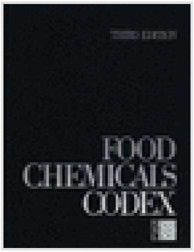

Food Chemicals Codex: Second Supplement to the Third Edition (1986)

Committee on Food Chemicals Codex; Food and Nutrition Board; Commission on Life Sciences; National Research Council

Pages
66

Size
8.5 x 10

ISBN
0309030900

SECOND SUPPLEMENT
TO THE
THIRD EDITION

FOOD
CHEMICALS
CODEX

COMMITTEE ON FOOD CHEMICALS CODEX

Food and Nutrition Board
Commission on Life Sciences
National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1986

NATIONAL ACADEMY PRESS 2101 CONSTITUTION AVENUE, NW WASHINGTON, DC 20418

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Financial support for the work of the Board is primarily provided by government contracts and grants. In addition, uncommitted support is provided by private foundations and industrial organizations.

Through members of its liaison panels, technical input in aspects of nutrition, food safety, food technology, and food processing is provided.

This study is supported by U.S. Food and Drug Administration Contract No. 223-78-2053 (formerly Grant No. FD 00213).

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LIBRARY OF CONGRESS CATALOG CARD NUMBER 81-38403
INTERNATIONAL STANDARD BOOK NUMBER 0-309-03090-0

Printed in the United States of America

First Printing, December 1983
Second Printing, June 1989
Third Printing, June 1990
Fourth Printing, July 1991
Fifth Printing, June 1992
Sixth Printing, November 1992

NATIONAL ACADEMY PRESS

The National Academy Press was created by the National Academy of Sciences to publish the reports issued by the Academy and by the National Academy of Engineering, the Institute of Medicine, and the Nationavesearch Council, all operating under the charter granted to the National Academy of Sciences by the Congress of the United States.

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Additions, Changes, and Corrections

Additions, changes, and corrections listed herein constitute revisions in the *Food Chemicals Codex*, Third Edition (FCC III). Page numbers refer to FCC III unless indicated by a reference to pages in THIS SUPPLEMENT.

NOTE

In order to bring the *Food Chemicals Codex* into concordance with current nomenclature, as used in analytical chemistry, *ppm* (parts per million) will be replaced by *mg/kg* (milligrams per kilogram) beginning with THIS SUPPLEMENT.

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

No Change.

2/ *Monographs*

Insert the following new monograph to precede the monograph entitled *β-Apo-8′-Carotenal*, page 25:

Annatto Extracts

DESCRIPTION

The extract prepared from annatto seed, *Bixa orellana* (L.), using a food-grade extraction solvent. Annatto extracts occur as dark red solutions, emulsions, or suspensions in water or oil or as dark red powders. Bixin is the principal pigment of oil-soluble annatto extracts. Norbixin is the principal pigment of alkaline water-soluble annatto extracts. Commercial preparations are usually mixtures of bixin, norbixin, and other carotenoids.

REQUIREMENTS

Identification

A. Oil-soluble annatto extract diluted with chloroform exhibits absorbance maxima at 439, 470, and 501 nm. Water soluble annatto extract diluted with water exhibits absorbance maxima at 453 and 482 nm.

B. *Carr-Price Reaction* Prepare a small chromatography column by filling a glass tube (e.g., 7 × 200 mm), stoppered with glass wool, with alumina (800-200 mesh) slurried in benzene so that the settled alumina fills about 2/3 of the tube. Adjust the flow rate to about 30 drops/min using a rubber outlet tube and clamp.

Oil-Soluble Annatto Add to the top of the alumina column 3 ml of a solution containing sufficient sample, in benzene, to impart a color equivalent to a 0.1% potassium dichromate solution; elute with benzene until a pale yellow fraction is washed from the column. Wash the column with three 10-ml volumes of dry chloroform; then add 5 ml of Carr-Price reagent and allow it to run onto the top of the column. The orange-red zone (bixin) at the top of the column immediately becomes blue-green.

Water-Soluble Annatto Transfer 2 ml or 2 g to a 50-ml separatory funnel and add sufficient 2 *N* sulfuric acid to make the solution acidic to pH test paper (pH 1-2). Dissolve the red precipitate of norbixin by mixing the solution with 50 ml of benzene. Discard the water layer and wash the benzene phase with water until it no longer gives an acid reaction. Remove any undissolved norbixin by centrifugation or filtration and dry the solution over anhydrous sodium sulfate. Transfer 3-5 ml of the dry solution to the top of an alumina column prepared as described above. Elute the column with benzene, three 10-ml volumes of dry chloroform, followed by 5 ml of Carr-Price reagent added to the top of the column. The orange-red band of norbixin immediately becomes blue-green.

Arsenic (as As) Not more than 3 mg/kg.

Color Intensity Meets the representations of the vendor.

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

Lead Not more than 10 mg/kg.

Residual Solvent *Acetone:* not more than 0.003%; *hexanes:* not more than 0.0025%; *isopropyl alcohol:* not more than 0.005%; *methyl alcohol:* not more than 0.005% in excess of that produced naturally; *methylene chloride:* not more than 0.003%.

TESTS

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

Color Intensity

Oil-Soluble Extracts Transfer an accurately weighed sample to a solution of 1% glacial acetic acid in chloroform and dilute to a suitable volume (absorbance of 0.5-1.0). Filter the sample to clarify if necessary. Measure the absorbance at 470 nm and calculate the color intensity (I) by the formula:

$$I = A/(b \times c),$$

in which A is the absorbance, b is the cell length in cm, and c is the concentration in g per L.

Water-Soluble Extracts Proceed as directed under *Oil-Soluble Extracts*, above, but dissolve the sample in 0.1 *M* sodium hydroxide and measure the absorbance at 453 nm.

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*).

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 Proceed as directed under *Residual Solvent*, page 528.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Color.

BHA, page 37

Replace the *Test* entitled *Assay* with the following:

Assay

Internal Standard Solution Dissolve about 500 mg of 4-*tert*-butylphenol, accurately weighed, in acetone in a 100-ml volumetric flask, add acetone to volume, and mix.

Standard Preparation Dissolve together, accurately weighed quantities of USP reference standards 3-*tert*-butyl-4-hydroxyanisole and 2-*tert*-butyl-4-hydroxyanisole to final concentrations of 9 mg/ml and 1 mg/ml, respectively, in *Internal Standard Solution*.

Assay Preparation Dissolve about 100 mg of butylated hydroxyanisole, accurately weighed, in the *Internal Standard Solution* in a 10-ml volumetric flask, dilute with the *Internal Standard Solution* to volume, and mix.

Chromatographic System The gas chromatograph is equipped with a flame-ionization detector and contains a 1.8-m × 2-mm (id) stainless-steel column packed with 10% silicone GE XE-60; the column is maintained isothermally at a temperature between 175° and 185°, and helium is used as the carrier gas, at a flow rate of 30 ml/min. Chromatograph a sufficient number of injections of the *Standard Preparation*, and record the areas as directed under *Procedure*, to ensure that the relative standard deviation does not exceed 2.0% for the 3-*tert*-butyl-4-hydroxyanisole isomer and 6.0% for the 2-*tert*-butyl-4-hydroxyanisole isomer; the resolution between the isomers is not less than 1.3, and the tailing factor does not exceed 2.0. (See *Chromatography*, p. 27, *First Supplement* to FCC III.)

Procedure Separately inject suitable portions (about 5 µl) of the *Standard Preparation* and the *Assay Preparation* into the gas chromatograph, and record the chromatograms. Measure the areas under the peaks for each isomer and the internal standard in each chromatogram, and calculate the quantity, (I), in mg, of each isomer in the butylated hydroxyanisole by the formula:

$$I = 10\, C_S (R_U/R_S),$$

in which C_S is the concentration, in mg/ml, of the isomer in the *Standard Preparation*, R_S is the ratio of the area of the isomer to that of the internal standard in the chromatogram from the *Standard Preparation*, and R_U is the ratio of the area of the isomer to that of the internal standard in the chromatogram from the *Assay Preparation*. Calculate the weight, in mg, of $C_{11}H_{16}O_2$ in the butylated hydroxyanisole by adding the quantities of the two isomers.

Brominated Vegetable Oil, page 40

Change the *Description* to read:

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 chloroform, in ether, in hexane, and in fixed oils. Brominated vegetable oil may contain a suitable stabilizer.

Calcium Carbonate, page 46

Delete the synonym, *Precipitated Calcium Carbonate*.

Calcium Chloride, Anhydrous, page 48

Insert the following under *Requirements*:

Acid-Insoluble Matter Not more than 0.02%; no particles per kg of sample greater than 2 mm in any dimension.

Insert the following under *Tests*:

Acid-Insoluble Matter Proceed as directed under *Acid-Insoluble Matter*, page 70 of THIS SUPPLEMENT.

Calcium Sulfate, page 66

Change the *Requirement* entitled *Assay* to read:

Assay Not less than 98.0% $CaSO_4$, calculated on the dried basis.

Carrageenan, page 74

Insert the following *Requirements* for *Acid-Insoluble Matter* and *Solubility in Water:*

Acid-Insoluble Matter Not more than 2.0%.
Solubility in Water Not more than 30 ml of water is required to completely dissolve 1 g at a temperature of 80°.

Insert the following *Test* for *Acid-Insoluble Matter* and *Solubility in Water:*

Acid-Insoluble Matter Transfer about 2 g, accurately weighed, to a 250-ml beaker containing 150 ml of water and 1.5 ml of sulfuric acid. Cover with a watch glass, and heat 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. Transfer about 500 mg of a suitable filter aid, accurately weighed, to the beaker, and filter through a tared filtering crucible containing a 2.4-cm glass fiber filter. Wash the residue several times with hot water, dry at 105° for 3 h, cool in a desiccator, and weigh. The difference between the total weight and the sum of the weights of the filter aid, crucible, and glass fiber filter is the weight of the acid-insoluble matter.
Solubility in Water Dissolve 1 g of carrageenan in 30 ml water at a temperature of 80°. The resulting solution when maintained at 80° is uniformly viscous, clear, or slightly opalescent.

Insert the following new monograph to precede the monograph entitled *Cassia Oil*, page 77:

Casein and Caseinate Salts

DESCRIPTION

Casein is an off-white to cream-colored granular or fine powder derived from the coagulum formed by treating skim milk with a food-grade acid (acid casein), enzyme (rennet casein), or other food-grade precipitating agent. After the precipitation, casein is separated from the soluble milk fraction, washed, and dried. Chemically, casein is a mixture of at least 20 electrophoretically distinct phosphoproteins. The main fractions—designated α-casein, β-casein, and κ-casein—are known to be mixtures, rather than single proteins. Casein contains all the amino acids known to be essential for human nutrition. It is insoluble in water and alcohol, but can be dissolved by aqueous alkalies to form caseinate salts. Caseinate salts are white to cream-colored granules or powders soluble or dispersible in water. They are prepared by treatment of casein with food-grade alkalies, neutralizing agents, enzymes, buffers, or sequestrants. Common counter ions are: NH_4^+, Ca^{++}, Mg^{++}, K^+, and Na^+.

REQUIREMENTS

Assay Not less than 90% protein for acid casein; not less than 86% protein for rennet casein; not less than 84% for caseinate salts; calculated on the dried basis.
Acid Value (Casein only) Not more than 1.8.
Aerobic Plate Count Not more than 100,000/g.
Arsenic Not more than 3 mg/kg.
Coliforms Not more than 2/0.1 g.
Fat Not more than 2.25%.
Heavy Metals (as Pb) Not more than 0.004%.
Lactose Not more than 2.0%.
Lead Not more than 5 mg/kg.
Loss on Drying Not more than 12%.
Salmonella sp. Negative.

TESTS

Assay Proceed as directed under *Nitrogen Determination*, page 521. Calculate the percent protein (*P*) by the formula:

$$P = N \times 6.38,$$

in which *N* is the percent nitrogen.
Acid Value Proceed as directed under *Method I*, page 503.
Aerobic Plate Count Proceed as directed in Chapter IV, *Bacteriological Analytical Manual*, Fifth Edition, Food and Drug Administration, 1978.
Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, page 464.
Coliforms Proceed as directed in section 46.016, *Official Methods of Analysis of the AOAC*, Thirteenth Edition, 1980, page 825.
Fat Transfer to a fat-extraction flask 1 g, accurately weighed; add 10 ml of water, and shake until homogeneous (warm if necessary). Add approximately 1 ml of ammonium hydroxide and heat in a water bath for 15 min at 60°-70°, shaking occasionally. Add 10 ml of alcohol and mix well. Add 25 ml of peroxide-free ether, stopper, and shake vigorously for 1 min; allow to cool if necessary; add 25 ml of petroleum ether and repeat vigorous shaking. Allow the layers to separate and clarify or centrifuge at 600 rpm to expedite the process. Decant the organic layer into a suitable flask or dish and repeat the extraction twice with 15 ml each of ether and petroleum ether for each extraction. Evaporate the combined ether extractions on a steam bath and dry the residue to a constant weight at 102°, or 70°-75° at less than 50 mm Hg. Calculate the percent fat (*F*) by the formula:

$$F = (R \times 100)/S,$$

in which, *R* is the weight of the residue and *S* is the weight of the sample, each in g.

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)*

Lactose

Apparatus Use a suitable absorption spectrophotometer capable of operating in the visible range.

Phenol Reagent Heat a mixture of 8 g of phenol and 2 g of water until the crystals dissolve.

Lactose Solution Transfer approximately 2 g of lactose monohydrate, accurately weighed, to a 100-ml volumetric flask; dissolve in and dilute to volume with water.

Sample Solution Transfer approximately 1 g of sample, accurately weighed, to a 150-ml beaker. If the sample is acid casein add 0.10 g of sodium hydrogen carbonate. If the sample is rennet casein add 0.10 g pentasodium triphosphate. Add 25 ml of water and dissolve the sample by gently swirling while warming to 60°-70° on a hot plate. Cool the solution to ambient temperature and add 15 ml water, 8 ml of 0.1 *N* hydrochloric acid, and 1 ml of a 10% solution of acetic acid. Mix well by swirling and after 5 min add 1 ml of 1 *M* sodium acetate; mix well.

After the precipitate has settled, filter and discard the first 5 ml of fitrate. Pipet 2 ml of the remaining filtrate into a test tube, add 0.2 ml *Phenol Reagent*, and mix well. Add 5 ml of concentrated sulfuric acid using an automatic dispenser or other means that permits mixing within 1-2 seconds. Assure that the solution has been thoroughly mixed and allow it to stand for 15 min, then cool to 20° in a water bath for 5 min.

Standard Solutions Transfer 10 ml of *Lactose Solution* to a 100-ml volumetric flask; dissolve in and dilute to volume with water (diluted *Lactose Solution*). Transfer respectively, 1, 2, 3, and 4 ml of diluted *Lactose Solution* to four 100-ml volumetric flasks; dilute to volume with water. These dilutions (standard dilutions) contain 20, 40, 60, and 80 µg of lactose per ml of solution, respectively. Into each of five test tubes add in sequence, 2 ml of water and, respectively, 2 ml each of the standard dilutions of lactose. Then to each test tube add *Phenol Reagent* and sulfuric acid as described under *Sample Solution*.

Calibration Determine the absorbance of each *Standard Solution* at 490 nm against the water blank. Calculate the slope of the curve obtained by plotting absorbance versus µg/ml of lactose. The slope of the curve is the absorptivity (*a*) of the lactose-reagent product, presuming a cell of 1-cm pathlength is used for absorbance readings.

Procedure Determine the absorbance of the *Sample Solution* at 490 nm against a blank prepared using identical reagents.

Calculation Calculate the percent lactose (*L*) in the casein sample by the formula:

$$L = (A \times 0.00475)/(a \times m),$$

in which *A* is the absorbance of the *Sample Solution* (at 490 nm, *a* is the absorptivity calculated under *Calibration*, *m* is the sample weight in g, and the numerical factor accounts for dilution and conversion to percent from µg/ml.

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 in the control.

Loss on Drying Proceed as directed under *Loss on Drying*, page 518.

Salmonella sp. Proceed as directed in Chapter VI, Procedure 2, *Bacteriological Analytical Manual*, Fifth Edition, Food and Drug Administration, 1978.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Binder; extender; clarifying agent; emulsifier; stabilizer.

Insert the following new monograph to precede the monograph entitled *Cognac Oil, Green*, page 89:

Coconut Oil (Unhydrogenated)

DESCRIPTION

A fat with a sweet nutty flavor obtained from the kernel of the fruit of the coconut palm *Cocos nucifera*. The crude oil is obtained by mechanically expressing dried coconut meat (copra) and is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. Compared with many natural fats, coconut oil has an abrupt melting range, changing from a rather firm, plastic solid at about 21° or below to a liquid at about 27°.

REQUIREMENTS

Identification

Coconut oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	6:0	8:0	10:0	12:0	14:0	16:0	16:1
Weight % (Range):	0-0.8	5-9	4-8	44-52	15-21	8-11	0-1
Fatty Acid:	18:0	18:1	18:2	20:0			
Weight % (Range):	1-4	5-8	0-2.5	0-0.4			

Arsenic (as As) Not more than 0.5 mg/kg.

Color (Lovibond) Not more than 20 yellow/2.0 red.

Free Fatty Acids (as oleic acid) Not more than 0.1%.
(as lauric acid) Not more than 0.07%.

Iodine Value Between 6 and 11.

Lead Not more than 0.1 mg/kg.

Melting Range Between 23.5° and 27°.

Peroxide Value Not more than 10 meq/kg.

Unsaponifiable Matter Not more than 1.5%.

Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test,* page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 μg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color,* page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids,* page 504, using the following equivalence factors (e) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$
Free fatty acids as lauric acid, $e = 20.0$

Iodine Value Proceed as directed under *Iodine Value,* page 505.

Lead

Apparatus Use a suitable atomic absorption spectrophotometer of either the flame or furnace type. Follow the manufacturer's directions for setting the appropriate instrument parameters for the determination of lead.

Butanol-Nitric Acid Slowly add 50 ml of concentrated nitric acid to approximately 500 ml of butyl alcohol in a 1-L volumetric flask. Dilute to volume with butyl alcohol and mix well. Each reagent should be sufficiently free of lead such that interference with the atomic absorption measurements is negligible.

Sample Solution Weigh accurately approximately 1 g of sample in a tared 10-ml volumetric flask. Samples are most easily transferred as liquids. Therefore, samples solid at room temperature should be melted on a steam bath. Stir liquified samples thoroughly with an acid-washed (10% HNO_3, 10% HCl) plastic spatula, being careful not to scrape the walls of metallic containers. Canned shortening or oils should be opened by shearing off the entire top of the can. Prepare a secondary standard with a sample that has been analyzed previously. Dilute each sample to volume with *Butanol-Nitric Acid.* Mix thoroughly and heat the sample on a steam bath to assist dissolution if necessary.

Standard Solutions Prepare a series of lead standards in *Butanol-Nitric Acid* such that their concentrations encompass that of the sample. Determine that this range defines a linear region of signal response by plotting absorption *vs.* the concentration of lead in the standards.

Procedure Determine the absorbance of the *Sample Solution* and calculate the concentration of lead (X) from the equation:

$$X = (Y - b)/m$$

in which Y is the absorbance of the sample, and m and b are linear regression parameters defining the slope and intercept of the standard curve obtained using the *Standard Solutions.* Calculate m and b for each series of *Standard Solutions* using the following equations:

$$m = \frac{N\Sigma X_iY_i - (\Sigma X_i)(\Sigma Y_i)}{N\Sigma X_i^2 - (\Sigma X_i)^2};$$

$$b = \frac{(\Sigma Y_i)(\Sigma X_i^2) - (\Sigma X_iY_i)(\Sigma X_i)}{N\Sigma X_i^2 - (\Sigma X_i)^2},$$

in which Y_i is the absorbance of an individual *Standard Solution* of lead concentration X_i, and N is the total number of standard solutions.

Melting Range Proceed as directed under *Melting Range,* page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value,* page 148 of the monograph for *Hydroxylated Lecithin.* However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter,* page 509.

