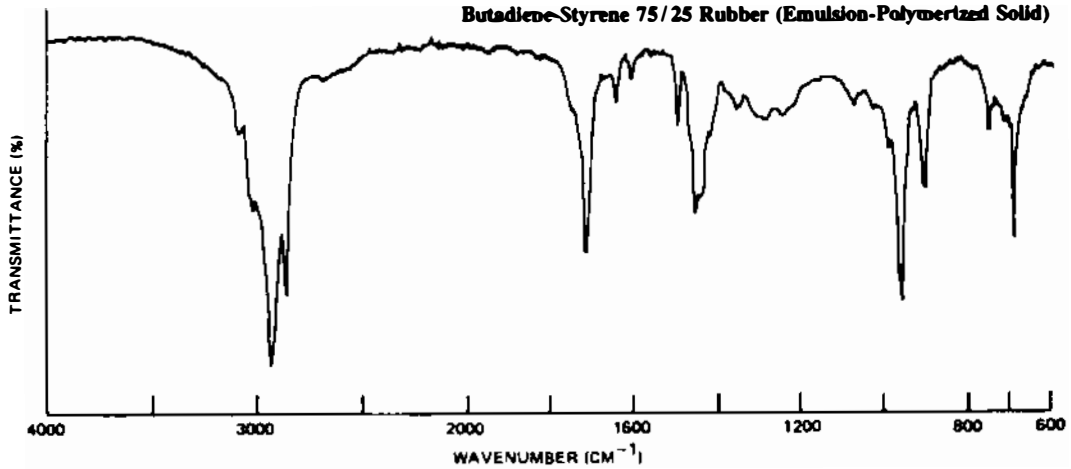
Series C: Other Substances

The spectra in this series are listed below alphabetically.

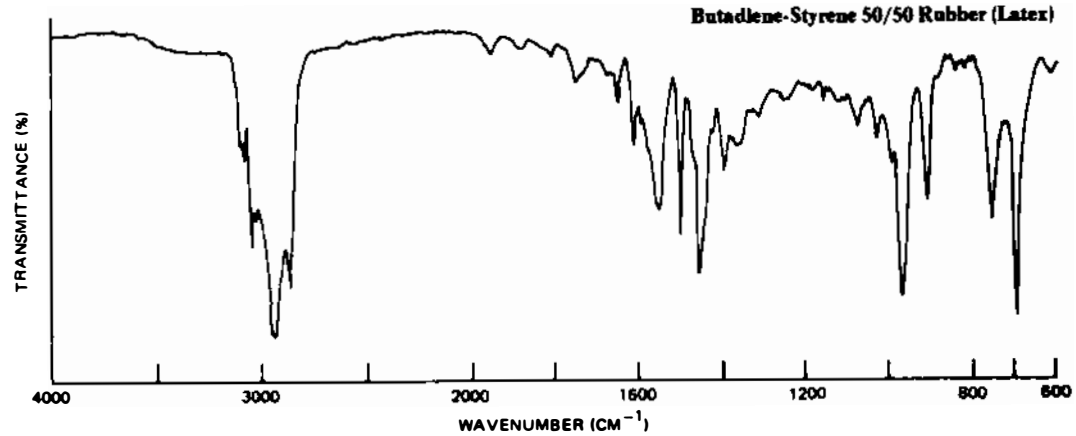
Butadiene-Styrene 75/25 Rubber (Emulsion-Polymerized Latex)



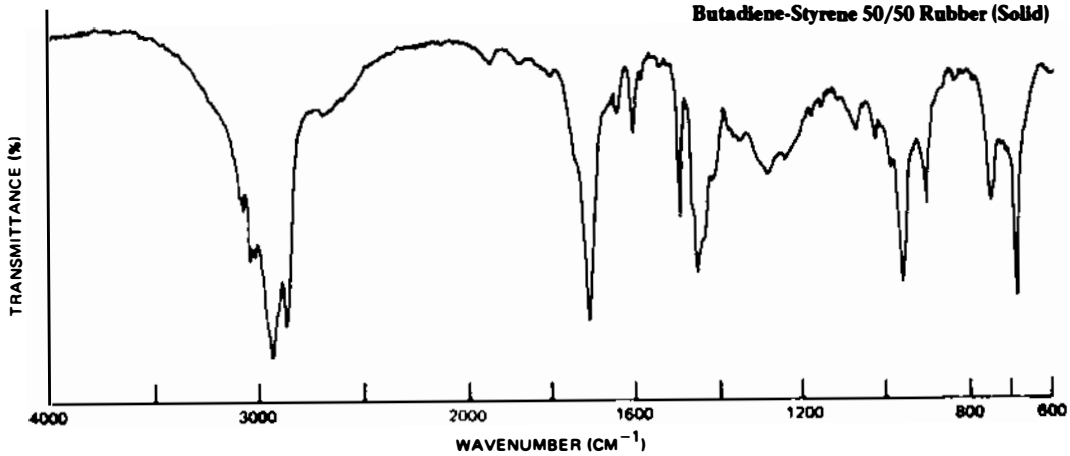
Butadiene-Styrene 75/25 Rubber (Emulsion-Polymerized Solid)