Water Proceed as directed under *Water Determination,* page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Insert the following new monograph to precede the monograph entitled *Costus Root Oil,* page 91:

Corn Oil (Unhydrogenated)

DESCRIPTION

An amber-colored oil with a characteristic slight corn flavor obtained from the seed of the corn plant *Zea mays,* usually by solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21°-27°, but traces of wax may cause the oil to cloud when cooled to low temperature, unless they are removed by winterization. It is free from visible foreign material (other than wax) at 21°-27°.

REQUIREMENTS

Identification

Corn oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition,* page 82 of THIS SUPPLEMENT.

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1
Weight % (Range):	<0.1	<1.0	8.0-19	<0.5	0.5-4.0	19-50
Fatty Acid:	18:2	18:3	20:0	20:1		
Weight % (Range):	38-65	<2.0	<1.0	<0.5		

Arsenic (as As) Not more than 0.5 mg/kg.
Color (Lovibond) Not more than 5.0 red.

Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 120 and 130.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 2.0%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factors (e) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Linolenic Acid Proceed as directed under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Insert the following new monograph to precede the monograph entitled *Cubeb Oil*, page 91:

Cottonseed Oil (Unhydrogenated)

DESCRIPTION

A dark, reddish brown oil with a slight nutty flavor obtained from the seed of the cotton plant *Gossypium hirsutum* (American) or *Gossypium barbadense* (Egyptian) by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21°-27°, will cloud at 21°, but partially solidifies at storage temperatures below 10°-16°. It is free from visible foreign material at 23°-27°.

REQUIREMENTS

Identification

Cottonseed oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	< 14:0	14:0	16:0	16:1	18:0	18:1	
Weight % (Range):	< 0.1	0.5-2.0	17-29	< 1.5	1.0-4.0	13-44	
Fatty Acid:	18:2	18:3	20:0	20:1	22:0	22:1	24:0
Weight % (Range):	40-63	0.1-2.1	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5

Arsenic (as As) Not more than 0.5 mg/kg.
Color (Lovibond) Not more than 70 yellow/4.5 red.
Free Fatty Acids (as oleic acid) Not more than 0.01%.
(as lauric acid) Not more than 0.07%.
Iodine Value Between 99 and 119.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 2.1%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factors (e) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$
Free fatty acids as lauric acid, $e = 20.0$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Linoleic Acid Proceed as directed under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Dimethylpolysiloxane, page 102

Add the synonym: Polydimethylsiloxane

Change the *Requirements* entitled *Refractive Index*, *Specific Gravity*, and *Viscosity* to read:

Refractive Index Between 1.4000 and 1.4005
Specific Gravity Between 0.96 and 0.98
Viscosity Between 300 and 1,500 centistokes

Insert the following new monographs to precede the monograph entitled *Fennel Oil*, page 115:

F D & C Blue No. 1*

Brilliant Blue FCF; CI 42090; CAS 3844-45-9; Class: Triphenylmethane

$C_{37}H_{34}N_2O_9S_3Na_2$ Mol wt 792.84

DESCRIPTION

F D & C Blue No. 1 is principally the disodium salt of ethyl [4-[*p*-[ethyl (*m*-sulfobenzyl) amino]-α-(*o*-sulfophenyl)benzylidene]-2,5-cyclohexadien-1-ylidene] (*m*-sulfobenzyl) ammonium hydroxide inner salt with smaller amounts of the isomeric disodium salts of ethyl [4-[*p*-[ethyl (*p*-sulfobenzyl) amino]-α-(*o*-sulfophenyl) benzylidene]-2,5-cyclohexadiene-1-ylidene] (*p*-sulfobenzyl) ammonium hydroxide inner salt and ethyl [4-[*p*-[ethyl (*o*-sulfobenzyl) amino]-α-(*o*-sulfophenyl) benzylidene]-2,5-cyclohexadiene-1-ylidene] (*o*-sulfobenzyl) ammonium hydroxide inner salt.

The colorant is a dark purple to bronze powder that dissolves in water to give a solution green-blue at neutrality, green in weak acid, and yellow in stronger acid. Addition of base to its neutral solution produces a violet color only on boiling. When dissolved in concentrated sulfuric acid, it yields a yellow solution that turns green when diluted with water. It is soluble in 95% ethanol.

REQUIREMENTS

Identification

An aqueous solution containing 10 mg/L exhibits absorbance intensities (*A*) and wavelength maxima as follows: at pH 7, *A* = 1.11 at 630 nm; at pH 1, *A* = 0.95 at 629 nm, and *A* = 0.2 at 410 nm; and at pH 13, *A* = 1.29 at 630 nm, and *A* = 0.15 at 408 nm.

Arsenic (as As) Not more than 3 mg/kg.
Chromium (as Cr) Not more than 0.005%.
Ether Extracts* (combined) Not more than 0.4%.
Lead Not more than 10 mg/kg.
Leuco Base Not more than 5.0%.
Mercury (as Hg) Not more than 1 mg/kg.
Subsidiary Colors Not more than 6.0%.
Total Color Not less than 85.0%.
Uncombined Intermediates and Products of Side Reactions
Sum of o-, m-, *and* p-*sulfobenzaldehydes* Not more than 1.5%.
N-*ethyl*,N-(m-*sulfobenzyl)sulfanilic acid* Not more than 0.3%.
Volatile Matter at 135°, Chlorides and Sulfates (as sodium salts) Not more than 15.0% in combination.
Water-Insoluble Matter Not more than 0.2%.

TESTS

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

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

Chromium Proceed as directed under *Chromium*, page 73 of THIS SUPPLEMENT.

Ether Extracts Proceed as directed under *Ether Extracts*, page 74 of THIS SUPPLEMENT.

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.

Leuco Base Transfer approximately 120 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. Proceed as directed under *Leuco Base*, page 74 of THIS SUPPLEMENT.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Subsidiary Colors

Apparatus Use a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. The solvent system is composed of acetonitrile (50 ml), isoamyl alcohol (50 ml), 2-butanone (15 ml), water (5 ml), and ammonium hydroxide (5 ml).

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

* Not required for certification in the United States.

Sample Solution Transfer approximately 1 g of colorant, accurately weighed, to a 100-ml volumetric flask. Fill the flask about 3/4 full with water and place in the dark for one h; then dilute to volume and mix well.

Procedure Spot 0.1 ml of *Sample Solution* in a line across the plate, approximately 3 cm from the bottom edge. Allow the plate to dry for about 20 min, in the dark; then develop with the solvent system in an unlined tank equilibrated for at least 20 min before the plate is inserted. Allow the solvent front to reach within about 3 cm of the top of the plate. Dry the developed plate in the dark.

When the plate has dried, scrape off all the colored bands above the F D & C Blue No. 1, which remains close to the origin, into a 30-ml beaker. Extract the subsidiary colors with three 6-ml portions of 95% ethanol, or until no color remains on the gel by visual inspection. Record the volume of ethanol used and record the spectrum of the solution between 400 and 700 nm. Calculate the percent of subsidiary colors (P) by the formula:

$$P = (A \times V \times 100)/(a \times W \times b),$$

in which A is the absorbance at the wavelength maximum, V is the volume of the solution in ml, a is the absorptivity (0.126 mg per L per cm), W is the sample weight in mg, and b is the pathlength of the cell.

Sulfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and II*, page 76 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 50-75 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for F D & C Blue No. 1 is 0.164 mg per L per cm at 630 nm.

Method II (Sample Prepatation) Transfer approximately 0.5 g of colorant, accurately weighed, to the titration flask. The stoichiometric factor (F_s) for F D & C Blue No. 1 is 2.52.

Uncombined Intermediates and Products of Side Reactions Proceed as directed under *Uncombined Intermediates and Products of Side Reactions, Method I*, page 77 of THIS SUPPLEMENT. Calculate the concentrations of *m*-sulfobenzaldehyde and *N*-ethyl-*N*-(3-sulfobenzyl)-sulfanilic acid using the following absorptivities:

m-Sulfobenzaldehyde, a = 0.0495 mg per L per cm at 246 nm (acid solution).

N-Ethyl-*N*-(3-sulfobenzyl)-sulfanilic acid, a = 0.078 mg per L per cm at 277 nm (alkaline solution).

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Color.

F D & C Blue No. 2*

Indigotine; Indigotine Disulfonate; Indigo Carmine; CI 73015; CAS 860-22-0; Class: Indigoid

$C_{16}H_8N_2O_8S_2Na_2$ Mol wt 466.35

DESCRIPTION

F D & C Blue No. 2 is principally the disodium salt of 2-(1,3-dihydro-3-oxo-5-sulfo-2*H*-indol-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid with smaller amounts of the isomeric disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2*H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid.

The colorant is a blue-brown to red-brown powder that dissolves in water to give a solution blue at neutrality, blue-violet in acid, and green to yellow-green in base. When dissolved in concentrated sulfuric acid, it yields a blue-violet solution that turns blue when diluted with water. It is sparingly soluble in 95% ethanol.

REQUIREMENTS

Identification

A solution containing 20 mg/L exhibits absorbance intensities (A) and wavelength maxima as follows: at pH 7, A = 0.82 at 610 nm; at pH 1, A = 0.81 at 610 nm; and at pH 13, A = 0.2 at 610 nm, and A = 0.31 at 442 nm.

Arsenic (as As) Not more than 3 mg/kg.

Ether Extracts† (combined) Not more than 0.4%.

Isomeric and Subsidiary Colors

*Disodium salt of 2-(1,3-dihydro-3-oxo-7-sulfo-2*H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid* Not more than 18.0%.

*Sodium salt of 2-(1,3-dihydro-3-oxo-2*H*-indole-2-ylidene)-2,3-dihydro-3-oxo-1*H*-indole-5-sulfonic acid* Not more than 2.0%.

Lead Not more than 10 mg/kg.

Mercury (as Hg) Not more than 1 mg/kg.

Total Color Not less than 85.0%.

Uncombined Intermediates and Products of Side Reactions

Isatin-5-sulfonic acid Not more than 0.4%.

5-Sulfoanthranilic acid Not more than 0.2%.

Volatile Matter at 135°, Chlorides and Sulfate (as sodium salts) Not more than 15.0% in combination.

Water-Insoluble Matter Not more than 0.4%.

TESTS

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

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

† Not required for certification in the United States.

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

Ether Extracts Proceed as directed under *Ether Extracts*, page 74 of THIS SUPPLEMENT.

Isomeric and Subsidiary Colors

Apparatus Use a 2.5- × 40-cm glass column packed with Celite (Johns Mansville No. 595 or the equivalent) prepared as described under *Procedure*. Dissolve 20 g of hydroxylamine hydrochloride in 500 ml of water, place the solution in a 2-L separatory funnel, and add 450 ml of butanol, 450 ml of chloroform, 300 ml of water, and 100 ml of concentrated HCl. Agitate the mixture well, periodically venting the funnel. After settling, the bottom layer (organic), called the *Mobile Phase*, and the top layer (aqueous), called the *Stationary Phase*, should be separated and stored.

Sample Solution Dissolve approximately 100 mg of colorant, accurately weighed, in 100 ml of *Stationary Phase*; warm on a steam bath, if necessary, to dissolve the sample.

Procedure Slurry 12 g of Celite with 7 ml of *Stationary Phase* and pour into the column. Mix 5 ml of *Sample Solution* with 10 g of Celite and pour into the column over the slurry, insuring that the sample is quantitatively transferred to the column.

Elute the column with the *Mobile Phase*. Collect the monosulfonated derivative, the first band eluting, in a 25-ml graduated cylinder and note the volume. Collect the next band, the isomeric (unsulfonated) derivative, in a similar manner.

Mix each aliquot collected with an equal volume of hexane and transfer to a separatory funnel. Extract this mixture with three 15-ml aliquots of water; combine the water extracts and calculate the percent concentration (P) of the monosulfonated derivative (a = 0.0513 mg per L per cm at 615 nm) and the isomeric derivative (a = 0.0478 mg per L per cm at 610 nm) by the formula:

$$P = (A \times V)/(a \times W \times 10),$$

in which A is the absorbance, V is the volume of extract, a is the absorptivity in mg per L per cm, and W is the sample weight in 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.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Salfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and II*, page 76 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 175-225 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for F D & C Blue No. 2 is 0.0478 mg per L per cm at 610 nm.

Method II (Sample Preparation) Transfer approximately 0.3 g of colorant, accurately weighed, to the titration flask. The stoichiometric factor (F_s) for F D & C Blue No. 2 is 4.29.

Uncombined Intermediates and Products of Side Reactions Proceed as directed under *Uncombined Intermediates and Products of Side Reactions, Method I*, page 77 of THIS SUPPLEMENT. Calculate the concentration of isatin-5-sulfonic acid using an absorptivity of 0.089 mg per L per cm at 245 nm.

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Color.

F D & C Green No. 3*

Fast Green FCF; CI 42053; CAS 2353-45-9; Class: Triphenylmethane

$C_{37}H_{34}N_2O_{10}S_3Na_2$ Mol wt 808.84

DESCRIPTION

F D & C Green No. 3 is principally the disodium salt of *N*-ethyl-*N*-4-[[4-ethyl[(3-sulfophenyl)methyl]amino]phenyl] (4-hydroxy-2-sulfophenyl)methylene]-2,5-cyclohexadiene-1-ylidene]-3-sulfobenzenemethanaminium hydroxide.

The colorant is a red to brown-violet powder or collection of crystals that dissolves in water to give a solution blue-green at neutrality, green in acid, and blue to blue-violet in base. When dissolved in concentrated sulfuric acid, it yields a brown-orange solution that turns green when diluted with water. When heated to 130° with glycerine triacetate and an excess of acetic anhydride, acetylation of its phenolic hydroxyl group causes a color change from green to light blue.

REQUIREMENTS

Identification

An aqueous solution containing 5 mg/L exhibits absorbance intensities (A) and wavelength maxima as follows: at pH 7, A = 0.80 at 624 nm, and A = 0.08 at 423 nm; at pH 1, A = 0.83 at 625 nm, and A = 0.09 at 423 nm; and at pH 13, A = 0.74 at 610 nm.

Arsenic (as As) Not more than 3 mg/kg.
Chromium (as Cr) Not more than 0.005%.
Ether Extracts† (combined) Not more than 0.4%.
Lead Not more than 10 mg/kg.
Leuco Base Not more than 5.0%.

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

† Not required for certification in the United States.

Mercury (as Hg) Not more than 1 mg/kg.
Subsidiary Colors Not more than 6.0%
Total Color Not less than 85.0%.
Uncombined Intermediates and Products of Side Reactions
Sum of 3- and 4-[[ethyl(4-sulfophenyl)amino]methyl] benzenesulfonic acid disodium salts Not more than 0.3%.
Sum of 2-, 3-, and 4-formyl benzenesulfonic acids sodium salts Not more than 0.5%.
2-Formyl-5-hydroxybenzenesulfonic acid Not more than 0.5%.
Volatile Matter at 135°, Chlorides and Sulfates (as sodium salts) Not more than 15.0% in combination.
Water-Insoluble Matter Not more than 0.2%.

TESTS

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

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

Chromium Proceed as directed under *Chromium*, page 73 of THIS SUPPLEMENT.

Ether Extracts Proceed as directed under *Ether Extracts*, page 74 of THIS SUPPLEMENT.

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.

Leuco Base Transfer approximately 130 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. Proceed as directed under *Leuco Base*, page 74 of THIS SUPPLEMENT.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Subsidiary Colors

Apparatus Use a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. The solvent system is composed of acetonitrile (50 ml), isoamyl alcohol (50 ml), 2-butanone (15 ml), water (10 ml), and ammonium hydroxide (5 ml).

Sample Solution Transfer approximately 1 g of colorant, accurately weighed, to a 100-ml volumetric flask. Fill the flask about 3/4 full with water and incubate in the dark for 1 h; dilute to volume and mix well.

Procedure Proceed as directed in the monograph for F D & C Blue No. 1 under *Subsidiary Colors*, page 44 of THIS SUPPLEMENT.

Sulfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and II*, page 76 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 50-75 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for F D & C Green No. 3 is 0.156 mg per L per cm at 625 nm.

Method II (Sample Preparation) Transfer approximately 0.5 g of colorant, accurately weighed, to the titration flask. The stoichiometric factor (F_s) for F D & C Green No. 3 is 2.47.

Uncombined Intermediates and Products of Side Reactions Transfer approximately 2 g of colorant, accurately weighed, to a 100-ml volumetric flask; dissolve in and dilute to volume with water. Proceed as directed under *Uncombined Intermediates and Products of Side Reactions, Method I*, page 77 of THIS SUPPLEMENT. Calculate the amounts of intermediates and other products present using the following absorptivities after identifying the unknowns by comparing their spectra with standards:

4-Hydroxy-2-sulfobenzaldehyde, a = 0.080 mg per L per cm at 335 nm (alkaline solution).
m-Sulfobenzaldehyde, a = 0.495 mg per L per cm at 246 nm (acid solution).
N-Ethyl-*N*-(3-sulfobenzyl)-sulfanilic acid, a = 0.078 mg per L per cm at 277 nm (alkaline solution).

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

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

F D & C Red No. 3*

Erythrosine; CI 45430; CAS 16423-68-0; Class: Xanthene

$C_{20}H_6O_5I_4Na_2$ Mol wt 879.86

DESCRIPTION

F D & C Red No. 3 is principally the disodium salt of the monohydrate of 9(*o*-carboxyphenyl)-6-hydroxy-2,4,5,7-tetraiodo-3*H*-xanthen-3-one, with smaller amounts of lower iodinated fluoresceins.

The colorant is a brown powder that dissolves in water to give a solution red at neutrality, with a yellow-brown precipitate in acid, and with a red precipitate in base. When dissolved in concentrated sulfuric acid, it yields a brown-yellow solution that evolves iodine and a precipitate of the free acid when heated.

REQUIREMENTS

Identification

A solution containing 2.8 mg/L exhibits absorbance intensities (A) and wavelength maxima as follows: in neutral (pH = 7) and

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

alkaline (pH = 13) solutions, A = 0.32 at 527 nm with a shoulder at 490 nm. In acid solution, a yellow-brown precipitate forms.

Arsenic (as As) Not more than 3 mg/kg.
Ether Extracts* (combined) Not more than 0.2%.
Lead Not more than 10 mg/kg.
Mercury (as Hg) Not more than 1 mg/kg.
Subsidiary Colors
Monoiodofluoresceins Not more than 1.0%.
Other lower iodinated fluoresceins Not more than 9.0%.
Total Color Not less than 87.0%.
Uncombined Intermediates and Products of Side Reactions
2-(2′,4′-Dihydroxy-3′,5′-diiodobenzoyl)benzoic acid Not more than 0.2%.
Sodium iodide Not more than 0.4%.
Triiodoresorcinol Not more than 0.2%.
Unhalogenated intermediates Not more than 0.1% in combination.
Volatile Matter at 135°, Chlorides and Sulfates (as sodium salts) Not more than 13.0% in combination.
Water-Insoluble Matter Not more than 0.2%.

TESTS

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

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

Ether Extracts Proceed as directed under *Ether Extracts*, page 74 of THIS SUPPLEMENT.

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.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Subsidiary Colors

Apparatus Use a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. The solvent system is composed of acetone (95 ml), chloroform (25 ml), butylamine (10 ml), and water (10 ml).

Sample Solution Transfer approximately 2 g of colorant, accurately weighed, to a 100-ml volumetric flask. Fill the flask about 3/4 full with water and incubate in the dark for 1 h; dilute to volume and mix well.

Procedure Spot 0.1 ml of *Sample Solution* in a line across the plate, approximately 3 cm from the bottom edge. Allow the plate to dry for about 20 min, in the dark; then develop with the solvent system in an unlined tank equilibrated for at least 20 min before inserting the plate. Allow the solvent front to reach to within about 3 cm of the top of the plate. Dry the developed plate in the dark.