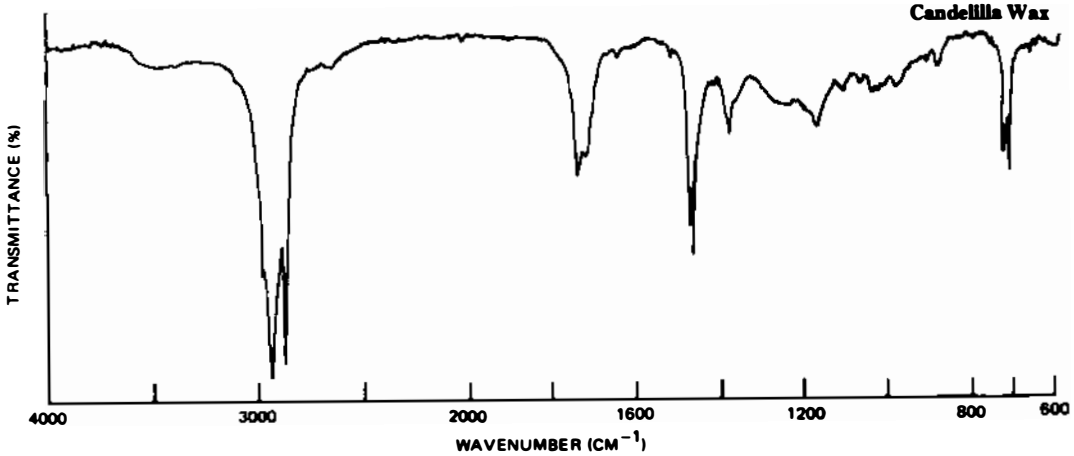
Butadiene-Styrene 50/50 Rubber (Latex)



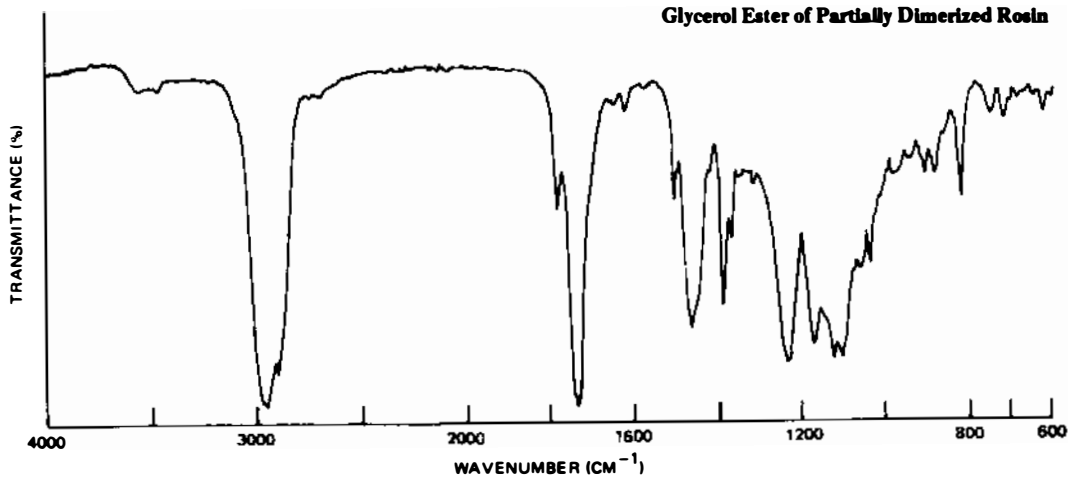
Butadiene-Styrene 50/50 Rubber (Solid)



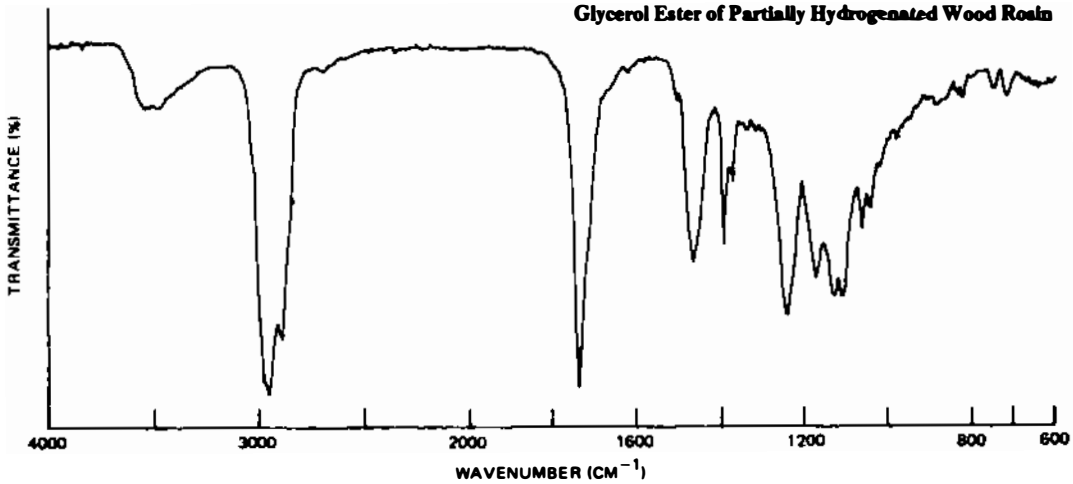
Candelilla Wax



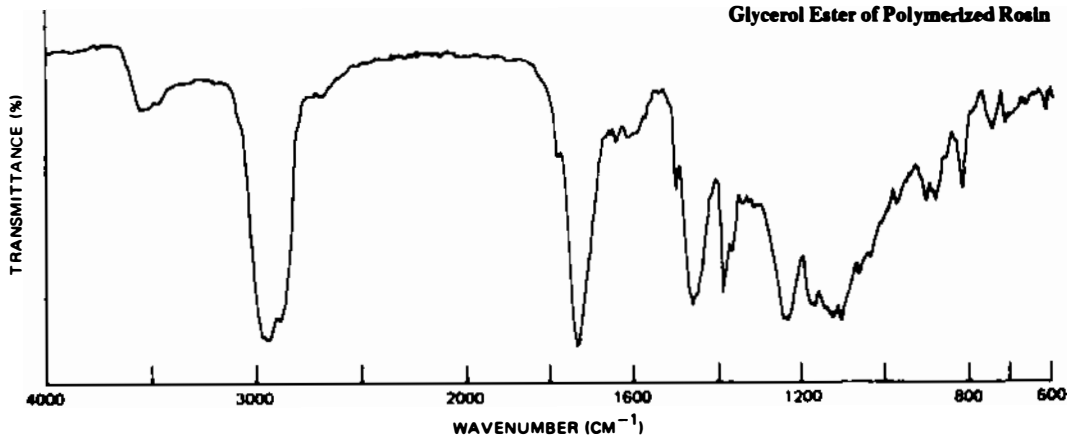
Glycerol Ester of Partially Dimerized Rosin



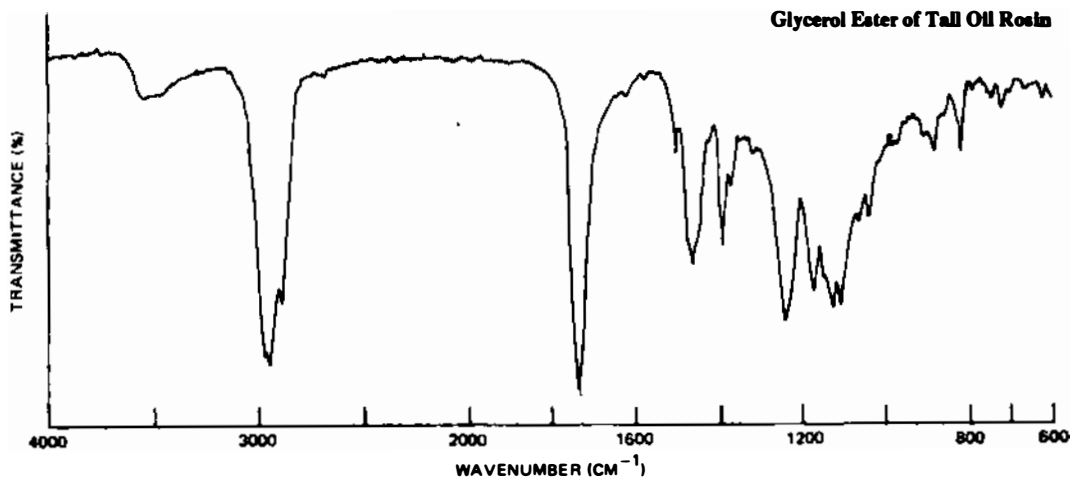
Glycerol Ester of Partially Hydrogenated Wood Rosin