Scrape off each subsidiary color and extract with 3-5-ml portions of 50% aqueous ethanol until no color remains on the gel by visual inspection. Dilute each sample to 13-15 ml, add a few drops of ammonium hydroxide and record the final volume. Repeat this procedure for the band of F D & C Red No. 3 using 10-20-ml portions of 50% ethanol; dilute the eluant to 250 ml in a volumetric flask after adding enough ammonium hydroxide to make the solution slightly alkaline. The approximate band positions (R_f), wavelengths of maximal absorbance (λ), and absorptivities (a) are as follows:

Color	R_f	λ	a
Unknown	0.84	524	0.110
Red-3	0.84	526	0.110
2,4,7	0.76	521	0.140
2,4,5	0.67	521	0.116
2,4/2,5	0.45	513	0.145
Unknown	0.45	524	0.110

Record the spectrum of each solution between 400 and 600 nm and calculate the quantity in percent (P) of each subsidiary color by the formula:

$$P = (A \times V \times 100)/(a \times W \times b),$$

in which A is the absorbance at the wavelength maximum, V is the volume of the solution in ml, a is the absorptivity in mg per L per cm as given above, W is the sample weight in mg, and b is the path length of the cell in cm.

Sulfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and III*, pages 76 and 77 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 75-100 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for F D & C Red No. 3 is 0.110 mg per L per cm at 527 nm.

Method III (Sample Preparation) Proceed as directed under *Total Color, Method III*, page 77 of THIS SUPPLEMENT. The gravimetric conversion factor (F) for F D & C Red No. 3 is 1.074.

Uncombined Intermediates and Products of Side Reactions Transfer 2 g of colorant, accurately weighed, to a 100-ml volumetric flask; dissolve in and dilute to volume with water. Proceed as directed under *Uncombined Intermediates and Products of Side Reactions, Method I*, page 76 of THIS SUPPLEMENT. Calculate the concentrations of 2-(2,4-dihydroxy-3,5-diiodobenzoyl)benzoic acid, iodine, phthalic acid, sodium iodide, and triiodoresorcinol, using the following absorptivities:

2-(2,4-Dihydroxy-3,5-diiodobenzoyl)benzoic acid, a = 0.047 mg per L per cm at 348 nm (alkaline);
Iodine, a = 0.082 mg per L per cm at 245 nm (acidic);
Phthalic acid, a = 0.045 mg per L per cm at 228 nm (acidic);
Sodium iodide, a = 0.091 mg per L per cm at 220 nm (acidic);
Triiodoresorcinol, a = 0.079 mg per L per cm at 223 nm (acidic).

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

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* Not required for certification in the United States.

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

F D & C Red No. 40*

Allura Red AC; CI 16035; CAS 25956-17-6; Class: Monoazo

$C_{18}H_{14}N_2O_8S_2Na_2$ Mol wt 496.42

DESCRIPTION

F D & C Red No. 40 is principally the disodium salt of 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-2-napthalenesulfonic acid.

The colorant is a red powder that dissolves in water to give a solution red at neutrality and in acid and dark red in base. It is slightly soluble in 95% ethanol.

REQUIREMENTS

Identification

A solution containing 16.4 mg/L exhibits absorbance intensities (*A*) and wavelength maxima as follows: at pH 7, *A* = 0.87 at 500 nm; at pH 1, *A* = 0.83 at 490 nm (both neutral and acid solutions exhibit a shoulder at about 410 nm); and at pH 13, *A* = 0.37 at 500 nm, and *A* = 0.41 at 450 nm.

Arsenic (as As) Not more than 3 mg/kg.

Lead Not more than 10 mg/kg.

Mercury (as Hg) Not more than 1 mg/kg.

Subsidiary Colors

Disodium 6-hydroxy-5-[(2-methoxy-5-methyl-4-sulfophenyl)azo]-8-(2-methoxy-5-methyl-4-sulfophenoxy)-2-napthalenesulfonic acid Not more than 1.0%.

Higher and lower sulfonated subsidiary colors (as sodium salts) Not more than 1.0% each.

Total Color Not less than 85.0%.

Uncombined Intermediates and Products of Side Reactions:

4-Amino-5-methoxy-o-toluenesulfonic acid Not more than 0.2%.

Disodium 6,6'-oxybis(2-napthalenesulfonic acid) Not more than 1%.

Sodium 6-hydroxy-2-napthalenesulfonic acid Not more than 0.3%.

Volatile Matter at 135°, Chlorides and Sulfates (as sodium salts) Not more than 14.0% in combination.

Water-Insoluble Matter Not more than 0.2%.

TESTS

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

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

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.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Subsidiary Colors

Apparatus Use a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. The solvent system is composed of acetonitrile (20 ml), dioxane (20 ml), ethyl acetate (20 ml), isoamyl alcohol (20 ml), water (20 ml), and ammonium hydroxide (4 ml).

Standard Solution Transfer approximately 1 g of purified F D & C Red No. 40 free of subsidiary colors, accurately weighed, to a 50-ml volumetric flask. Add 50 mg each of lower and higher sulfonated subsidiary colors, accurately weighed; dissolve in and dilute to volume with water. Store in the dark.

Sample Solution Transfer approximately 2 g of colorant, accurately weighed, to a 100-ml volumetric flask; dissolve in and dilute to volume with water.

Procedure Spot 3-µl aliquots of *Sample Solution* and *Standard Solution* side by side 3 cm from the bottom of the plate. Up to seven samples and standards may be run simultaneously.

When the plate has air-dried for 15 min, develop it in an unlined tank equilibrated with the solvent system for at least 20 min. Allow the solvent front to reach to within about 3 cm from the top of the plate.

Allow the plate to dry in a fume hood and by visual inspection compare the intensities of the lower and higher sulfonated subsidiary colors with those in the *Standard Solution*. If the subsidiary colors in the *Sample Solution* appear more concentrated than those in the *Standard Solution*, determine the quantity of each, using a densitometer set to monitor the absorbance maximum of each. Calculate the concentrations of the subsidiary colors in percent (*P*), if present above 0.1%, by the formula:

$$P = (A \times p)/A_s,$$

in which *A* is the area of the densitometer curve, *p* is the percent of subsidiary color in the *Standard Solution*, and A_s is the area of the densitometer curve for the subsidiary color in the *Standard Solution*.

Sulfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and II*, page 76 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 175-225 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (*a*) for F D & C Red No. 40 is 0.052 mg per L per cm at 502 nm.

Method II (Sample Preparation) Transfer approximately 0.2 g of colorant, accurately weighed, to the titration flask. The stoichiometric factor (F_s) for F D & C Red No. 40 is 8.06.

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

Uncombined Intermediates and Products of Side Reactions

Sample Preparation Transfer 0.25 g of colorant, accurately weighed, to a 100-ml volumetric flask. Dissolve in and dilute to volume with 0.1 M $Na_2B_4O_7$. Inject 20 µl of this solution as directed under *Uncombined Intermediates and Products of Side Reactions, Method II*, page 78 of THIS SUPPLEMENT.

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Color.

F D & C Yellow No. 5*

Tartrazine; CI 19140; CAS 1934-21-0; Class: Pyrazalone

$C_{16}H_9N_4O_9S_2Na_3$ Mol wt 534.36

DESCRIPTION

F D & C Yellow No. 5 is principally the trisodium salt of 5-oxo-1-(*p*-sulfophenyl)-4-[(*p*-sulfophenyl)azo]-2-pyrazoline-3-carboxylic acid.

The colorant is a yellow-orange powder that dissolves in water to give a solution golden yellow at neutrality and in acid. When dissolved in concentrated sulfuric acid, it yields an orange-yellow solution that turns yellow when diluted with water.

REQUIREMENTS

Identification

A solution (A) containing 19.9 mg/L exhibits absorbance intensities and wavelength maxima as follows: at pH 7, $A = 1.4$ at 425 nm; at pH 1, $A = 1.1$ at 426 nm; and at pH 13, the absorbance maximum is shifted below 400 nm.

Arsenic (as As) Not more than 3 mg/kg.

Ether Extracts† (combined) Not more than 0.2%.

Lead Not more than 10 mg/kg.

Mercury (as Hg) Not more than 1 mg/kg.

Subsidiary Colors Not more than 1.0%.

Total Color Not less than 87.0%.

Uncombined Intermediates and Products of Side Reactions:

*Phenylhydrazine-*p*-sulfonic acid* Not more than 0.1%.

Other uncombined intermediates Not more than 0.2%.

Volatile Matter at 135°, Chlorides and Sulfates (as sodium salts) Not more than 13.0% in combination.

Water-Insoluble Matter Not more than 0.2%.

TESTS

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

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

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.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Subsidiary Colors

Apparatus Use a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. The solvent system is composed of dioxane (50 ml), isoamyl alcohol (50 ml), water (20 ml), and ammonium hydroxide (5 ml).

Standard Solution Transfer approximately 1 g of purified F D & C Yellow No. 5 free of subsidiary colors, accurately weighed, to a 50-ml volumetric flask. Add 150 mg of the lower sulfonated subsidiary color, accurately weighed; dissolve in and dilute to volume with water. Store in the dark.

Sample Solution Transfer approximately 1 g of colorant, accurately weighed, to a 250-ml volumetric flask; dissolve in and dilute to volume with water.

Procedure Proceed as directed in the monograph for F D & C Red No. 40 under Subsidiary Colors, page 48 of THIS SUPPLEMENT.

Sulfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and II*, page 76 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 175-250 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (a) for F D & C Yellow No. 5 is 0.053 mg per L per cm at 428 nm.

Method II (Sample Preparation) Transfer approximately 0.2 g of colorant, accurately weighed, to the titration flask. The stoichiometric factor (F_s) for F D & C Yellow No. 5 is 7.49.

Uncombined Intermediates and Products of Side Reactions

Sample Preparation Transfer 0.15 g of colorant, accurately weighed, to a 100-ml volumetric flask. Dissolve in and dilute to volume with 0.1 *M* $Na_2B_4O_7$. Inject 50 µl of this solution as directed under *Uncombined Intermediates and Products of Side Reactions, Method II*, page 76 of THIS SUPPLEMENT.

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Color.

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

† Not required for certification in the United States.

F D & C Yellow No. 6*

Sunset Yellow FCF; CI 15985; CAS 2783-94-0; Class: Monoazo

$C_{16}H_{10}N_2O_7S_2Na_2$ Mol wt 452.36

DESCRIPTION

F D & C Yellow No. 6 is principally the disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid.

The colorant is an orange powder that dissolves in water to give a solution yellow-orange at neutrality or in acid and red-brown in base. When dissolved in concentrated sulfuric acid, it yields an orange solution that turns yellow when diluted with water. It is slightly soluble in 95% ethanol.

REQUIREMENTS

Identification

A solution (*A*) containing 18.5 mg/L exhibits absorbance intensities and wavelength maxima as follows: at pH 1, $A = 1.1$ at 480 nm; and at pH 13, $A = 0.46$ at 443 nm, with a shoulder at about 500 nm.

Arsenic (as As) Not more than 3 mg/kg.

Ether Extracts† (combined) Not more than 0.2%.

Lead Not more than 10 mg/kg.

Mercury (as Hg) Not more than 1 mg/kg.

Subsidiary Colors Not more than 5.0%.

Total Color Not less than 85.0%.

Uncombined Intermediates and Products of Side Reactions

4,4'-(Diazoamino)-dibenzenesulfonic acid Not more than 0.1%.

Disodium 6,6'-oxybis(2-napthalenesulfonic acid) Not more than 0.5%

Sulfanilic acid Not more than 0.2%

Sodium 6-hydroxy-2-napthalenesulfonic acid Not more than 0.3%.

Volatile Matter at 135°, Chlorides and Sulfates (as sodium salts) Not more than 13.0% in combination.

Water-Insoluble Matter Not more than 0.2%.

TESTS

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

Chloride Proceed as directed under *Sodium Chloride*, page 76 of THIS SUPPLEMENT.

Ether Extracts Proceed as directed under *Ether Extracts*, page 74 of THIS SUPPLEMENT.

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.

Mercury Proceed as directed under *Mercury*, page 75 of THIS SUPPLEMENT.

Subsidiary Colors

Apparatus Use a 20- × 20-cm glass plate coated with a 0.25-mm layer of Silica Gel G. The solvent system is composed of acetone (50 ml), isoamyl alcohol (65 ml), water (20 ml), and ammonium hydroxide (5 ml).

Standard Solution Transfer accurately weighed quantities of the following to a 50-ml volumetric flask:

Purified F D & C Yellow No. 6, free from subsidiary colors; 1 g.
1-(4-Sulfophenylazo)-2-napthol-3,6-disulfonic acid; 30 mg.
1-(4-Sulfophenylazo)-2-napthol; 10 mg.
Dissolve in and dilute to volume with water.

Sample Solution Transfer approximately 2 g of colorant, accurately weighed, to a 100-ml volumetric flask; dissolve in and dilute to volume with water.

Procedure Proceed as directed in the monograph for F D & C Red No. 40 under *Subsidiary Colors*, page 48 of THIS SUPPLEMENT.

Sulfate Proceed as directed under *Sodium Sulfate*, page 76 of THIS SUPPLEMENT.

Total Color Determine the total color strength as the weight percent of colorant using *Methods I and II*, page 76 of THIS SUPPLEMENT. Express the *Total Color* as the average of the two results.

Method I (Sample Preparation) Transfer 200-225 mg of colorant, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water. The absorptivity (*a*) for F D & C Yellow No. 6 is 0.054 mg per L per cm at 484 nm.

Method II (Sample Preparation) Transfer approximately 0.2 g of colorant, accurately weighed, to the titration flask. The stoichiometric factor (F_s) for F D & C Yellow No. 6 is 8.84.

Uncombined Intermediates and Products of Side Reactions

Sample Preparation Transfer 0.25 g of colorant, accurately weighed, to a 100-ml volumetric flask. Dissolve in and dilute to volume with 0.1 *M* $Na_2B_4O_7$. Inject 20 μl of this solution as directed under *Uncombined Intermediates and Products of Side Reactions, Method II*, page 78 of THIS SUPPLEMENT.

Volatile Matter Proceed as directed under *Volatile Matter*, page 80 of THIS SUPPLEMENT.

Water-Insoluble Matter Proceed as directed under *Water-Insoluble Matter*, page 80 of THIS SUPPLEMENT.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Color.

Insert the following new monograph to precede the monograph entitled L-*Histidine*, page 143:

Hexanes

Mixed Paraffinic Hydrocarbons

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* To be used or sold in the United States this colorant must be certified by the U.S. Food and Drug Administration.

† Not required for certification in the United States.

DESCRIPTION

Hexanes constitute a clear, colorless, flammable liquid composed predominantly of C_6, with some C_5 and C_7, isomeric paraffins. The relative proportion of isomers varies with the producer and the production lot. It is soluble in alcohol, acetone, and ether and insoluble in water.

REQUIREMENTS

Benzene Not more than 0.05%.
Color (APHA) Not more than 10.
Distillation Range Between 64° and 71°.
Heavy Metals (as Pb) Not more than 1 mg/kg.
Nonvolatile Residue Not more than 10 mg/kg.
Specific Gravity Between 0.655 and 0.675.
Sulfur Not more than 5 mg/kg.

TESTS

Benzene Proceed as directed under *Benzene* (in Paraffinic Hydrocarbon Solvents), page 71 of THIS SUPPLEMENT.

Color (APHA) Dilute 2.0 ml of platinum-cobalt stock solution (APHA No. 500) with water in a 100-ml volumetric flask. Compare this solution (APHA No. 10) with 100 ml of the sample in 100-ml Nessler tubes, viewed vertically over a white background.

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 one drop of hydrochloric acid, add 10 ml of hot water, and digest for 2 min. Cool, and dilute to 25 ml with water in a volumetric flask. 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 µg) of the sample to dryness 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).

Sulfur Proceed as directed under *Sulfur*, page 85 of THIS SUPPLEMENT.

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

Functional Use in Foods Extraction solvent.

Insert the following new monograph to precede the monograph entitled L-*Histidine*, page 143:

High-Fructose Corn Syrup

DESCRIPTION

High-fructose corn syrup is a sweet, nutritive saccharide mixture prepared as a clear aqueous solution from high-dextrose-equivalent corn starch hydrolysate by the partial enzymatic conversion of glucose (dextrose) to fructose, using an insoluble glucose isomerase preparation complying with *21* CFR 184.1372 that has been grown in a pure culture fermentation that produces no antibiotics.

It is a water-white to light yellow, somewhat viscous liquid that darkens at high temperatures. It is miscible in all proportions with water.

REQUIREMENTS

Identification

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.

Assay Not less than 97% total saccharides, expressed as a percent of solids, of which not less than 40% nor more than 44% consists of fructose, not less than 51% nor more than 55% consists of glucose, and not more than 7% consists of other saccharides.
Arsenic (as As) Not more than 1 mg/kg.
Color Within the range specified by the vendor.
Heavy Metals (as Pb) Not more than 1 mg/kg.
Residue on Ignition Not more than 0.05%.
Solids Not less than 70%.
Sulfur Dioxide Not more than 0.003%.

TESTS

Assay

Apparatus Use a suitable high-performance liquid-chromatography (HPLC) system, such as described in Standard Analytical Methods of the Corn Industries Research Foundation (A division of the Corn Refiners Association) (also, see page 476) equipped with a 22-31-cm stainless-steel column, a strip-chart recorder, and a differential refractometer detector maintained at 45° ± 0.005°.

Stationary Phase Prepacked macroreticular polystyrene sulfonate divinylbenzene cation-exchange resin (2-8% cross-linked, 8-25-µm particle size), preferably in the calcium or silver form. Examples of acceptable resins are Bio-Rad Aminex HPX-87C (or the equivalent) for separating DP_1-DP_4 saccharides and Aminex HPX-42C and HPX-42A (or the equivalent) for separating DP_1-DP_7 saccharides. Maintain the column at 85° during operation.

Mobile Phase Degassed purified water passed through a 0.22-µm filter before use; maintain at 85° during operation of the chromatograph.

Standardization Prepare a standard solution containing a total of about 10% solids using sugars of known purity (e.g., fructose, dextrose, and maltose) that approximates, on the dry basis, the composition of the sample to be analyzed. Dissolve each standard sugar, accurately weighed, in 20 ml of purified water contained in a 50-ml beaker. Heat on a steam bath until all sugars are dissolved; then cool and transfer to a 100-ml volumetric flask. Dilute to volume with water and mix. Freeze the solution if it is to be reused.

If a corn syrup or maltodextrin is used to supply a DP_{4+}

fraction, take care to include all saccharides in the standard composition calculation.

Compute the dry-basis concentration (C), in percent, of each individual component in the standard solution by the formula:

$$C = (W_c / \Sigma W_i) \times 100,$$

in which W_c is the weight of the sugar of interest and ΣW_i is the sum of all sugar components. Standardize by injecting 10-20 μl (about 1.0-2.0 mg solids) of the standard sugar solution. Integrate the peaks and normalize. Sum the individual DP_{4+} responses from the normalized printout to obtain the total DP_{4+} normalized response. Calculate the response factors as follows (see page 476, and page 73 of THIS SUPPLEMENT):

$$R_i = \frac{\text{known concentration, dry basis \%,}}{\text{measured concentration, normalized \%}},$$

in which R_i is the response factor for component i.

Compute the response factor for each component relative to glucose (R'_i) using the following equation:

$$R'_i = R_i / R_G,$$

in which R_G is the response factor for glucose. The R'_i for DP_{4+} should be programmed as a default value (if automated equipment is used) and used to compute the concentration of higher saccharides.

Sample Analysis Determine the solids content (see below) of the sample and dilute to approximately 10% solids with water. Inject a volume (10-50 μl) appropriate for the specific solids content.

Calculation Calculate the concentration of each component as follows:

$$C_i = (A_i \times R_i \times 100)/(\Sigma A_n R_n),$$

in which A_i is the area recorded for that component and $\Sigma A_n R_n$ is the sum of the product of the areas (A) and response factors (R) for all components detected.

Arsenic A *Sample Solution* prepared as directed for organic compounds meets the requirements of the *Arsenic Test*, using 1 ml of *Standard Arsenic Solution* (1 μg of As).

Color

Apparatus Use a suitable variable-wavelength spectrophotometer capable of measuring percent transmittance throughout the visible spectrum and designed to permit the use of sample and reference cells with pathlengths of 2-4 cm. The transmittance of all paired cells should agree within 0.5%.

Standard Solution Dissolve 0.10 g of reagent grade potassium dichromate ($K_2Cr_2O_7$) in 1 L of water and mix thoroughly.