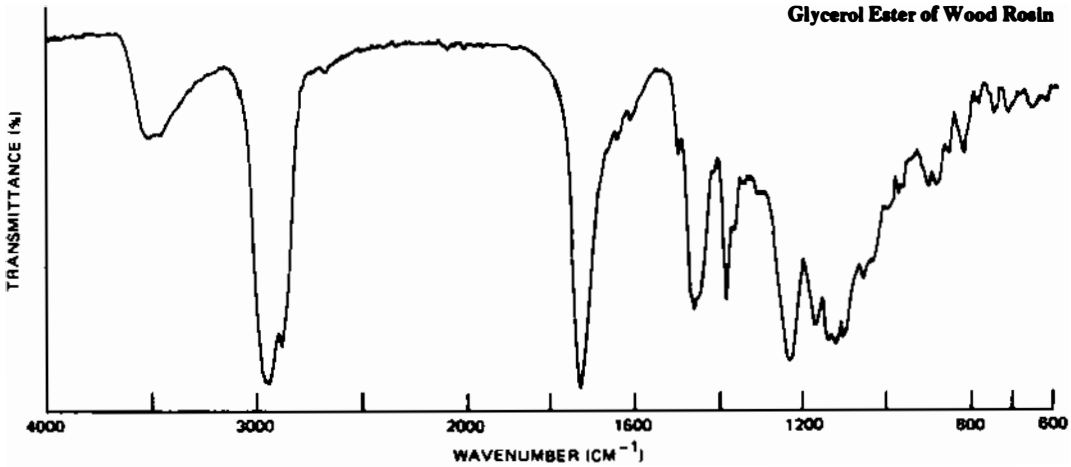
Glycerol Ester of Polymerized Rosin



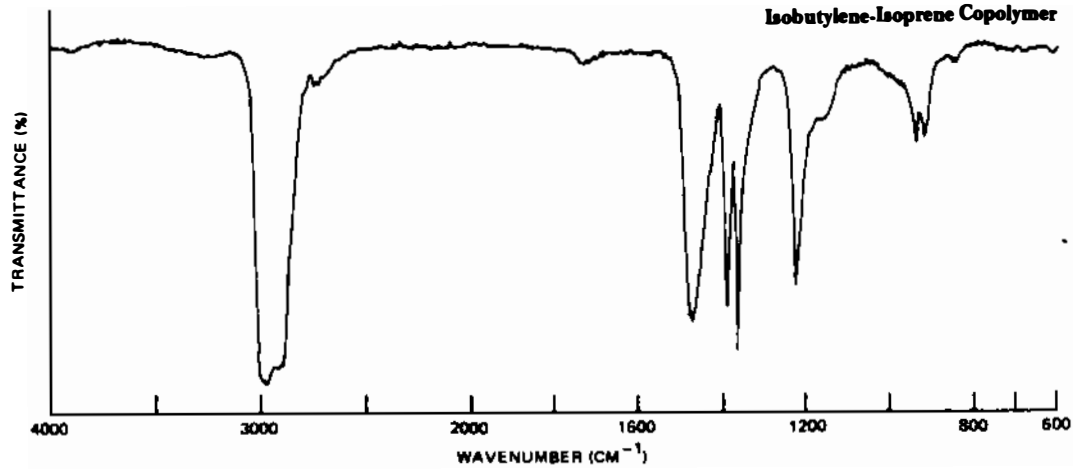
Glycerol Ester of Tall Oil Rosin



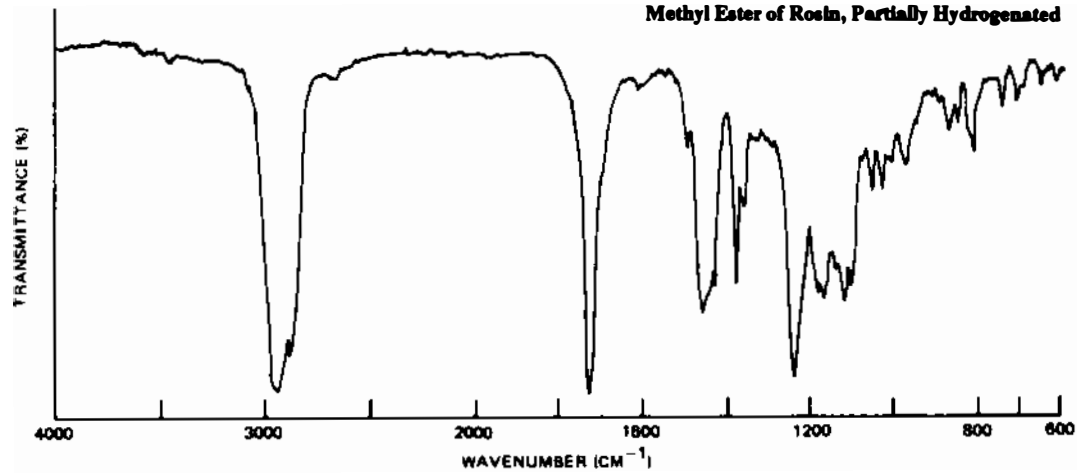
Glycerol Ester of Wood Rosin



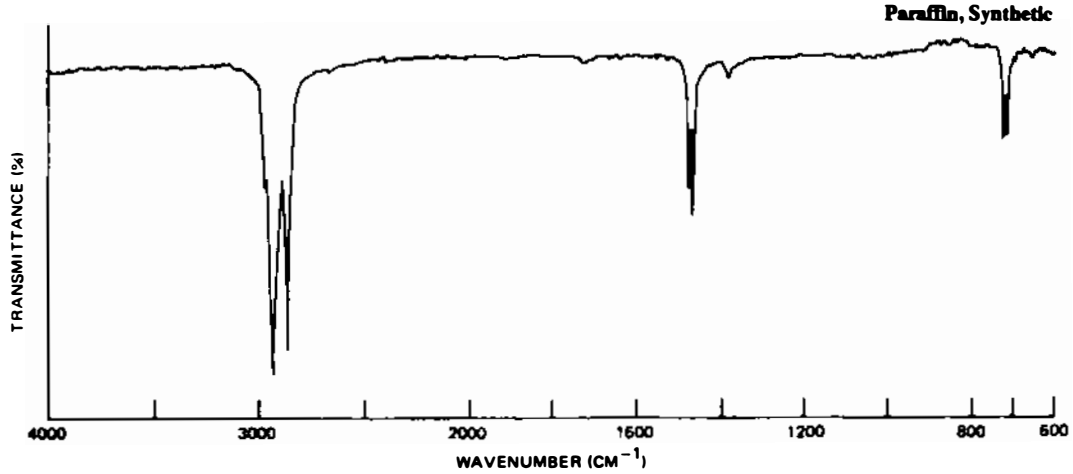
Isobutylene-Isoprene Copolymer



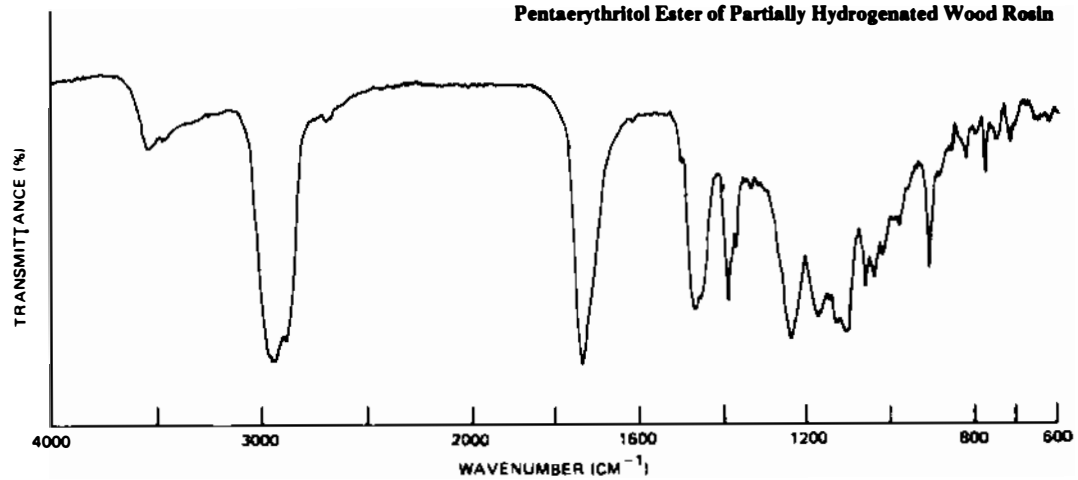
Methyl Ester of Rosin, Partially Hydrogenated