Procedure With water in sample and reference cells of 2-cm pathlength adjust the percent transmittance scale of the spectrophotometer to 100%. Leave the reference cell in place and replace the water in the sample cell with the *Standard Solution;* determine the wavelength at which it exhibits exactly 54.5 percent transmittance. This wavelength is defined as λ_c, the corrected 450-nm wavelength. Remove the 2-cm cells from the spectrophotometer and with water in the sample and reference cells of 4-cm pathlength adjust the percent transmittance scale to 100% with the spectrophotometer set at λ_c. Leave the reference cell in place and replace the water in the sample cell with the sample of high-fructose corn syrup. Measure the percent transmittance (T_{450}). Remove the sample cell, set the wavelength at 600 nm, replace the sample with water, and adjust the percent transmittance scale to 100%; then determine the percent transmittance at 600 nm (T_{600}) with the same sample of high-fructose corn syrup in the sample cell. Calculate the *Color* (C) of the sample with the following formula:

$$C = (\log T_{600} - \log T_{450})/4,$$

in which T_{600} is the percent transmittance at 600 nm and T_{450} is the percent transmittance at 450 nm.

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*) and 500° as the ignition temperature.

Lead Transfer 10 g of the sample to an evaporating dish, add 5 ml of sulfuric acid solution (1 in 4), mixing it thoroughly with 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*, using 10 g of lead ion (Pb) in the control.

Residue on Ignition Ignite 5 g as directed under *Residue on Ignition*, page 533.

Solids Determine the percent solids from the refractive index (see page 533) as directed under *High-Fructose Corn Syrup Solids*, page 84 of THIS SUPPLEMENT.

Sulfur Dioxide Proceed as directed under *Sulfur Dioxide*, page 546, using a 100 g sample.

Packaging and Storage Store in tight containers.
Functional Use in Foods Nutritive sweetener.

Hydrochloric Acid, page 144

Replace the *Description* and *Requirements* with the following:

DESCRIPTION

A water solution of hydrogen chloride of varied 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 hydrochloric acid 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 13° Be (19.6%) 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 by various methods that might impart trace amounts of organic compounds as impurities. The manufacturer, vendor, or user is responsible for identifying the specific organic compounds which are present and for meeting the *Requirements* for *Extractable Organic Compounds*. Methods are provided for their determination. In applying the procedures any necessary standards should be used to quantitate the organic compounds present in each specific product.
>
> The variety of organic impurities that might conceivably be found in hydrochloric acid is such that it is impossible to provide a comprehensive and accurate list here. Therefore, the manufacturer, vendor, or user is responsible for establishing the suitability of such hydrochloric acid for its intended application in foods or food processing in accordance with the provision on *Trace Impurities*, page 3.

REQUIREMENTS

Identification

It gives positive tests for *Chloride*, page 516.

Assay Within the range of Baumé degrees specified or implied by the vendor.
Arsenic (as As) Not more than 1 mg/kg.
Color Passes test.
Concentration of HCl Within the range specified or implied by the vendor.
Extractable Organic Compounds
Total Organic Compounds (Non-Fluorine-Containing) Not more than 5 mg/kg, including:
Benzene Not more than 0.05 mg/kg.
Vinyl Chloride Not more than 0.05 mg/kg.

Fluorinated Organic Compounds (total) Not more than 25 mg/kg.
Heavy Metals (as Pb) Not more than 5 mg/kg.
Iron Not more than 5 mg/kg.
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 Within the range specified or implied by the vendor.
Sulfate Not more than 0.5%.

TESTS

Extractable Organic Compounds Proceed as directed under *Extractable Organic Compounds* (in Hydrochloric Acid), page 80 of THIS SUPPLEMENT.

Insert the following new monograph to precede the monograph entitled *Iron, Carbonyl*, page 151:

Invert Sugar

Invert Sugar Syrup, Invert

DESCRIPTION

Invert sugar is a mixture of glucose and fructose that results from the hydrolysis of sucrose in accordance with good manufacturing practices. Invert sugar is marketed as a component of invert sugar syrup that also contains sucrose in various amounts as represented by the manufacturer.

Invert sugar syrup is a hygroscopic liquid that has a sweet taste, is very soluble in water, glycerin and glycols, and is very sparingly soluble in acetone and ethanol.

REQUIREMENTS

Identification

Prepare a 10% solution in purified water and inject 7.5 µl into a high-performance liquid chromatographic system equipped with a cation exchange resin maintained at 85° and a differential refractometer detector; the liquid phase is purified water eluting at a flow rate of 0.7 ml per min. The chromatogram of the sample gives appropriate elution times for fructose, glucose, and sucrose when compared to a standard solution containing 1 g of each saccharide in 100 ml of purified water.

Assay Sucrose and invert sugar content shall be as represented by the manufacturer.
Arsenic (as As) Not more than 3 mg/kg.
Heavy Metals (as Pb) Not more than 7 mg/kg.
Lead Not more than 5 mg/kg.
pH Not less than 3 nor more than 5.5.
Residue on Ignition Not more than 0.2%.
Total Solids As represented by the vendor.
Total Sugars Not less than 99.5% of the total solids content.

TESTS

Assay

Apparatus Mount a ring support on a ringstand 1-2 in. above a gas burner, and mount a second ring 6-7 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 50-ml buret to the ringstand so that the tip just passes through the watch glass centered above the flask. Alternatively, a buret with an offset tip may be used in place of a buret with a straight tip extending through the hole in the center of the watch glass. Place an indirectly lighted white surface behind the assembly for observing the end point.

Soxhlet Solution

Copper Sulfate Solution Dissolve 34.639 g of $CuSO_4 \cdot 5H_2O$ in water, dilute to 500 ml, and filter.

Alkaline Tartrate Solution Dissolve 173 g of potassium sodium tartrate ($KNaC_4H_4O_6{\cdot}4H_2O$) and 50 g of NaOH in water and dilute to 500 ml; allow to stand 2 d and filter before use.

Just prior to use, prepare the *Soxhlet Solution* by mixing equal volumes of *Copper Sulfate Solution* and *Alkaline Tartrate Solution*.

Standard Solution Transfer approximately 9.5 g of USP-grade sucrose, accurately weighed, to a 1-L volumetric flask; dissolve in 100 ml of water, add 5 ml of concentrated hydrochloric acid, and store 3 d at 20°-25°. Dilute to volume with water. This solution is stable for several months.

Sample Solution Transfer 10 g of sample, accurately weighed, to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Standardized Soxhlet Solution Pipet 25.0 ml of *Soxhlet Solution* into a 400-ml Erlenmeyer flask containing a few boiling chips, and titrate with the *Standard Solution* as directed under *Procedure*. Accurately dilute the *Standard Solution* so that the titration requires more than 15 but less than 50 ml.

Procedure

Invert Sugar Pipet 25.0 ml of *Standardized Soxhlet Solution* into a 400-ml Erlenmeyer flask containing a few boiling chips. Rapidly add *Sample Solution* from a buret to within 0.5 ml of the end point (determined by a 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 2 min. As boiling proceeds, add 1 ml of a 0.2% 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.

Find the invert sugar factor (F_I) in the table of conversion factors, page 84 of THIS SUPPLEMENT, corresponding to the volume of titrant (V) used. When using the table interpolation might be required to obtain correct factors corresponding to titrant volumes not shown and for solutions of invert sugar containing between 0 and 1 g of sucrose per 100 ml of solution. Calculate the concentration (C_I) of invert sugar, in mg/ml, used in the titration with the formula:

$$C_I = F_I/V.$$

Calculate the percent of invert sugar (P_I) in the sample using the following formula:

$$P_I = (C_I \times 100)/C_S,$$

in which C_I is the concentration of invert sugar in mg/ml, as defined above, and C_S is the concentration of sample in mg/ml which is calculated from the quantity of sample used to prepare the *Sample Solution*.

Sucrose Pipet 100 ml of *Sample Solution* into a 200-ml volumetric flask and add slowly 10 ml of hydrochloric acid (TS), diluted 1:1, while gently swirling the solution; place in a constant temperature bath maintained at 60°; agitate continuously for 3 min; and allow to sit in the bath for an additional 7 min. Remove the flask from the bath and cool to 20° as rapidly as possible; dilute to volume with water and mix well. Continue as directed in the *Procedure* above under *Invert Sugar*. Calculate the percent invert sugar present after hydrolysis (P_H) using the formulas:

$$C_H = F_S/V, \qquad P_H = C_H/C_S,$$

in which C_H is the concentration of invert sugar in mg/ml after hydrolysis, F_S is the sucrose factor from the conversion table corresponding to the volume of titrant V, and C_S is the concentration of sample in mg/ml in the sample solution, as defined above. Calculate the percent sucrose using the formula:

$$P_S = (P_H - P_I)\ 1.90,$$

in which P_H and P_I are the percentages of invert sugar determined after and prior to hydrolysis, 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 1.0 g sample as directed in *Method II* under the *Heavy Metals Test*, page 513, using 7 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 in the control.

pH Determine by the *Potentiomentric Method*, page 531.

Residue on Ignition Determine as directed in *Method II* under *Residue on Ignition*, page 533.

Total Solids Proceed as directed under *Total Solids*, page 84 of THIS SUPPLEMENT.

Total Sugars Calculate the *Total Sugars* (T_S) as the sum of the concentrations of invert sugar (P_I) and sucrose (P_S) determined under *Assay*:

$$T_S = P_I + P_S.$$

Packaging and Storage Store in tight containers.

Functional Use in Foods Nutritive sweetener.

Insert the following new monograph to precede the monograph entitled *Laurel Leaf Oil*, page 164:

Lard (Unhydrogenated)

DESCRIPTION

An off-white fat obtained by dry or wet (steam) rendering of fresh fatty porcine tissues (cuttings and trimmings) shortly after slaughtering. Rendered lard may be bleached, or bleached and deodorized. It is soft to semisolid at 27° and melts completely at 42°.

Rendered, bleached, and bleached-deodorized lard are off-white semisolids at 21°-27°. Bleached, and bleached-deodorized lard, which are pale yellow and clear at 54°, differ from rendered lard, which is pale yellow, clear to hazy, and may contain extraneous matter.

SPECIFIC REQUIREMENTS

	Rendered Lard	*Bleached Lard*	*Bleached and Deodorized Lard*
Color (Lovibond)	Not more than 3.0 red	Not more than 1.5 red	Not more than 1.5 red
Free Fatty Acids (as oleic acid)	Not more than 1.0%	Not more than 1.0%	Not more than 0.1%
Insoluble Matter	Not more than 0.1%	Not more than 0.05%	Not more than 0.05%
Iodine Value	Between 46 and 70	Between 46 and 70	Between 46 and 70
Water	Not more than 0.5%	Not more than 0.1%	Not more than 0.1%

GENERAL REQUIREMENTS

Identification

Lard exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition,* page 82 of THIS SUPPLEMENT.

Fatty Acid:	< 14:0	14:0	14:1	15:0	16:0
Weight % (Range):	< 0.5	0.5-2.5	< 0.2	< 0.1	20-32

Fatty Acid:	16:1	17:0	17:1	18:0	18:1
Weight % (Range):	1.7-5	< 1.0	< 0.7	5.0-24	35-62

Fatty Acid:	18:2	18:3	20:0	20:1
Weight % (Range):	3.0-16	< 2.0	< 1.0	< 1.0

Arsenic (as As) Not more than 0.5 mg/kg.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test,* page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 μg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color,* page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids,* page 504, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Proceed as directed under *Iodine Value,* page 505.

Lead Proceed as directed under *Coconut Oil,* page 41 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value,* page 148 of the monograph for *Hydroxylated Lecithin.* However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter,* page 509.

Water Proceed as directed under *Water Determination,* page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Methyl Formate, page 198

Replace the last sentence of the *Test* entitled *Assay* with the following:

Each ml of 0.5 *N* sodium hydroxide is equivalent to 30.03 mg of $C_2H_4O_2$.

Insert the following two new monographs to precede the monograph entitled *Palmarosa Oil,* page 212:

Palm Kernel Oil (Unhydrogenated)

DESCRIPTION

A fat with a slight, characteristic sweet nutty flavor obtained from the kernel of the fruit of the oil palm *Elaeis guineensis* by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. Like coconut oil it has a more abrupt melting range than other fats and oils.

REQUIREMENTS

Identification

Palm kernel oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition,* page 82 of THIS SUPPLEMENT.

Fatty Acid:	6:0	8:0	10:0	12:0	14:0	16:0	16:1
Weight % (Range):	0-1.5	3-5	2.5-6	40-52	14-18	7-10	0-1

Fatty Acid:	18:0	18:1	18:2	20:0
Weight % (Range):	1-3	11-19	0.5-4	tr.-1

Arsenic (as As) Not more than 0.5 mg/kg.

Color (Lovibond) Not more than 20 yellow/2.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
(as lauric acid) Not more than 0.07%.
Iodine Value Between 13 and 23.
Lead Not more than 0.1 mg/kg.
Melting Range Between 27° and 29°.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factors (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$
Free fatty acids as lauric acid, $e = 20.0$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Melting Range Proceed as directed under *Melting Range*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.
Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Palm Oil (Unhydrogenated)

DESCRIPTION

A deep orange-red fat with a nutty flavor obtained from the pulp of the fruit of the oil palm *Elaeis guineensis* usually by boiling, centrifugation, and mechanical expression. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a semisolid at 21°-27°.

REQUIREMENTS

Identification

Palm oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	14:0	16:0	18:0	18:1	18:2
Weight % (Range):	0.5-5.9	32-47	2-8	34-44	7-12

Arsenic (as As) Not more than 0.5 mg/kg.
Color (Lovibond) Not more than 35 yellow/5.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
(as palmitic acid) Not more than 0.09%.
Iodine Value Between 50 and 55.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Stability (AOM) Not less than 50 h.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factors (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$
Free fatty acids as palmitic acid, $e = 25.6$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Stability (Active Oxygen Method) Proceed as directed under *Stability*, page 83 of THIS SUPPLEMENT.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Insert the following new monograph to precede the monograph entitled *Pectin*, page 215:

Peanut Oil (Unhydrogenated)

DESCRIPTION

A pale yellow oil with a bland flavor obtained from the kernel of the peanut plant *Arachis hypogaea* by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21°-27°, but solidifies to a gel-like consistency at refrigerator temperatures (2°-4°). It is free from visible foreign matter at 21°-27°, but sometimes clouds at temperatures above 21°.

REQUIREMENTS

Identification

Peanut oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	< 14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	< 0.1	< 0.2	6-15	< 1.0	1.3-6.5	36-72	13-45

Fatty Acid:	18:3	20:0	20:1	22:0	22:1	24:0
Weight % (Range):	< 2.0	< 1.0-2.5	0.5-2.1	1.5-4.8	< 0.1	1.0-2.5

Arsenic (as As) Not more than 0.5 mg/kg.
Color (Lovibond) Not more than 5.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 84 and 100.
Lead Not more than 0.1 mg/kg.
Linolenic Acid Not more than 1.5%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 μg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factor (e) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$.

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Linolenic Acid Proceed as directed under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Poloxamer 331, page 227

Change the *Test* entitled *pH*, page 228, to read:

pH Prepare a 2.5% solution in water and determine the pH by the *Potentiomentric Method*, page 531.

Poloxamer 407, page 228

Change the *Test* entitled *pH*, page 229, to read:

pH Prepare a 2.5% solution in water and determine the pH by the *Potentiomentric Method*, page 531.

Insert the following two new monographs to precede the monograph entitled *Polyethylene*, page 229:

Polydextrose

DESCRIPTION

A randomly bonded condensation polymer of D-glucose with some bound sorbitol and citric acid. The 1,6-glycosidic linkage predominates in the polymer but all other possible bonds are present. The product contains small quantities of free glucose, sorbitol and 1,6-anhydro-D-glucose (levoglucosan) with a trace of citric acid. It may be neutralized and/or decolorized. It is an off-white to light tan colored solid, very soluble in water.

GENERAL REQUIREMENTS

Identification

A. To 1 drop of a 1-in-10 aqueous solution of sample, add 4 drops of 5% aqueous phenol solution, then rapidly add 15 drops of concentrated sulfuric acid. A deep yellow to orange color is produced.

B. With vigorous swirling (vortex mixer), add 1.0 ml of acetone to 1.0 ml of a 1-in-10 aqueous solution of sample. The solution remains clear.

C. With vigorous swirling, add 2.0 ml of acetone to the solution from B. A heavy, milky turbidity develops immediately.

D. To 1 ml of a 1-in-50 aqueous solution of sample, add 4 ml of alkaline cupric citrate TS. Boil vigorously 2-4 min. Remove from heat and allow the precipitate (if any) to settle. The supernatant is blue or blue-green.

Assay Not less than 90.0% polymer calculated on the anhydrous, ash free basis.

Arsenic (as As) Not more than 3 mg/kg.

Heavy Metals (as Pb) Not more than 10 mg/kg.

5-Hydroxymethylfurfural Not more than 0.1% calculated on the anhydrous, ash free basis.

Molecular Weight Limit Passes test.

Monomers

1,6-Anhydro-D-Glucose Not more than 4.0% calculated on the anhydrous, ash free basis.

Glucose Not more than 4.0% calculated on the anhydrous, ash free basis.

Sorbitol Not more than 2.0% calculated on the anhydrous, ash free basis.

Water Not more than 4.0%.

ADDITIONAL REQUIREMENTS

Polydextrose (untreated)

pH of a 10 Percent Solution Between 2.5 and 3.5.

Residue on Ignition Not more than 0.3%.

Polydextrose (neutralized/decolorized)

pH of a 10 Percent Solution Between 5.0 and 6.0.

Residue on Ignition Not more than 3.0%.

TESTS

General Requirements

Assay

Glucose Standard Solutions Weigh accurately 100 mg of α-D-glucose (National Bureau of Standards) into a 500-ml volumetric flask and make up to volume with distilled water. Dilute five aliquots of the solution with distilled water to obtain the following concentrations of standard: 50, 40, 20, 10, and 5 μg/ml.

Phenol Solution Add 20 ml of water to 80 g of phenol.

Procedure Weigh accurately approximately 250 mg of the sample into a 250-ml volumetric flask and make up to volume with distilled water. Transfer a 10.0-ml aliquot to a 250-ml volumetric flask and dilute to volume with distilled water. Proceed as in *Standard Curve*. Calculate the percentage of polymer (P) by the formula:

$$P = 1.05\left[100\,\frac{(A - Y)}{S \times C}\right] - P_g - 1.11\,P_l,$$

in which A is the sample absorbance, Y is the y intercept of the standard curve, S is the slope of absorbance versus glucose concentration in g/ml obtained from the *Standard Curve* ($S \simeq 0.02$), C is the concentration of the sample solution in g/ml (adjusted for ash and moisture), and P_g and P_l are the percentages of glucose and levoglucosan determined respectively in the *Assay for Monomers*.

Standard Curve On a daily basis, pipette 2.0 ml of each of the *Glucose Standard Solutions* into 4-dram acetone-free screw-cap vials. Add 0.12 ml of the phenol solution and mix gently. Uncap each vial and add rapidly 5.0 ml of concentrated sulfuric acid. Immediately recap each vial and shake vigorously. (Caution: rubber gloves and a safety shield should be used in the sulfuric acid addition step.) Let the vials stand at room temperature for 45 min then determine the absorbance of each sample at 490 nm in a suitable spectrophotometer, using a *Phenol Solution* sulfuric acid reagent blank in the reference cell. Plot mean absorbances versus concentrations in μg/ml obtained from triplicate samples.

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 2.0-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)*.

5-Hydroxymethylfurfural Transfer approximately 1 g of the sample, accurately weighed, to a 100-ml volumetric flask and make up to volume with distilled water. Read the absorbance of this solution against a water blank at 283 nm in a 1-cm quartz cell in a spectrophotometer. Calculate the percentage of 5-hydroxymethyl furfural by the formula:

$$\%\ \mathrm{HMF} = (0.749 \times A)/C,$$

in which A is the absorbance of the sample solution and C is the concentration of the sample solution in mg/ml corrected for ash and moisture.