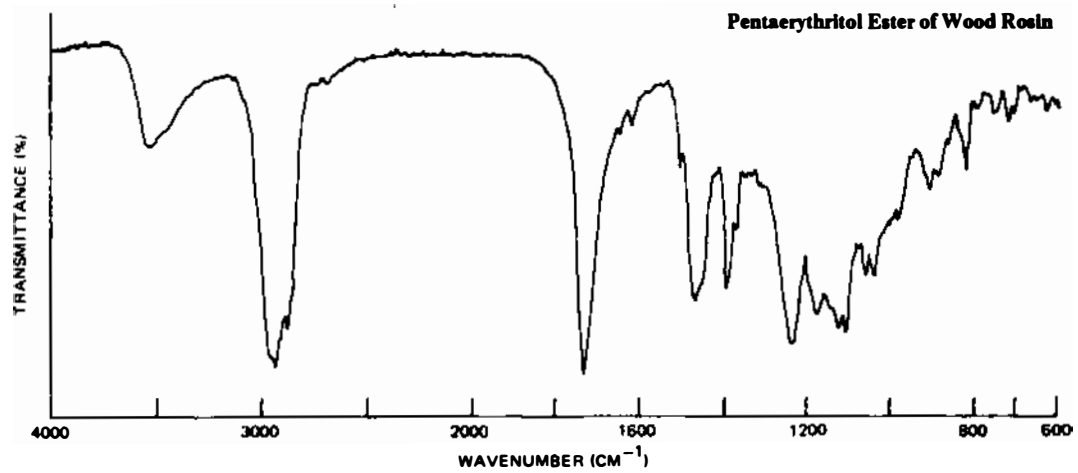
Paraffin, Synthetic



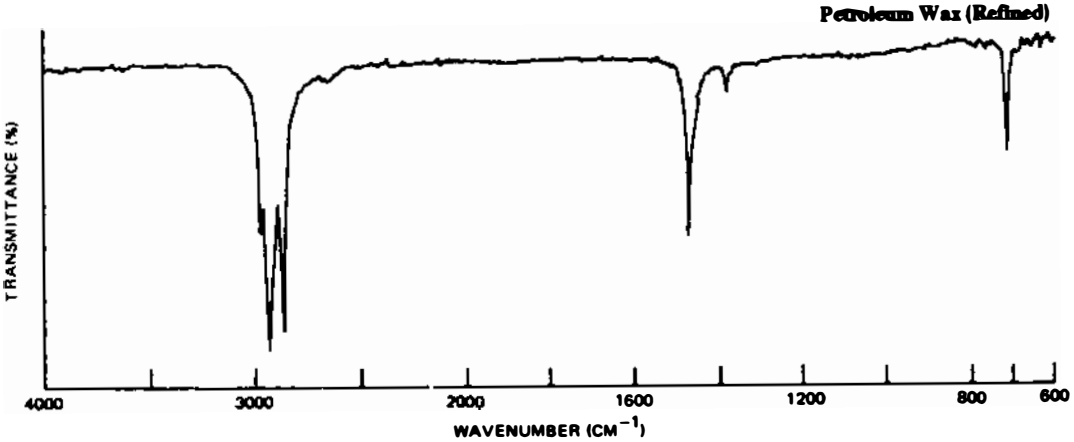
Pentaerythritol Ester of Partially Hydrogenated Wood Rosin



Pentaerythritol Ester of Wood Rosin



Petroleum Wax (Refined)



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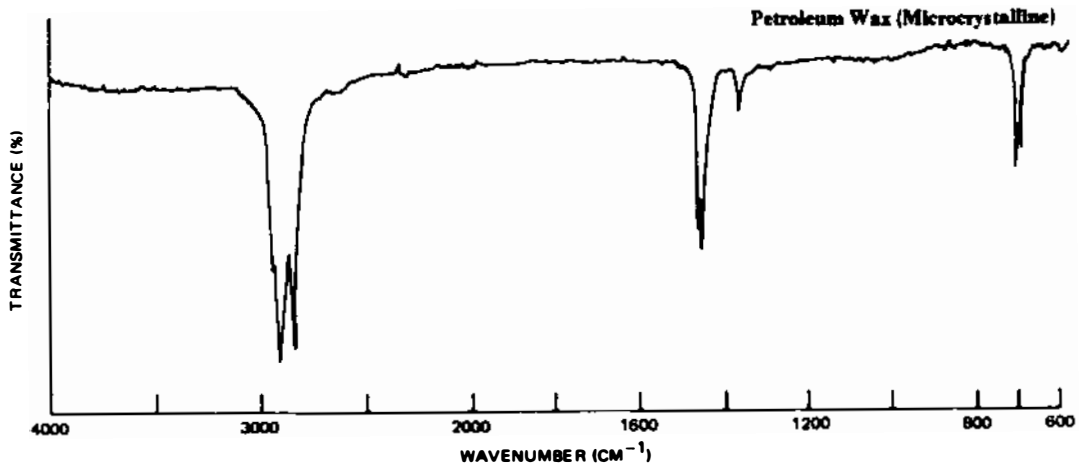
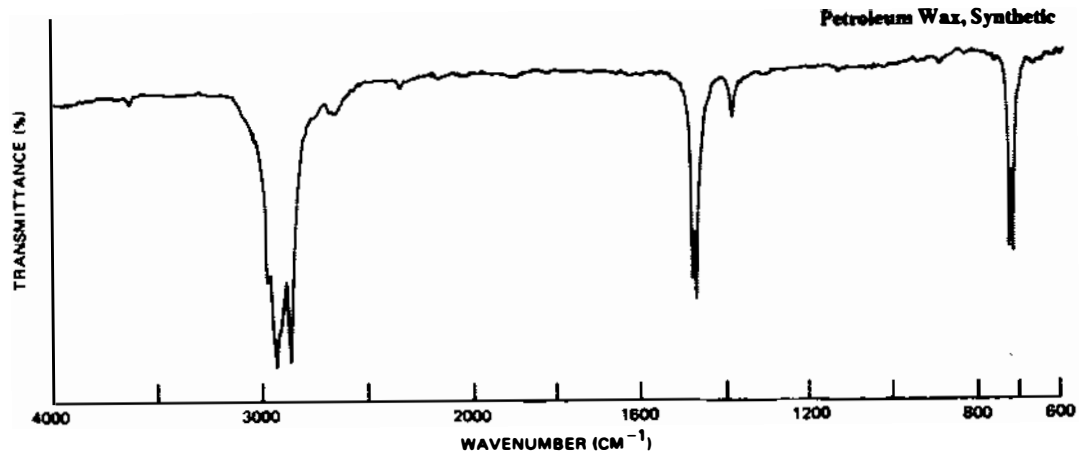
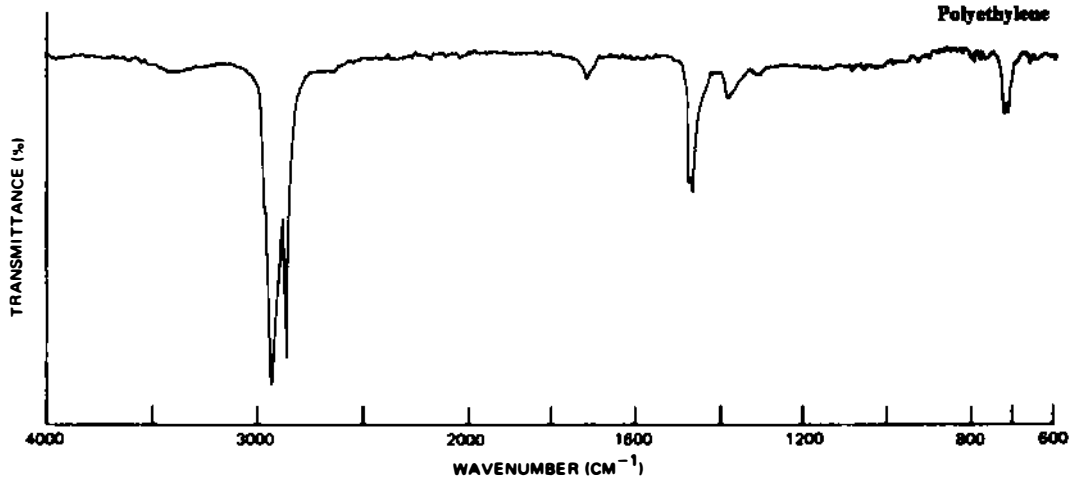
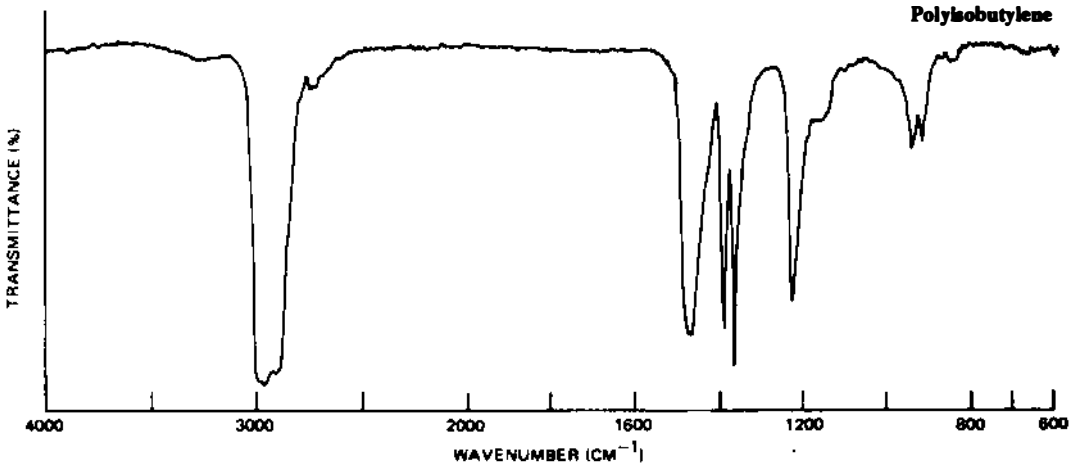