Molecular Weight Limit

Apparatus Chromatographic separations are conducted in a 25-mm × 250-mm glass chromatographic column equipped at the upper end with a removable 1-L solvent reservoir and at the lower end with a low dead-volume fitting accommodating 1-mm id tubing. Eluent is supplied to the column by gravity and pumped from the bottom of the column with a precision, low dead-volume, reciprocating piston pump capable of providing flow rates between 2 ml/min and 3 ml/min reproducible to one percent. Flow pulsations are damped by installing a blanked-off 1/8-in. by 36-in. stainless steel tube on the pump outlet line. Connections to and from the pump are made with 1.0 mm id silicone, Teflon, or stainless steel tubing. Eluent is pumped to a low dead-volume recording differential refractometer having a sensitivity of at least 4 × 10^{-4} refractive index units full scale. Noise attributable to the detector and electronics should be less than 0.5% of full scale. Maintain the detector cell at a constant temperature of 30 ± 0.1°.

Buffer 0.05 *N* Ammonium carbonate containing 0.02% sodium azide in degassed distilled water (pH 8.8). Dissolve 14.25 g of ammonium carbonate and 1.0 g of sodium azide in 5 L of degassed water.

Standard Solution Transfer 20 mg of Blue Dextran 2000 and 20 mg of sodium chloride to a 10-ml volumetric flask; dissolve in and dilute to volume with *Buffer.*

Column Preparation Swell 15 g of Sephadex G-50 in 300 ml of water for 3 h. Pack the chromatographic column with the gel suspension to a height of 230 mm. Equilibrate by eluting with *Buffer* for 24 h at 1.0 ml/min. After adjusting the gel height to 220 mm, place a circle of filter paper on the upper surface.

Column Standardization With the eluent reservoir disconnected, drain the column slowly until the liquid level falls just below the top of the gel. Add a 1-ml aliquot of *Standard Solution* to the column and start the pump and recorder. After the *Standard Solution* flows into the column, add a 1-ml aliquot of *Buffer* twice, allowing each to flow completely into the gel. Add an additional 1 ml of *Buffer* to the top of the column, connect the eluent reservoir, and record the monitoring curve. Adjust the refractometer so that elution of each standard peak causes a maximum recorder deflection of about 50% of full scale. Set the pump to give a flow rate of 2.3-2.4 ml/min. Mark on the curve the positions where Blue Dextran can first be detected (V_o), where the peak recorder deflection for sodium chloride occurs (S_p), and *M*, a point 23.0% of the distance from V_o to S_p, corresponding to a molecular weight of 22,000 Daltons.

System Suitability Carry out the *Column Standardization* five times, record the value of *M* in minutes and calculate an average value, $\bar{M}$. Calculate the volume of eluent corresponding to a molecular weight of 22,000 Daltons, V_e, by multiplying $\bar{M}$ by the flow rate in ml/min. The analytical system is suitable for conducting the *Procedure* if the value of V_e is within the range of 92.0 ± 9.2 ml.

Procedure Weigh accurately 500 mg of sample into a 50-ml volumetric flask; dissolve in and dilute to volume with *Buffer*. Apply a 1-ml aliquot of the *Sample Solution* using the same conditions and procedure under *Column Standardization* above and record the chromatogram. Peak recorder deflection should be 50-80% of full scale. Mark the positions V_o and *M* on the chromatogram as described under *Column Standardization*, above. There is no significant peak exhibiting a ratio of baseline signal to peak height less than 0.02 between V_o and *M*.

Monomers

Apparatus Use a suitable gas chromatograph equipped with a 250-cm × 2-mm (id) glass column packed with 3 percent OV-1 stationary phase on 100/120 mesh Gas Chrom Q and a flame ionization detector. Maintain the column at 175°, the injection port at 210°, and the detector at 230°. Relative retention times (min): 1,6-anhydro-D-glucose (levoglucosan), pyranose form (3.7), furanose form (not present in standard) (4.3); *n*-octadecane (5.1); α-D-glucose (8.7); D-sorbitol 11.3; β-D-glucose (13.3).

Standard Solution Weigh accurately 50 mg of α-D-glucose (National Bureau of Standards), 40 mg of anhydrous D-sorbitol, and 35 mg of 1,6-anhydro-D-glucose into a 100-ml volumetric flask; dissolve in and dilute to volume with pyridine.

Octadecane Solution Transfer 50 mg of *n*-octadecane, accurately weighed, to a 100-ml volumetric flask; dissolve in and dilute to volume with pyridine.

Silylation of Standard Solution Transfer 1.0 ml of *Standard Solution* to a screw-cap vial and add 1.0 ml of *Octadecane Solution*, and 0.5 ml of *N*-trimethylsilylimidazole. Cap the vial and immerse it in an ultrasonic bath at 70° for 60 min.

Procedure Accurately weigh 20 mg of the sample into a screw-cap vial and add 1.0 ml of *Octadecane Solution*, 1 ml of pyridine, and 0.5 ml of *N*-trimethylsilylimidazole. Cap the vial and immerse it in an ultrasonic bath at 70° for 60 min. Prior to sample analysis, inject 3 µl of the silylated *Standard Solution* into the gas chromatograph. Repeat twice, then inject duplicate 3-µl portions of the sample solution. Calculate the percentage of each monomer (P_M) by the formula:

$$P_M = (R \times W_s)/(R_s \times W)$$

in which W_s is the weight of the sample in mg, adjusted for ash and moisture; W is the weight, in mg, of the respective monomer in the *Standard Solution; R* is the ratio of the area of the monomer peak to the area of the octadecane peak in the sample injection; R_s is the mean ratio of the area of the monomer peak to the area of the octadecane peak in the standard injections. In the case of glucose, the peak areas for the α and β epimers, and in the case of levoglucosan, the peak areas for the pyranose form and furanose form are combined.

Water Determine by the *Karl Fischer Titrimetric Method*, page 552, using pyridine instead of methanol in the titration vessel.

Additional Requirements

pH of a Solution of 10% Solids Content Determine by the *Potentiometric Method*, page 531.

Residue on Ignition Determine as directed in *Method I* under *Residue on Ignition*, page 533.

Packaging and Storage Store in tight, light-resistant containers.

Functional Use in Foods Bulking agent; formulation aid; humectant; texturizer.

Polydextrose Solution

DESCRIPTION

A neutralized, decolorized water solution of polydextrose. It is a clear, straw-colored liquid.

REQUIREMENTS

Identification

A. To 1 drop of a 1-in-10 aqueous solution of sample, add 4 drops of 5% aqueous phenol solution, then rapidly add 15 drops of concentrated sulfuric acid. A deep yellow to orange color is produced.

B. With vigorous swirling (vortex mixer), add 1.0 ml of acetone to 1.0 ml of a 1-in-10 aqueous solution of sample. The solution remains clear.

C. With vigorous swirling, add 2.0 ml of acetone to the solution from B. A heavy, milky turbidity develops immediately.

D. To 1 ml of a 1-in-50 aqueous solution of sample, add 4 ml of alkaline cupric citrate TS. Boil vigorously 2-4 min. Remove from heat and allow the precipitate (if any) to settle. The supernatant liquid is blue or blue-green.

Assay Not less than 90.0% polymer calculated on the anhydrous, ash free basis.

Arsenic (as As) Not more than 3 mg/kg.

Heavy Metals (as Pb) Not more than 10 mg/kg.

5-Hydroxymethylfurfural Not more than 0.1% calculated on the anhydrous, ash free basis.

Molecular Weight Limit Passes test.

Monomers

1,6-Anhydro-D-Glucose Not more than 4.0% calculated on the anhydrous, ash free basis.

Glucose Not more than 4.0% calculated on the anhydrous ash free basis.

Sorbitol Not more than 2.0% calculated on the anhydrous, ash free basis.

pH of a 10 Percent Solution Between 5.0 and 6.0.

Residue on Ignition Not more than 2.0%.

Water Within the range 27.5-32.5%.

TESTS

Assay Weigh accurately approximately 360 mg of *Polydextrose Solution* and proceed as in *Assay* under *Polydextrose,* page 58 of THIS SUPPLEMENT.

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 2.0 g sample as directed in *Method II* under the *Heavy Metals Test,* page 920, using 20 µg of lead ion (Pb) in the control *(Solution A).*

5-Hydroxymethylfurfural Proceed as directed under *Polydextrose* using a 1.4-g sample, accurately weighed, page 58 of THIS SUPPLEMENT.

Molecular Weight Limit Weigh accurately 720 mg of the sample and proceed as under *Molecular Weight Limit* in the *Polydextrose* monograph, page 58 of THIS SUPPLEMENT.

Monomers

1-6,Anhydro-D-glucose; Glucose; and Sorbitol Weigh accurately 30 mg of the sample into a screw-cap vial and add about 2 ml of pyridine. While flushing the vial with a stream of dry air or nitrogen, heat at 80°-90° until the solution volume is reduced to 0.2-0.5 ml. Add a second portion of pyridine and repeat the evaporation procedure. Continue as described under *Monomers* in the *Polydextrose* monograph, page 59 of THIS SUPPLEMENT.

pH of a 10 Percent Solution Dilute a 1.4-g sample to 10 ml with water and mix. Proceed as directed under the *Potentiomentric Method,* page 531.

Residue on Ignition Determine as directed in *Method II* under *Residue on Ignition,* page 533.

Water Transfer 1-2 ml of the sample into a dropper vial and accurately weigh dropper, vial and sample combined. Add 50 ml of pyridine to a clean, dry, reaction jar previously flushed with dry air for 1 min. Titrate the pyridine with Karl Fischer reagent to the endpoint to consume any water present. Transfer one drop of sample (50-100 mg) from the weighed sample vial to the reaction jar. Accurately reweigh the dropper, vial and remaining sample. Stir the pyridine-sample mixture for 5-10 min. Titrate with Karl Fischer reagent to the endpoint. For each determination calculate the percentage water (W) in the sample by the formula:

$$W = (V \times F \times 100)/S,$$

in which S is the sample weight, in mg, equal to the difference between the initial and final weighings of the dropper, vial and sample combination; V is the volume of Karl Fischer reagent consumed in the second titration, in ml; F is the Karl Fischer reagent standardization factor, in mg/ml. Calculate the water content of the sample as the average of two determinations.

Functional Use in Foods Bulking agent; formulation aid; humectant; texturizer.

Packaging and Storage Store in tight, light-resistant containers.

Insert the following new monograph to precede the monograph entitled *Sage Oil, Dalmatian Type,* page 267:

Safflower Oil (Unhydrogenated)

DESCRIPTION

A light yellow oil obtained from the plant *Carthamus tinctorius* by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21°-27°, but traces of wax may cause the oil to cloud unless removed by winterization. Safflower oil has the highest linoleic acid *(cis, cis*-9,12-octadecadienoic acid) content (typically about 78% of total fatty acids) of any known oil. It is free from visible foreign matter at 21°-27°.

REQUIREMENTS

Identification

Safflower oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition,* page 82 of THIS SUPPLEMENT.

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<1.0	2-10	<0.5	1-10	7.0-42	72-81

Fatty Acid:	18:3	20:0	20:1
Weight % (Range):	<1.5	<0.5	<0.5

Arsenic (as As) Not more than 0.5 mg/kg.
Cold Test Passes test.
Color (Lovibond) Not more than 1.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 135 and 150.
Lead Not more than 0.1 mg/kg.
Linoleic Acid Not less than 72% of total fatty acids.
Linolenic Acid Not more than 1.5%.
Peroxide Value Not more than 10 meq/kg.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 μg As) when treated in the same manner and under the same conditions as the sample.

Cold Test Proceed as directed under *Cold Test*, page 82 of THIS SUPPLEMENT.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factor (e) in the formula given in the procedure:

$$\text{Free fatty acids as oleic acid, } e = 28.2$$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Linoleic Acid Proceed as directed under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Linolenic Acid Proceed as directed under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Insert the following new monograph to precede the monograph entitled *Spearmint Oil*, page 309:

Soybean Oil (Unhydrogenated)

DESCRIPTION

A light amber-colored oil that is obtained from the seed of the legume *Glycine max*, usually by solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21°-27° and remains so even at refrigerator temperatures (2°-4°). It is free from visible foreign matter at 21°-27°.

REQUIREMENTS

Identification

Soybean oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<0.5	7.0-12	<0.5	2.0-5.5	19-30	48-58

Fatty Acid:	18:3	20:0	20:1
Weight % (Range):	5-9	<1.0	<1.0

Arsenic (as As) Not more than 0.5 mg/kg.
Cold Test Passes test.
Color (Lovibond) Not more than 20 yellow/2.0 red.
Free Fatty Acids (as oleic acid) Not more than 0.1%.
Iodine Value Between 120 and 143.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.
Stability (Active Oxygen Method) Not less than 7 h.
Unsaponifiable Matter Not more than 1.5%.
Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 μg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Cold Test Proceed as directed under *Cold Test*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factor (e) in the formula given in the procedure:

$$\text{Free fatty acids as oleic acid, } e = 28.2$$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Stability (Active Oxygen Method) Proceed as directed under *Stability*, page 83 of THIS SUPPLEMENT.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Insert the following new monograph to precede the monograph entitled *Talc*, page 318:

Sunflower Oil (Unhydrogenated)

DESCRIPTION

A light amber-colored oil obtained from the seed of the sunflower plant *Helianthus annuus* by mechanical expression or solvent extraction. It is refined, bleached, and deodorized to substantially remove free fatty acids, phospholipids, color, odor and flavor components, and miscellaneous other non-oil materials. It is a liquid at 21°-27°, but traces of wax may cause the oil to cloud, unless removed by winterization.

REQUIREMENTS

Identification

Sunflower oil exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	<14	14:0	16:0	16:1	18:0	18:1	18:2
Weight % (Range):	<0.1	<0.5	3.0-10	<1.0	1.0-10	14-65	20-75

Fatty Acid:	18:3	20:0	20:1
Weight % (Range):	<1.5	<1.0	<0.5

Arsenic (as As) Not more than 0.5 mg/kg.

Cold Test Passes test.

Color (Lovibond) Not more than 1.3 red.

Free Fatty Acids (as oleic acid) Not more than 0.1%.

Iodine Value Between 110 and 143.

Lead Not more than 0.1 mg/kg.

Linolenic Acid Not more than 1.5%.

Peroxide Value Not more than 10 meq/kg.

Unsaponifiable Matter Not more than 1.5%.

Water Not more than 0.1%.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 μg As) when treated in the same manner and under the same conditions as the sample.

Cold Test Proceed as directed under *Cold Test*, page 82 of THIS SUPPLEMENT.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factor (e) in the formula given in the procedure:

$$\text{Free fatty acids as oleic acid, } e = 28.2$$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Linolenic Acid Proceed as directed under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

Insert the following new monograph to precede the monograph entitled *Tangerine Oil, Coldpressed*, page 319:

Tallow

DESCRIPTION

An off-white fat obtained by heat rendering of tissues (cuttings and trimmings) from beef and, to a lesser degree, mutton shortly after slaughter. Rendered tallow may be alkali refined and bleached, or bleached and deodorized without prior refining. It is a firm fat containing a high proportion of saturated fatty acids and exhibiting greater flavor stability than lard or unhydrogenated vegetable oils.

Rendered, alkali refined, and bleached-deodorized tallow are white to off-white solids at 21°-27°. Alkali refined and bleached-deodorized tallow, which are pale yellow to colorless and free of extraneous matter at 54°, differ from rendered tallow, which is clear to hazy and may contain extraneous matter.

SPECIFIC REQUIREMENTS

	Rendered Tallow	*Alkali Refined Tallow*	*Bleached and Deodorized Tallow*
Color (Lovibond)	Not more than 3.0 red	Not more than 1.5 red	Not more than 1.5 red
Free Fatty Acids (as oleic acid)	Not more than 1.5%	Not more than 0.5%	Not more than 0.1%
Insoluble Matter	Not more than 0.1%	Not more than 0.01%	Not more than 0.01%
Iodine Value	Between 37 and 50	Between 37 and 50	Between 37 and 50
Water	Not more than 0.5%	Not more than 0.2%	Not more than 0.1%

GENERAL REQUIREMENTS

Identification

Tallow exhibits the following composition profile of fatty acids as determined under *Fatty Acid Composition*, page 82 of THIS SUPPLEMENT.

Fatty Acid:	< 14:0	14:0	14:1	15:0	15:0 iso	16:0
Weight % (Range):	< 0.1	1.4-6.3	0.5-1.5	0.5-1.0	< 1.5	20-37
Fatty Acid:	16:0 iso	16:1	16:2	17:0	17:1	18:0
Weight % (Range):	< 0.5	0.7-8.8	< 1.0	0.5-2.0	< 1.0	6-40
Fatty Acid:	18:1	18:2	18:3	20:0	20:1	20:4
Weight % (Range):	26-50	0.5-5.0	< 2.5	< 0.5	< 0.5	< 0.5

Arsenic (as As) Not more than 0.5 mg/kg.
Lead Not more than 0.1 mg/kg.
Peroxide Value Not more than 10 meq/kg.

TESTS

Arsenic A *Sample Solution* prepared using 2 g of sample, accurately weighed, meets the requirements of the *Arsenic Test*, page 464. The absorbance due to any red color from the solution of the sample does not exceed that produced by 1.0 ml of *Standard Arsenic Solution* (1 µg As) when treated in the same manner and under the same conditions as the sample.

Color Proceed as directed under *Color*, page 82 of THIS SUPPLEMENT.

Free Fatty Acids Proceed as directed under *Free Fatty Acids*, page 504, using the following equivalence factor (*e*) in the formula given in the procedure:

Free fatty acids as oleic acid, $e = 28.2$

Iodine Value Proceed as directed under *Iodine Value*, page 505.

Lead Proceed as directed under *Coconut Oil*, page 41 of THIS SUPPLEMENT.

Melting Range Proceed as directed under *Melting Range*, page 82 of THIS SUPPLEMENT.

Peroxide Value Proceed as directed under *Peroxide Value*, page 148 of the monograph for *Hydroxylated Lecithin*. However, after the addition of saturated potassium iodide and mixing, instead of allowing the solution to stand for 10 min, mix the solution for 1 min and begin the titration immediately.

Unsaponifiable Matter Proceed as directed under *Unsaponifiable Matter*, page 509.

Water Proceed as directed under *Water Determination*, page 553. However, in place of 35-40 ml of methanol use 50 ml of chloroform to dissolve the sample.

Packaging and Storage Store in well-closed containers.

Functional Use in Foods Coating agent; emulsifying agent; formulation aid; texturizer.

TBHQ, page 321

In the first sentence of the *Test* entitled *2,5-Di-t-butylhydroquinone and Hydroquinone*, page 322, replace page 000 with page 475.

Replace the first sentence of the last paragraph of the *Test* entitled *Ultraviolet Absorbance*, page 323, with the following:

Quantitatively wash the residue from the distillation flask into a 50-ml volumetric flask with isooctane, dilute to volume with isooctane, and mix.

Triethyl Citrate, page 339

Replace the last sentence of the *Test* entitled *Assay* with the following:

Each ml of 0.5 *N* sodium hydroxide is equivalent to 46.05 mg of $C_{12}H_{20}O_7$.

3/ *Specifications for Flavor Aromatic Chemicals and Isolates*

3-Acetyl-2,5-dimethyl Furan, page 354

(2,5-Dimethyl-3-acetylfuran)

[FEMA No. 3391]

Change the name to *3-Acetyl-2,5-dimethylfuran.*

Anethole, page 356

(*p*-Propenylanisole)

[FEMA No. 2086]

Change the *Ref. Index,* page 357, from 1.557-1.561 to 1.557-1.562.

Butyl Alcohol, page 360

(1-Butanol)

[FEMA No. 2178]

Under *Assay Min. %,* page 361, insert 99.5%.

Under *Other Requirements,* page 361, insert *Butyl Ether* — 0.15% (M-19).

Cinnamyl Formate, page 364

[FEMA No. 2299]

Change the *Sp. Gr.,* page 365, from 1.074-1.079 to 1.077-1.082.

Cinnamyl Propionate, page 364

[FEMA No. 2301]

Change the *Sp. Gr.,* page 365, from 1.029-1.033 to 1.029-1.035.

Citronellal, page 366

(3,7-Dimethyl-6-octen-1-al)

[FEMA No. 2307]

Change the *Synonym* to (3,7-Dimethyl-6-octenal).

Cuminic Aldehyde, page 366

(*p*-Cuminic Aldehyde; Cumaldehyde; *p*-Isopropylbenzaldehyde; Cuminal)

[FEMA No. 2957]

Change the FEMA No. to 2341.