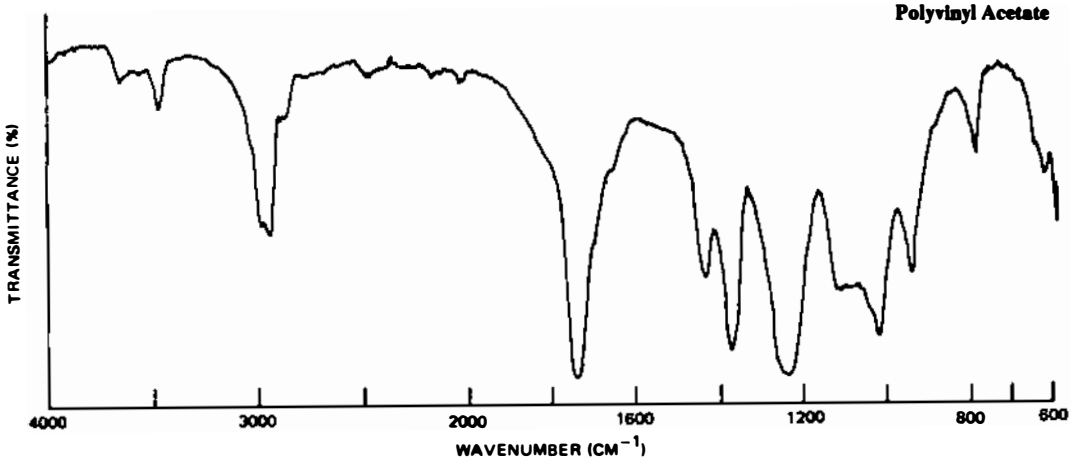
Petroleum Wax, Synthetic



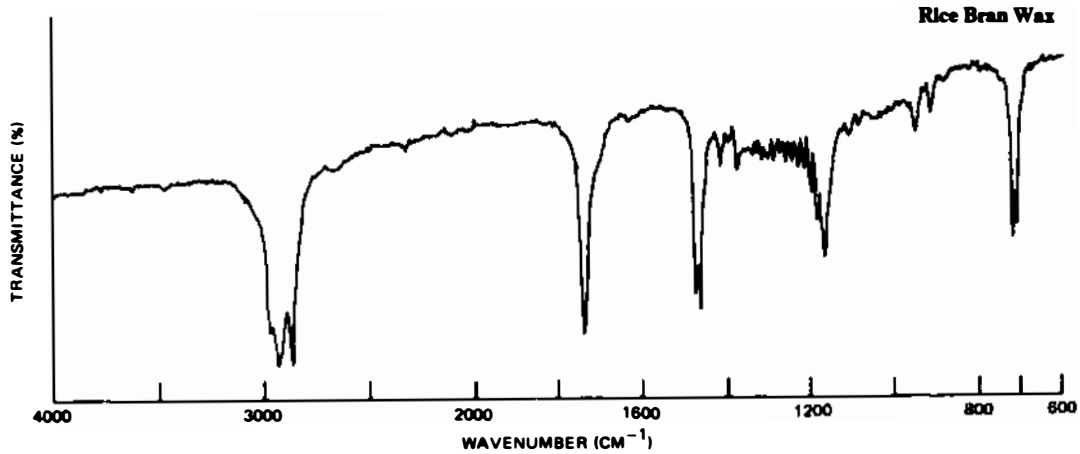




Polyvinyl Acetate



Rice Bran Wax



Index

Titles of monographs in *Section 2*, as well as of flavor aromatic chemicals and isolates in *Section 3*, are shown in boldface type. An asterisk (*) indicates a new monograph for the third edition. A dagger (†) indicates a monograph included in one of the supplements to the second edition but not in the bound volume.