Cyclamen Aldehyde, page 368

[2-Methyl-3-(*p*-isopropylphenyl)-propionaldehyde]

[FEMA No. 2743]

Change the *Synonym* to 2-Methyl-3-(*p*-isopropylphenyl)propionaldehyde.

Δ-Decalactone, page 368

[FEMA No. 2361]

Change the name to δ-*Decalactone*.

trans-2-Decen-1-al, page 368

[FEMA No. 2366]

Change the name to *trans-2-Decenal*.

Change the *Assay Min, %*, page 369, from 99% of $C_{10}H_{18}O$ (M-8a) to 92% of $C_{10}H_{18}O$ (M-8a).

Change the *Ref. Index*, page 369, from 1.454-1.458 to 1.452-1.457.

cis-4-Decen-1-al, page 368

[FEMA No. 3264]

Change the name to *cis-4-Decenal*.

Change the *Assay Min, %*, page 369 from 95% $C_{10}H_{18}O$ (M-8a) to 90% of $C_{10}H_{18}O$ (M-8a).

Δ-Dodecalactone, page 372

[FEMA No. 2401]

Change the name to δ-*Dodecalactone*.

Estragole, page 372

(*p*-Allylanisole)

[FEMA No. 2411]

Change the *Ref. Index*, page 373, from 1.517-1.522 to 1.519-1.524.

Change the *Sp. Gr.*, page 373, from 0.957-0.965 to 0.960-0.968.

2-Ethyl Fenchol, page 376

[FEMA No. 3491]

Change the *Assay Min, %*, page 377, from 98% $C_{12}H_{22}O$ (M-8a) to 95% $C_{12}H_{22}O$ (M-8a).

Change the *Ref. Index*, page 377, from 1.470-1.491 (26°) to 1.470-1.491.

Isobornyl Acetate, page 388

[FEMA No. 2160]

Change the *Sp. Gr.*, page 389, from 0.980-0.984 to 0.979-0.984.

Change *Other Requirement*, page 389, from *Angular rotation*—between −1° and +1° to between −4° and 0°.

Isobutyl Alcohol, Page 390

[FEMA No. 2179]

Insert 98% (M-8a) under *Assay Min, %*, page 391.

2-Methylbutyl Isovalerate, page 398

(2-Methylbutyl-3-methylbutanoate)

[FEMA No. 2753]

Change the FEMA No. to 3506.

Terpineol, page 416

(Menthen-1-ol-8)

[FEMA No. 3045]

Change the name to α-*Terpinol*.

Change the *Synonym* to *p*-Menth-1-en-8-ol.

New flavor aromatic chemicals and isolates follow on page 66.

General Information and Description

Name of Substance (Synonyms)	Mol Wt/Formula/ Structure	Physical Form/Odor	Solubility/B.P.	GLC Profile	Solubility in Alcohol
2-Acetyl Pyrazine [FEMA No. 3126]	122.13/$C_6H_6N_2O$	Colorless to pale yellow crystals/popcornlike			
Amyl Heptanoate [FEMA No. 2073]	200.32/$C_{12}H_{24}O_2$	Colorless to pale yellow liq/fruity			
Benzaldehyde Glyceryl Acetal [FEMA No. 2129]	180.20/$C_{10}H_{12}O_3$	Colorless to pale yellow liq/mild almond odor			
Butyl Phenylacetate [FEMA No. 2209]	192.26/$C_{12}H_{16}O_2$	Colorless to pale yellow liq/honey, roselike			
***p*-Cymene** [FEMA No. 2356]	134.22/$C_{10}H_{14}$	Colorless to pale yellow liq no odor			
2,6-Dimethyl-5-heptenal [FEMA No. 2389]	140.23/$C_9H_{16}O$	Pale yellow liq/melon-like			
Fusel Oil Refined [FEMA No. 2497]		Colorless to pale yellow liq no odor			
Isoamyl Benzoate [FEMA No. 2058]	192.26/$C_{12}H_{16}O_2$	Colorless to pale yel liq/pungent fruitlike odor			
4-*p*-Methoxyphenyl-2-butanone (Anisylacetone) [FEMA No. 2672]	178.23/$C_{11}H_{14}O_2$	Colorless to pale yel liq/sweet, floral, fruity			
6-Methylcoumarin [FEMA No. 2690]	160.17/$C_{10}H_8O_2$	White crystalline solid/coconutlike			
Methyl Hexyl Ketone (2-Octanone) [FEMA No. 2802]	128.22/$C_8H_{16}O$	Colorless to pale yel liq/applelike			
δ-Nonalactone (5-Hydroxynonanoic acid, lactone) [FEMA No. 3356]	156.23/$C_9H_{16}O_2$	Colorless to pale yel liq/coconutlike			
δ-Octalactone (5-Hydroxyoctanoic acid, lactone) [FEMA No. 3214]	142.20/$C_8H_{14}O_2$	Colorless to pale yellow liquid/coconutlike			

Requirements

I.D. Test	Assay Min. %	A.V. Max	Ref. Index	Sp. Gr.	Other Requirements
	99% (M-8a)				Melting Range—75° to 78° (p. 519)
	97% (M-8a) sum of isomers	1.0	1.422–1.426		0.859–0.863
	95% (M-8a) sum of isomers	2.0	1.535–1.541		1.183–1.193
	98% (M-8a)	1.0	1.488–1.492		0.990–0.997
	98% (M-8a)		1.489–1.491		0.853–0.855
	85% (M-8a)	1.0	1.443–1.448		0.852–0.858
	95% Min. 2 & 3 Methyl butanol (M-8a)		1.405–1.410	0.807–0.813	Angular Rotation— −0.5 to −2.0 (p. 530)
	98% (M-8a) sum of isomers	1.0 max.	1.492–1.496		0.986–0.992
	98% (M-8a)		1.517–1.521		1.042–1.048
	99% (M-8a)				Melting Range—73° to 76° (p. 519)
	95% (M-8a)	1.0 max.	1.414–1.418		0.813–0.818
	98% (M-8a)		1.452–1.458		0.980–0.985
	98% (M-8a)		1.452–1.458		0.994–0.999

4/ Test Methods for Flavor Aromatic Chemicals and Isolates

M-19 Gas Chromatographic Analysis of Butyl and Isobutyl Alcohols, page 430

Replace the test *M-19, Gas Chromatographic Analysis of Butyl and Isobutyl Alcohols,* with the following:

Determine the percentages of butyl ether, isobutyl alcohol, butyl alcohol, and other alcohols in the sample by gas-liquid chromatography (see page 475), using an instrument containing a flame ionization detector. Prepare a 6-m × 3.2-mm column consisting of 15 percent Igepal CO-990 on 60/80-mesh Chromosorb W (AW) and program the temperature from 80° to 240° at 4° per min. Use a removable glass or aluminum tubing liner in the injection port. With the inlet pressure adjusted to 90 psi, establish a helium flow rate which will elute butyl alcohol at approximately 10 min and inject a sample of about 0.5 µl. Under the conditions described, butyl ether will elute at about 6 min, butyl alcohol at about 10 min, 3-methylbutanol at about 12 min, and *n*-pentanol at about 14 min. Chromatograph standards containing about 1% each of the minor components in isobutyl alcohol and 1% each of the minor components in butyl alcohol to verify retention times. *In butyl alcohol samples,* the area of the butyl alcohol peak is not less than 99.5% of the total area of all peaks, the area of the butyl ether peak is not more than 0.15% of the total area of all peaks, and the sum of the areas of the other alcohols is not more than 0.20% of the total area of all peaks. *In isobutyl alcohol samples,* the area of the isobutyl alcohol peak is not less than 99.4% of the total of all peaks.

5/ GLC Analysis of Flavor Aromatic Chemicals and Isolates

No Change.

6/ General Tests and Apparatus

Insert the following new test to precede the test entitled *Alginates Assay*, page 463:

Acid-Insoluble Matter in Anhydrous Calcium Chloride

Place a lintine disc filter* in a suitable filter assembly (Fig. 33), and wash with 100 ml of diluted acetic acid (1 in 300), followed by 100 ml of water. Remove the disc from the assembly, place on a watch glass, and dry the combination at 105° for 2 h.

Dissolve 1 kg of sample in 3 L of water containing 10 ml of glacial acetic acid. Allow to cool, and filter through the lintine disc. Rinse the walls of the filter assembly so that all insoluble matter is transferred to the disc, and wash with 100 ml of water. Place the disc on the same watch glass mentioned above, and dry at 105° for 2 h, being careful at all times not to lose any particles which may be on the disc. The difference in the two weights is the weight of the acid-insoluble matter.

Place the disc under a low power magnifier (4× to 10× magnification). Using a millimeter rule, measure the largest dimension of each particle (or as many as may be necessary) on the disc. No particles greater than 2 mm in any dimension are present.

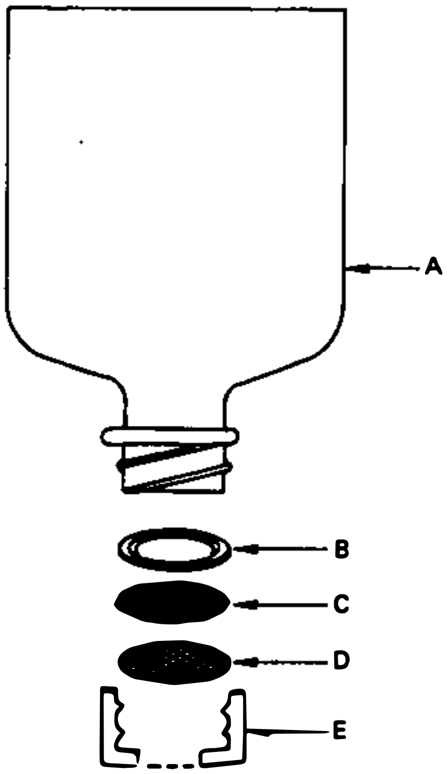

FIGURE 33 Filter Assembly for the Determination of Acid-Insoluble Matter (A, 2.5 L bottle cut in half; B, rubber washer-1 3/8" OD, 7/8" ID; C, lintine disc 1 1/4" diam; D, 20 mesh stainless steel screen-1 3/8" OD; E, bottle cap with 7/8" hole in top).

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* Available from Filter Fabrics, Inc., 814 E. Jefferson, Goshen, Indiana 46526

Insert the following new test to precede the test entitled *Calcium Pantothenate Assay*, page 466.

Benzene (in Paraffinic Hydrocarbon Solvents)

(Based on ASTM Method D2600-72)

Apparatus Use a suitable gas chromatograph (see page 475), equipped with a column that will elute *n*-decane before benzene under the conditions of the *System Suitability Test* (see below). Column materials and conditions that have been found suitable for this method are listed in the accompanying Tables. See Figure 34 for a typical chromatogram obtained with column No. 5.

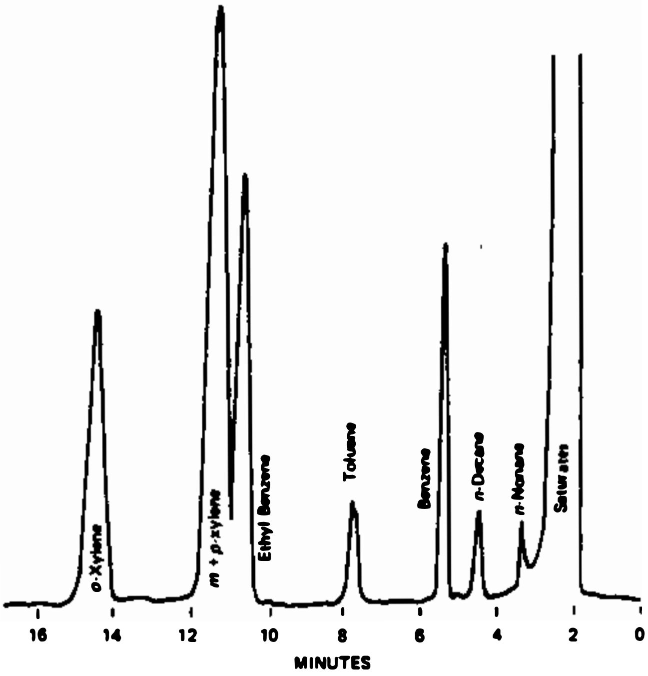

FIGURE 34 Typical Chromatogram for the Determination of Benzene in Hexanes Using Column No. 5.

Reagents

Isooctane 99 mole percent minimum containing less than 0.05 mole percent aromatic material.

Benzene 99.5 mole percent minimum.

Internal Standard *n*-Decane and either *n*-undecane or *n*-dodecane according to the requirement of the *System Suitability Test*.

Reference Solution A Prepare a standard solution containing 0.5% by weight each of the *Internal Standard* and benzene in isooctane.

Reference Solution B Prepare a standard solution containing about 0.5% by weight each of *n*-decane, *Internal Standard*, and benzene in isooctane.

Calibration Select the instrument conditions necessary to give the desired sensitivity. Inject a known volume of *Reference Solution A* and change the attenuation, if necessary, so that the benzene peak is measured with a chart deflection of not less than 25% nor more than 95% of full scale. When choosing the attenuation, consider all unresolved peaks to represent a single compound. There may be tailing of the nonaromatic peak, but do not use any conditions that lead to a depth of the valley ahead of the benzene peak (A) less than 50% of the height of the benzene peak (B) as depicted in Figure 35.

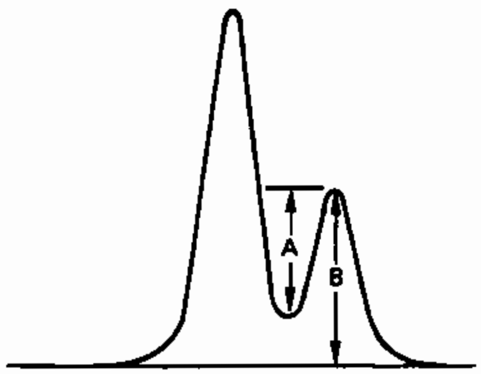

FIGURE 35 Illustration of *A/B* Ratio.

If there is tailing of the nonaromatic material, construct a baseline by drawing a line from the bottom of the valley ahead of the benzene peak to the point of tangency after the peak (see Fig. 36). Measure the areas of the benzene peak and the internal standard peak by any of the following means: triangulation, planimeter, paper cutout, or mechanical or electronic integrator. Do not use integrators on peaks without a constant baseline, unless the integrator has provision for making baseline corrections with accuracy at least as good as that of manual methods.

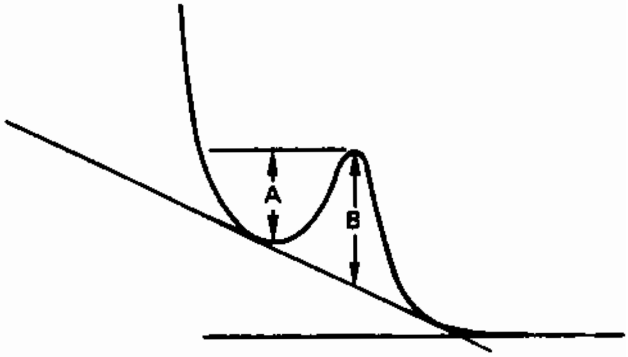

FIGURE 36 Illustration of *A/B* Ratio for a Small Component Peak on the Tail of a Large Peak.

Calculate a response factor for benzene (R_b) relative to the internal standard by the formula:

$$R_b = A_i/W_i \times W_b/A_b,$$

in which A_i is the area of the internal standard peak in arbitrary units corrected for attenuation; W_i is the weight percent of internal standard in *Reference Solution A;* A_b is the area of the benzene peak in arbitrary units corrected for attenuation; and W_b is the weight percent of benzene in *Reference Solution A.*

Procedure Place approximately 0.1 ml of *Internal Standard* in a tared 25-ml volumetric flask, weigh on an analytical balance; dissolve in and dilute to volume with the sample to be analyzed.

Using the exact instrumental conditions that were used in the calibration, inject the same volume of sample containing the *Internal Standard.* Before measuring the area of the *Internal Standard* and benzene peaks change the attenuation to assure at least 25% chart deflection.

Measure the area of the internal standard and benzene peaks in the same manner as was used for the calibration. Calculate the weight percent of benzene in the sample (W_B) by the formula:

$$W_B = (A_b \times R_b \times W_i \times 100)/(A_i \times S),$$

in which A_b is the area of the benzene peak corrected for attenuation; R_b is the relative response factor for benzene; W_i is the weight of *Internal Standard* added, in g; A_i is the area of the *Internal Standard* peak corrected for attenuation; and S is the weight of sample, in g.

System Suitability Test Inject the same volume of *Reference Solution B* as in the *Calibration* and record the chromatogram. *n*-Decane must be eluted before benzene, and the ratio of *A* to *B* (Figure 35) must be at least 0.5 where *A* is equal to the depth of the valley between the *n*-decane and benzene peaks and *B* is equal to the height of the benzene peak.

Column Materials and Conditions for the Determination of Benzene in Hexanes

Column No.	1	2	3	4	5	6	7
Liquid phase	CEF	PEF 200	CEF	DEGS	TCEPE	TCEPE	DEGS
Length, ft	15	6	16	10	15	100	12
m	4.5	2	5	3.1	—	31	3.7
Diameter, in (mm)							
Inside	0.07(1.8)	—	0.07	0.18(4.5)	0.06(1.5)	0.01(.254)	—
Outside	1/8(3.2)	1/4(6.4)	1/8	—	—	—	1/8
Weight, percent	17	30	20	20	10	—	20
Solid support	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Chromosorb P	Capillary	Chromosorb P
Mesh	60–80	60–80	60–80	80–100	60–80	—	80–100
Treatment	AW	AW	AW	none	AW	none	AW Sil
Inlet, deg	200	210	250	260	250	275	260
Detector, deg	200	155	250	200	175	250	240
Column, deg	115	95	90	100	115	95	65
Carrier gas	N_2	He	He	He	N_2	N_2	He
Flow rate, cm^3/min	30	60	60	60	1	3	52
Detector	Fl	TC	Fl	Fl	Fl	Fl	Fl
Recorder, mV	5	1	1	1	10	1	1
Sample, l	5	10	1	2	5	0.8	5
Split	9 + 1	—	—	—	100 + 1	100 − 1	—
Area	Tri	El	Dl	Tri Plan	El	El	Tri

Abbreviations used in Table

DEGS—Diethylene glycol succinate
Dl—Disk integrator
El—Electronic integrator
Fl—Flame ionization
CEF—*N,N*-Bis(2-cyanoethyl) formamide
Sil—Silanized
TC—Thermal conductivity
TCEPE—Tetracyanoethylated pentaerythritol
Tri—Triangulation
AW—Acid washed

Retention Times in Minutes for Selected Hydrocarbons Under the Conditions for the Determination of Benzene in Hexanes

Column No.	1	2	3	4	5	6	7
Benzene	3.4	2.0	6.5	6.7	5.4	6.1	6.7
Toluene	4.4	3.2	9.0	10.3	7.8	7.0	10.3
Ethylbenzene	5.4	5.2	11.5	14.8	10.8	8.0	14.8
p-m-Xylenes	5.8	—	12.5	—	11.4	8.5	—
o-Xylene	7.5	6.8	17.0	16.1	14.5	10.0	—
n-Undecane	3.0	2.8	3.5	—	—	—	—
n-Dodecane	—	—	—	12.8	8.5	6.5	—

Chromatography, page 471

Insert the following at the end of the section for *Quantitative Analysis*, page 476, immediately preceding *High-Pressure Liquid Chromatography* in the section on *Gas Chromatography:*

CALCULATION OF RESPONSE FACTORS

Area Normalization or *External Standard* When the use of a response factor (R) is stipulated, proceed as follows: Using a reference mixture containing those compounds likely to be found in the sample to be analyzed in proportions as close as possible to those expected, obtain a chromatogram under operating conditions identical with those used for the sample. Ideally, the sample and standard should be chromatographed one after the other. For this reference mixture:

$$P_W = (W_i/\Sigma W_i) \times 100,$$

in which P_W is the weight percent of component i, W_i is the weight of that component in the reference mixture, and ΣW_i is the sum of the weights of all components in the reference mixture.

Calculate the area percent of component (P_A) by the formula:

$$P_A = (A_i/\Sigma A_i) \times 100,$$

in which A_i is the area under the peak corresponding to component i and ΣA_i is the sum of the areas under all peaks. The response factor for component (R_i) is then found by the equation:

$$R_i = P_W/P_A = (W_i/A_i) \times (\Sigma A_i/\Sigma W_i).$$

Often, the concentrations of components in a mixture analyzed by GC are expressed relative to one component already present in the mixture; for example, to the amount of palmitic acid in a mixture of fatty acids, or dextrose in a mixture of saccharides. In such a case a relative response factor (R') is defined as R_i/R_r, in which R_i is the response factor of one component and R_r is the response factor of the reference component. R'_r will obviously be 1.0. Then the concentration (in weight percent) of each component of a mixture relative to the reference compound (P_i) is given by the formula:

$$P_i = (R'_i \times A_i \times 100)/\Sigma(R'_n \times A_n),$$

in which R'_i is the relative response factor for component i and A_i is the corresponding peak area; the denominator gives this product summed over all components in the mixture. This equation applies whether response factors (R_i) or relative response factors (R'_i) are used, but all components of the mixture must elute from the column and be detected.

Internal Standard For a reference mixture containing a weight proportion of internal standard (W_r) approximately equal to that of the component or compounds to be quantitated, the relative response factors (R'_i) are calculated from the equation:

$$R'_i = (W_i/W_r) \times (A_r/A_i),$$

in which W_i is the weight of component i in the reference mixture, W_r is the weight of internal standard added to the reference mixture, A_i is the area under the peak corresponding to component i, and A_r is the area under the peak corresponding to the internal standard.

For the sample, an appropriate weight of internal standard (W_{is}) is weighed into an amount of sample (W_{sp}) and the mixture is chromatographed. Calculate the weight percent (P_i) of component i using the formula:

$$P_i = [(A_i \times R'_i \times W_{is})/(A_{is} \times W_{sp})] \times 100,$$

in which A_i is the area under the peak corresponding to component i, R'_i is the relative response factor for component i, A_{is} is the area under the peak corresponding to the internal standard, W_{is} is the weight of internal standard, and W_{sp} is the weight of sample. Only components of interest need be calculated, and it is not necessary that all components elute from the column.

Colors, F D & C

Insert the following new tests to precede the test for *1,4-Dioxane Limit Test*, page 477:

CHROMIUM

Standards

Standard Chromium Solution (1,000 mg/kg) Transfer 2.829 g of $K_2Cr_2O_7$, accurately weighed (National Bureau of Standards No. 136) to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Standard Colorant Solution Transfer 62.5 g of colorant previously shown to be free of chromium to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Apparatus Use any suitable atomic absorption spectrophotometer equipped with a fast response recorder and capable of measuring the radiation absorbed at 357.9 nm.

Instrument Parameters Wavelength setting: 357.9 nm; optical passes: 5; lamp current: 8 ma; lamp voltage: 500 v; fuel: hydrogen; oxidant: air; recorder: 1 mv with a scale expansion of 5 or 10. Alternatively, follow the instructions supplied with the instrument.

Procedure Set the instrument at the optimum conditions for measuring chromium as directed by the manufacturer's instructions. Prepare a series of seven standard chromium solutions containing Cr at approximately 5, 10, 15, 20, 40, 50 and 60 mg/kg by appropriate dilutions of the *Standard Chromium Solution* into 100-ml volumetric flasks; add 80 ml of the *Standard Colorant Solution* and dilute each flask to volume with water.

Transfer 5 g of the colorant to be analyzed to a 100-ml volumetric flask; dissolve in and dilute to volume with water.

Prepare a calibration curve using the series of standards and determine the chromium content of the colorant samples using the curve.

ETHER EXTRACTS

> *Caution:* Isopropyl ether forms explosive peroxides. To assure the absence of peroxides the following test should be carried out: Prepare a colorless solution of ferrous thiocyanate by mixing equal volumes of 0.1 *N* ferrous sulfate and 0.1 *N* ammonium thiocyanate. Carefully discharge any red coloration due to ferric ions with titanous chloride. To 50 ml of the solution add 10 ml of ether and shake vigorously for 2-3 min. A red color indicates the presence of peroxides. If redistillation is necessary, the usual precautions against peroxide detonation should be observed. Immediately before use, the ether should be passed through a 30-cm column of chromatography grade aluminum oxide in order to remove peroxides and inhibitors.

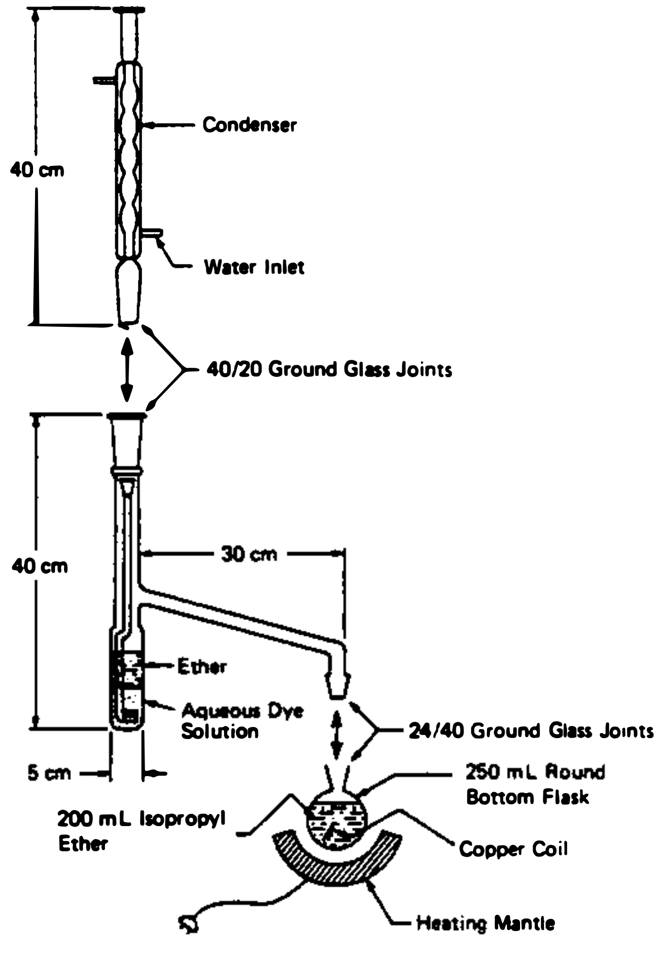

FIGURE 37 Upward Displacement Type Liquid-Liquid Extractor with Sintered Glass Diffuser.

Apparatus Use an upward displacement type liquid-liquid extractor, as shown in Figure 37, with a sintered glass diffuser and a working capacity of 200 ml. Suspend a piece of bright copper wire through the condenser and place a small coil of copper wire (about 0.5 g) in the distillation flask.

Alkaline Ether Extract

Transfer 5 g of the colorant to a beaker and dissolve in 150 ml of water. Add 2 ml of 2.5 *N* NaOH solution and transfer the solution to the extractor; dilute to approximately 200 ml with water. Add 200 ml of ether to the distillation flask and extract for 2 h with a reflux rate of about 15 ml/min. Set the extracted colorant solution aside. Transfer the ether extract to a separatory funnel and wash with two 25-ml portions of 0.1 *N* NaOH followed by two 25-ml portions of water. Reduce the volume of the ether extract to about 5 ml by distillation (in portions) from a tared flask containing a small piece of clean copper coil.

Acid Ether Extract

To the extracted colorant solution set aside in the alkaline ether extract procedure above, add 5 ml of 3 *N* HCl; mix and extract with ether as directed above. Wash the ether extract with two 25-ml portions of 0.1 *N* HCl and water. Transfer the washed ether in portions to the flask containing the evaporated alkaline extract and carefully remove all the ether by distillation. Dry the residue in an oven at 85° for 20 min. Then allow the flask to cool in a desiccator for 30 min and weigh. Repeat drying and cooling until a constant weight is obtained. The increase in weight of the tared flask, expressed as a percentage of the sample weight, is the combined ether extract.

LEUCO BASE

Reagents and Solutions

Cupric Chloride Solution Transfer 10.0 g of $CuCl_2 \cdot 2H_2O$ to a 1-L volumetric flask; dissolve in and dilute to volume with dimethylformamide (DMF).

Sample Solution Prepare as directed in the individual monograph.

Procedure Solution (1): Pipet 50 ml of DMF into a 250-ml volumetric flask, cover and place in the dark. Solution (2): Pipet 10 ml of the sample solution into a 250-ml volumetric flask, add 50 ml of DMF, and place in the dark. Solution (3): Pipet 50 ml of *Cupric Chloride Solution* into a 250-ml volumetric flask; gently bubble air through the solution for one-half hour. Solutions (4)a and (4)b: Pipet 10 ml of the sample solution into each of two 250-ml volumetric flasks, add 50 ml *Cupric Chloride Solution* to each, and bubble air gently through the solutions for 30 min. Dilute all the solutions nearly to volume with water, incubate for 5-10 min, but no longer, in a water bath cooled with tap water, and then dilute to volume. Record the spectrum for each solution between 500 nm and 700 nm using an absorbance range of 0-1 and a 1-cm pathlength cell; record all spectra on the same spectrogram.

Curve No.	*Solution in Sample Cell*	*Solution in Reference Cell*
I	(1)	(1)
II	(1)	(2)
III	(3)	(3)
IVa	(3)	(4)a
IVb	(3)	(4)b

Calculation

$$\% \text{ Leuco Base} = \frac{[(IV - III) - (II - I)] \times 2500}{a \times W \times r},$$

in which the Roman numerals I-IV represent the absorbance reading for the curve of the same Roman numeral (above) at the wavelength maximum; a is the absorptivity (for F D & C Green No. 3, $a = 0.156$ at 625 nm; for F D & C Blue No. 1, $a = 0.164$ at 630 nm), W is the weight of the sample in g, and r is the ratio of the molecular weights of colorant and leuco base (for F D & C Green No. 3, $r = 0.9712$; for F D & C Blue No. 1, $r = 0.9706$).

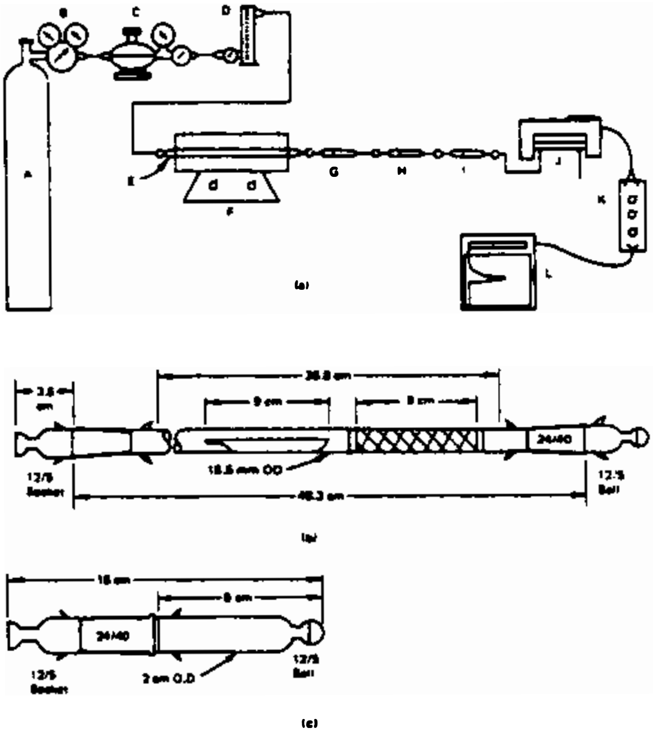

FIGURE 38 (a) Schematic Diagram of Apparatus for Photometric Mercury Vapor M Method:

A. Tank of nitrogen
B. Two-stage pressure regulator
C. Low-pressure regulator
D. Flowmeter
E. Combustion tube
F. Combustion-tube furnace
G. Dehydrite trap
H. Ascarite trap
I. Aluminum oxide trap
J. Mercury vapor meter
K. Attenuator
L. Recorder

(b) Quartz Combustion Tube with Boat and Copper Oxide Packing; (c) Schematic Diagram of Trap Used to Contain Ascarite, Dehydrite, and Aluminum Oxide.

MERCURY

Apparatus The apparatus used for the direct microdetermination of mercury is shown in Figure 38. It consists of a quartz combustion tube designed to hold a porcelain combustion boat (60 × 10 × 8 mm) and a small piece of copper oxide wire. The combustion tube is placed in a heavy duty hinged combustion tube furnace (Lindberg Type 70T, or equivalent) and connected by clamped ball joints at one end to a source of nitrogen and at the other to a series of three traps. The traps are constructed of a linear array of 18-mm × 2-mm Pyrex tubes connected by clamped ball joints and extend from the connection at the combustion tube. Trap I contains anhydrous calcium sulfate packed between quartz-wool plugs, trap II contains Ascarite packed between cotton plugs, and trap III contains aluminum oxide packed between cotton plugs. The nitrogen flow forces the mercury through the combustion tube, the three traps, and a section of Tygon tube to a mercury vapor meter (Beckman model K-23, or equivalent). The mercury released from a sample during combustion is quantitated by comparing the recorder response with that given by a series of mercury standards.

Reagents and Equipment

Absorbent Cotton

Aluminum Oxide, Anhydrous

Anhydrous Calcium Sulfate (Dehydrite, or equivalent)

Asbestos Pads (1 × 0.5 × 1 cm) preheated at 800° for one h

Ascarite, 20.30 mesh

Copper Oxide Wire preheated at 850° for two h

Nitrogen, purified grade

Quartz Wool

Sodium Carbonate, anhydrous, fine granular

Standard Solution Transfer approximately 1.35 g of reagent grade mercurous chloride, accurately weighed, to a 1-L volumetric flask. Dissolve in and dilute to volume with water. When diluted 100-fold the solution contains 0.01 µg Hg per µl.

Procedure Preheat the furnace to 650° and adjust the nitrogen flow to 1 L per min.

Blank Analysis Place a square piece of preheated asbestos pad in the combustion boat and cover it with sodium carbonate. Stop the nitrogen flow, disconnect the ball-joint, quickly insert the boat into the combustion tube with large forceps and reconnect the joint. Note the time and allow the boat to sit in the tube with no nitrogen flow for exactly one min, then restart the flow of nitrogen. Mercury elutes almost immediately with the reinstated nitrogen flow; note the recorder response. Allow about 30 s between runs.

Calibration Determine the recorder response after the application to the asbestos pad of 1, 2, and 3 µl of the *Standard Solution* diluted 100-fold.

Sample Analysis Transfer 25 mg of colorant, accurately weighed, to the combustion boat and cover the sample completely with sodium carbonate. Follow the procedure used for the blank analysis above and calculate the mercury content using the standard curve.

Trap Problems (1) Some colorants (e.g., Blue No. 1 and Green No. 3) may give a response that is symmetrically dissimilar to the Hg peak. If such a response "carries over" to the next sample, then the aluminum oxide trap may need to be changed.

(2) If the recorder response is of inadequate sensitivity (peak height induced by 0.01 μg less than 0.5 cm) then the traps are packed too tightly. Remove or redistribute packing first in the aluminum oxide trap, then try the other traps. (3) The traps will need changing periodically as indicated by a change in the physical appearance of the trap material or by chart responses of different retention times or different symmetry from that of mercury standards.

SODIUM CHLORIDE

Dissolve approximately 2 g of colorant, accurately weighed, in 100 ml of water and add 10 g of activated carbon that is free of chloride and sulfate. Boil gently for 2-3 min. Cool to room temperature, add 1 ml of 6 *N* nitric acid, and stir. Dilute to volume with water in a 200-ml volumetric flask, and then filter through dry paper. Repeat the treatment with 2-g portions of carbon until no color is adsorbed onto filter paper dipped into the filtrate.

Transfer 50 ml of filtrate to a 250-ml flask. Add 2 ml of 6 *N* nitric acid, 5 ml of nitrobenzene, and 10 ml of standardized 0.1 *N* silver nitrate solution. Shake the flask until the silver chloride coagulates. Prepare a saturated solution of ferric ammonium sulfate and add just enough concentrated nitric acid to discharge the red color; add 1 ml of this solution to the 250-ml flask to serve as the indicator. Titrate with 0.1 *N* ammonium thiocyanate solution that has been standardized against the silver nitrate solution until the color persists after shaking for 1 min. Calculate the weight percent of the sodium chloride (*P*) by the formula:

$$P = [(V \times N/W] \times 22.79;$$

in which *V* is the net volume of silver nitrate solution required, in ml; *N* is the normality of the silver nitrate solution; and *W* is the weight of the sample, in g. The factor 22.79 incorporates a total volume of 195 ml, because 10 g of activated carbon occupies 5 ml.

SODIUM SULFATE

Place 25 ml of the decolorized filtrate obtained from the *Sodium Chloride* test above in a 125-ml Erlenmeyer flask, and add 1 drop of a 0.5% phenolphthalein solution in 50% ethanol. Add 0.05 *N* sodium hydroxide dropwise until the solution is indicated to be alkaline with pH paper, and then add 0.002 *N* hydrochloric acid until the indicator is decolorized. Add 25 ml of ethanol and about 0.2 g of tetrahydroquinone sulfate indicator. Titrate with 0.03 *N* barium chloride solution to a red end point. Make a blank determination.

Calculate the weight percent (*P*) of sodium sufate by the formula:

$$P = [(V - B) \times N/W] \times 55.4,$$

in which *V* is the volume of barium chloride solution required to titrate the sample, in ml; *B* is the volume of barium chloride solution required for the blank, in ml, *N* is the normality of the barium chloride solution; and *W* is the sample weight, in g. The factor 55.4 incorporates a total volume of 195 ml, because 10 g of activated carbon occupies 5 ml.

TOTAL COLOR

Method I (Spectrophotometric)

Pipet 10.0 ml of the dissolved colorant into a 250-ml Erlenmeyer flask containing 90 ml of 0.04 *N* ammonium acetate; mix well. Determine the net absorbance of the solution relative to water at the wavelength maximum given for each color. Calculate the percentage of colorant present using the following formula, which presumes a 1-cm pathlength cell:

$$\% \text{ total color} = (A \times 100)/(a \times W),$$

in which *A* is the absorbance, *a* is the absorptivity, and *W* is the weight of the sample, in g.

Method II (Titration with Titanium Chloride)

Apparatus The apparatus for determining total color by titration with $TiCl_3$ is shown in Figure 39. It consists of a

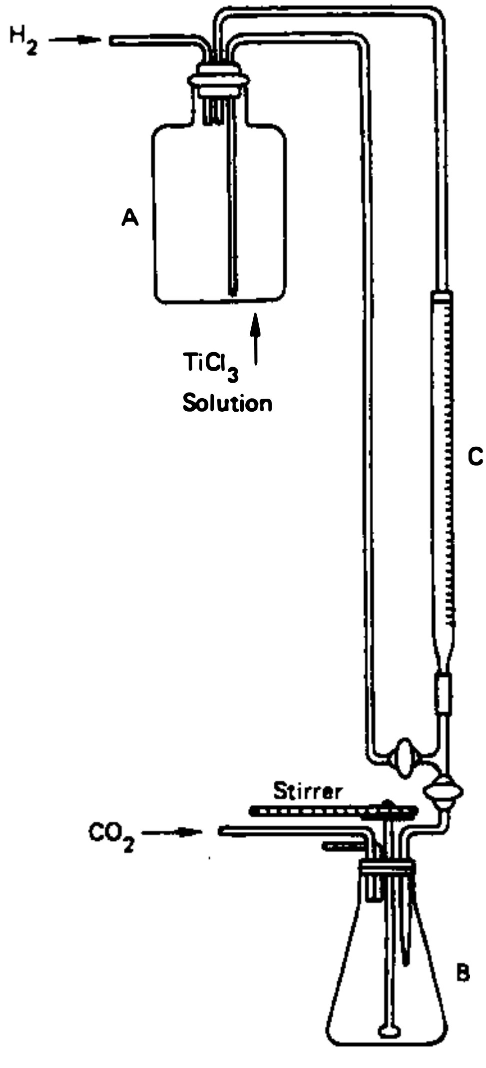

FIGURE 39 Titanous Chloride Titration Apparatus.

storage bottle (A) of 0.1 *N* $TiCl_3$ titrant maintained under hydrogen produced by a Kipp generator; an Erlenmeyer flask (B) in which the reaction takes place equipped with a source of CO_2 or N_2 to maintain an inert atmosphere; a stirrer; and the tip of the buret (C).

Reagents and Solutions

Titanium Chloride Solution (0.1 *N*) Transfer to a storage bottle 73 ml of commercially prepared 20% $TiCl_3$ solution and carefully add 82 ml of concentrated HCl per L of final solution. Mix well and bubble CO_2 or N_2 through the solution for 1 h. Before standardizing, maintain the solution under a hydrogen atmosphere for at least 16 h using a Kipp generator.

Potassium Dichromate (0.1 *N*, primary standard) Transfer 4.9032 g of $K_2Cr_2O_7$ (National Bureau of Standards No. 136) to a 1-L volumetric flask; dissolve in and dilute to volume with water.

Ammonium Thiocyanate (50%) Transfer 500 g of NH_4SCN (ACS certified) to a 1-L volumetric flask; dissolve in about 600 ml of water, warming if necessary; and dilute to volume.

Ferrous Ammonium Sulfate $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, ACS certified.

Sodium Bitartrate

Standardization of the Titanium Chloride Solution Drain any standing $TiCl_3$ from the feed lines and buret and refill with fresh solution. Add 3.0 g of ferrous ammonium sulfate to a wide-mouth Erlenmeyer flask followed by 200 ml of water, 25 ml of 50% H_2SO_4, 25 ml of 0.1 *N* $K_2Cr_2O_7$ (by pipet), and 2 or 3 boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air; then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the CO_2 flow and stirrer. Pass CO_2 over the solution for 1 min before beginning the titration.

Add the $TiCl_3$ solution at a fast steady drip to within 1 ml of the estimated end point (about 20 ml). Reduce the CO_2 flow, remove the solid glass rod from the stopper assembly, pipet 10 ml of *ammonium thiocyanate* (50%) into the flask, insert the glass rod, and increase the CO_2 flow. Continue titrating slowly until the endpoint, a color change from brown-red to light green, is observed. Perform a blank determination using the same reagents and quantities, and calculate the normality (*N*) of the $TiCl_3$ solution on the basis of three titrations by the formula:

$$N = (V_r \times N_r/V_t - V_b),$$

in which V_r is the volume, in ml, of $K_2Cr_2O_7$ solution used; N_r is the normality of the $K_2Cr_2O_7$ solution; V_t is the volume, in ml, of $TiCl_3$ solution used; and V_b is the volume, in ml, of $TiCl_3$ used in the blank titration.

Procedure Transfer the quantity of colorant prescribed in the individual monograph to a 500-ml wide-mouth Erlenmeyer flask and add 21-22 g of sodium bitartrate (sodium citrate for Yellow No. 6), 275 ml water, and two or three boiling chips. Boil the solution vigorously on a hot plate for 30 s to remove dissolved air; then quickly transfer the flask to the titration apparatus, securely connect the stopper assembly, and start the CO_2 flow and stirrer. Pass CO_2 over the solution for 1 min before beginning the titration.

Titrate the sample until the color lightens, wait 20 s, and then continue the addition with about 2 s between drops. When the color is almost completely bleached wait 20 s, then continue the addition with 5 s between drops. A complete color change indicates the end point. Perform a blank determination using the same reagents and quantities, and calculate the total color (*T*) in percent, on the basis of three titrations by the formula:

$$T = [(V_t - V_b)/(W \times F_s)] \times 100 \times N,$$

in which V_t is the volume of titrant used; V_b is the volume of titrant required to produce the endpoint in a blank; *N* is the normality of the titrant; *W* is the sample weight, in g; and F_s is a factor derived from the stoichiometry of the reaction characteristics of each colorant and is given in the individual monograph.

Method III (Gravimetric)

Transfer approximately 0.5 g of colorant, accurately weighed, to a 400-ml beaker, add 100 ml of water and then heat to boiling. Add 25 ml of dilute HCl (1 in 50) and bring to a boil. Wash down the sides of the beaker with water and then cover and keep on a steam bath for several hours or overnight. Cool to room temperature and then quantitatively transfer the precipitate to a tared Gooch crucible with dilute HCl (1 in 100). Wash the precipitate with two 15-ml portions of water and then dry the crucible for 3 h at 135°. Cool in a desiccator and weigh. Calculate the total color (*P*), in weight percent, by the formula:

$$P = [(W_p \times F)/W_s] \times 100,$$

in which W_p is the weight of the precipitate, in g; *F* is the gravimetric conversion factor given in the individual monograph; and W_s is the original sample weight, in g.

UNCOMBINED INTERMEDIATES AND PRODUCTS OF SIDE REACTIONS

Method I

Sample Solution Transfer approximately 2 g of colorant to a 100-ml volumetric flask; dissolve in and dilute to volume with water.

Apparatus Pack a 2.5- × 45-cm glass column with approximately 20 g of cellulose (Whatman CF-11 grade or the equivalent) that has been slurried in the eluant and from which the fines have been removed by decantation. Equilibrate the column thoroughly with the eluant, 35% ammonium sulfate.

Procedure Pipet 5 ml of *Sample Solution* into a beaker containing 5 g of cellulose that has been slurried in eluant and from which the fines have been removed by decantation. Stir the mixture thoroughly, add 10 g of ammonium sulfate and stir until uniformly mixed. Mix the slurry with 15 ml of eluant and apply to the column. Allow the fluid to enter the column and then wash the beaker with eluant until the sample is quantitatively transferred. Elute the column with approximately 500 ml of 35% ammonium sulfate and collect a total of eight 60-ml fractions. Divide each collected fraction in half and add 0.5 ml of NH_4OH to one half and 0.5 ml of HCl to the other.

Calculation After identifying each intermediate and side product by comparing spectra of the fractions with commercial standards calculate the concentration (C) of each using the formula:

$$C = A/(a \times b),$$

in which A is the absorbance at the wavelength of maximal absorption; b is the cell pathlength, in cm; and a is the absorptivity given in the individual monograph.

Method II

Apparatus Use a suitable high-performance liquid chromatography system (see page 476) equipped with a dual wavelength detector system such that the effluent can be monitored serially at 254 nm and 325-385 nm (wide band pass). Use a 1-m × 2.1-mm (id) column packed with a strong anion-exchange resin (Dupont No. 830950405, or the equivalent).

Operating Conditions The operating conditions required may vary depending on the system used. The following conditions have been shown to give suitable results for F D & C Red No. 40, F D & C Yellow No. 5, and F D & C Yellow No. 6.

F D & C Red No. 40

Primary eluant 0.01 M aqueous $Na_2B_4O_7$.

Secondary eluant 0.20 M $NaClO_4$ in aqueous 0.01 M $Na_2B_4O_7$.

Sample size 20 µl of a 0.25% solution.

Flow rate 0.60 ml/min.

Gradient Linear, in two phases: 0-18% in 40 min, 18-62% in 8 min more, then hold for 18 min more at 62%.

Temperature 50°.

Pressure 1,000 psi.

Order of elution (1) Cresidinesulfonic acid (CSA), (2) unknown, (3) Schaeffer's salt (SS), (4) unknown, (5) 4,4'-diazoaminobis(5-methoxy-2-methylbenzenesulfonic acid) (DMMA), (6) unknown, (7) F D & C Red No. 40, (8) 6,6'-oxybis(2-naphthalenesulfonic acid) (DONS).

F D & C Yellow No. 5

Primary eluant 0.01 M aqueous $Na_2B_4O_7$.

Secondary eluant 0.10 M $NaClO_4$ in aqueous 0.01 M $Na_2B_4O_7$.

Sample size 50 µl of a 0.15% solution, prepared within 13 min of injection.

Flow rate 1.00 ml/min.

Gradient Exponential at 4%/min: 0.95%.

Temperature 50°.

Pressure 1,000 psi.

Order of elution (1) Phenylhydrazine-*p*-sulfonic acid (PHSA), (2) sulfanilic acid (SA), (3) 1-(4-sulfophenyl)-3-ethylcarboxy-5-hydroxypyrazolone (PY-T), (4) 1-(4-sulfophenyl)-3-carboxy-5-hydroxypyrazolone (EEPT), (5) 4,4'-(diazoamino)-dibenzenesulfonic acid (DAADBSA).

F D & C Yellow No. 6

Primary eluant 0.01 M aqueous $Na_2B_4O_7$.

Secondary eluant 0.20 M $NaClO_4$ in aqueous 0.01 M $Na_2B_4O_7$.

Sample size 5 µl of a 1% solution.

Flow rate 0.50 ml/min.

Gradient Linear in four phases: 0-11% in 10 min; hold 25 min; 11-38% in 10 min; 38-42% in 10 min; 42-98% in 20 min; hold 20 min.

Temperature 50°.

Pressure 1,000 psi.

Order of elution (1) Sulfanilic acid (SA), (2) Schaeffer's salt (SS), (3) 4,4'-(diazoamino)-dibenzenesulfonic acid (DAADBSA), (4) *R*-salt dye, (5) F D & C Yellow No. 6, (6) 6,6'-oxybis(2-naphthalenesulfonic acid) (DONS).

Standard Solutions

F D & C Red No. 40 Prepare a solution containing 0.25 g colorant, 0.5 mg CSA, 0.75 mg SS, 0.25 mg DMMA, and 1.25 mg DONS in a 100-ml volumetric flask. Dissolve in, and dilute to volume with 0.1 M $Na_2B_4O_7$.

F D & C Yellow No. 5 Prepare a solution containing 0.15 g colorant and 0.3 mg each of PHSA, SA, PY-T, EEPT, and DAADBSA in a 100-ml volumetric flask. Dissolve in and dilute to volume with 0.1 M $Na_2B_4O_7$.

F D & C Yellow No. 6 Prepare a solution containing 0.25 g colorant, 0.5 mg SA, 0.75 mg SS, 0.25 mg DAADBSA, and 1.25 mg DONS in a 100-ml volumetric flask. Dissolve in and dilute to volume with 0.1 M $Na_2B_4O_7$.

Test Solutions Prepare at least four test solutions, each containing the colorant, and one impurity, accurately weighed, dissolved in 0.1 M $Na_2B_4O_7$, and diluted to volume in a 100-ml volumetric flask. The solutions should encompass the range of concentrations, evenly spaced, given below for each constituent:

F D & C Red No. 40 (250 mg): CSA (0.05-0.5 mg); SS (0.05-0.75 mg); DONS (0.5-2.5 mg); DMMA (0.025-0.25 mg). Inject 20 µl of each solution.

F D & C Yellow No. 5 (150 mg): SA (7.5-300 µg); PY-T (7.5-300 µg): EEPT (7.5-300 µg); DAADBSA (7.5-300 µg). Inject 50 µl of each solution.

F D & C Yellow No. 6 (250 mg): SA (0.05-0.5 mg); SS (0.05-0.75 mg); DONS (0.5-2.5 mg); DAADBSA (0.05-0.25 mg). Inject 20 µl of each solution.

System Suitability

Resolution Elute the column with the gradient specified under *Operating Conditions* until a smooth baseline is obtained. Inject an aliquot of the *Standard Solution*. The resolution of the eluted components matches or exceeds that shown for the corresponding colorant (see Figures 40, 41 and 42). After determining that the column will give the required resolution allow it to rest two weeks before use.

Calibration Inject the designated volume of each *Test Solution* onto a conditioned column and prepare a standard

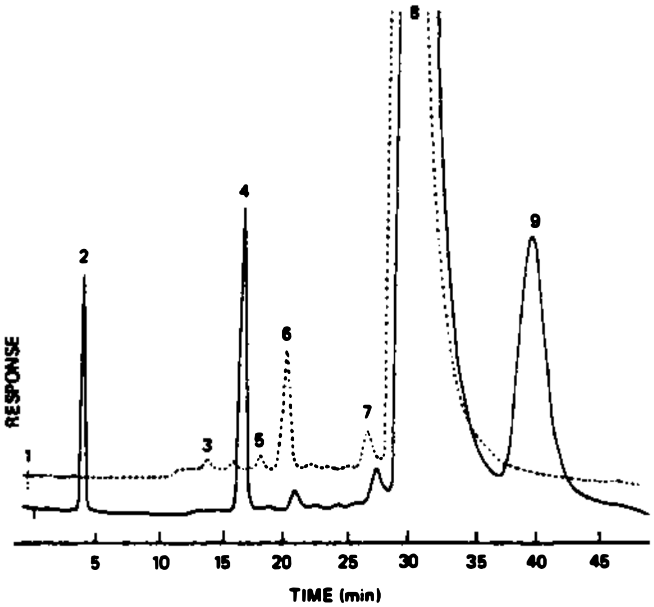

FIGURE 40 FD & C Red No. 40 Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375-385 nm.

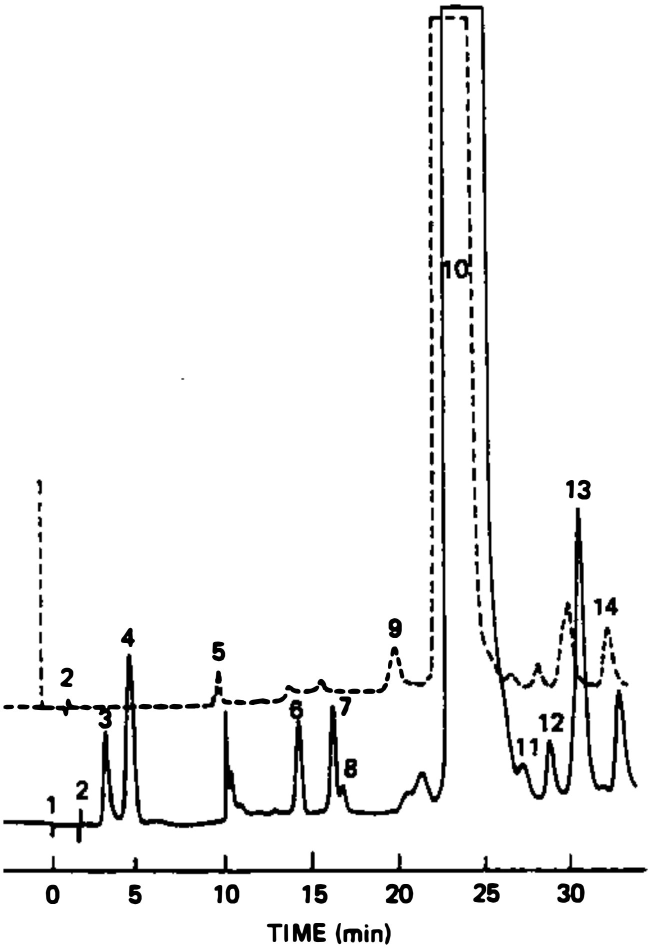

FIGURE 41 FD & C Yellow No. 5 Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375-385 nm.

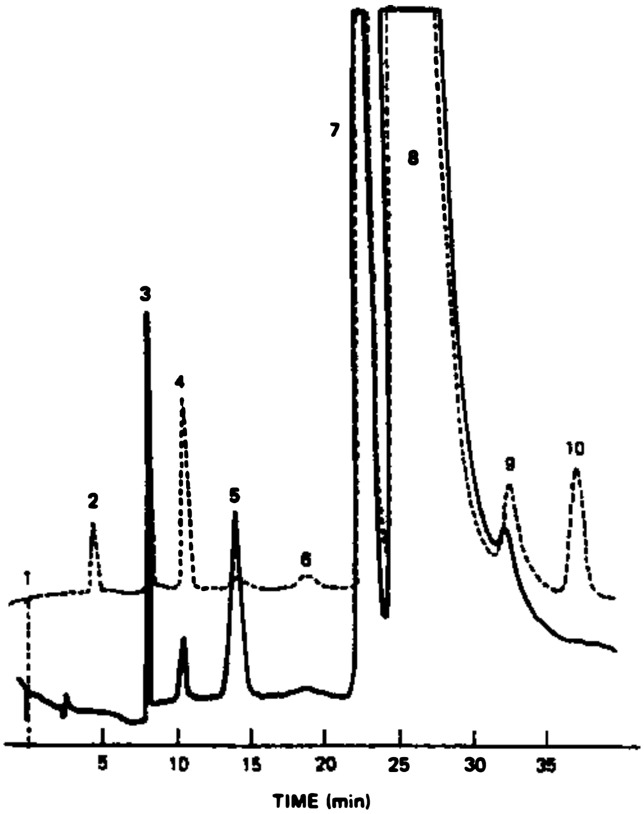

FIGURE 42 FD & C Yellow No. 6 Top Trace: Eluant Monitored at 254 nm; Bottom Trace: Eluant Monitored at 375-385 nm.

curve corresponding to each unreacted intermediate and side reaction product. Determine the area (A) for each peak from the integrator if an automated system is used or by multiplying the peak height by the width at one-half the height. The peak height alone may be used for EEPT, PY-T, and DAADBSA. Calculate the concentration (C_i) of each intermediate or side product using the formula:

$$A_i = mC_i + b,$$

in which A_i is the area of its corresponding chromatographic peak. Calculate the slope (m) and intercept (b) using the following linear regression formulas:

$$m = \frac{N\Sigma C_iA_i - \Sigma C_i\Sigma A_i}{N\Sigma A_i^2 - (\Sigma A_i)^2},$$

$$b = [\bar{A}]_i - m[\bar{C}]_i;$$

in which $\bar{C}$ and $\bar{A}$ are the calculated averages of the concentrations and peak areas, respectively, used to construct the standard curve for one intermediate or side reaction product. Calculate the correlation coefficient (r) from the following formula:

$$r = \frac{\Sigma(C_i - \bar{C})(A_i - A)}{[\Sigma(C_i - \bar{C}^2) \times \Sigma(A_i - \bar{A})^2]}.$$

Each time the system is calibrated add the new data to that accumulated from previous analyses. The correlation coefficient must be between 0.95 and 1.00 for any single experiment or from accumulated data.

Recalibrate the system after every ten determinations or two days; whichever occurs first.

Sample Preparation Prepare as directed in the individual monograph.

Procedure Inject the volume of *Sample Preparation* as designated in the monograph onto the column. Determine the concentration of intermediates and side reaction products from the peak areas using the slope (m) and intercept (b) calculated under *Calibration* by the equation:

$$A_s = mC_s + b,$$

in which C_s is the concentration of the unknown in the *Sample Preparation* and A_s its corresponding peak area.

VOLATILE MATTER

Transfer 1.5-2.5 g of colorant, accurately weighed, to a tared crucible. Heat in a vacuum oven at 135° for 12-15 h. Lower the pressure in the oven to minus 125 mm Hg and continue heating for an additional two h. Cover the crucible, and allow to cool in a desiccator. Reweigh the crucible when cool. The loss of weight is defined as the volatile matter.

WATER-INSOLUBLE MATTER

Transfer about 1 g of colorant, accurately weighed, to a 250-ml beaker and add 200 ml of boiling water; stir to facilitate dissolution of the color.

Tare a Gooch crucible equipped with a glass fiber filter (Reeve Angel, No. 5270, or equivalent). Filter the solution with the aid of suction when it has cooled to ambient temperature. Rinse the beaker three times pouring the rinse through the crucible. Wash the filter with water until the filtrate is colorless.

Dry the crucible and filter in an oven at 135° for at least 3 h, cool them in a desiccator and reweigh to the nearest 0.1 mg. Calculate the percent water-insoluble matter (I) by:

$$I = (W_c/W_s) \times 100,$$

in which W_c is the difference in crucible weight and W_s is the sample weight.

Extractable Organic Compounds (in Hydrochloric Acid)

Extractable Organic Compounds Analyses are to be carried out by gas chromatography employing *Vapor Partitioning* or *Solvent Extraction*, depending upon the characteristics of the compound being determined. It is necessary, however, to use the *Vapor Partitioning Method* for the determination of benzene and vinyl chloride.

Vapor Partitioning Method This method is suitable for the determination of extractable organic compounds at 0.05-100 mg/kg, but is most appropriate for organic compounds with a vapor pressure greater than 10 mm Hg at 25°. Use a gas chromatograph equipped with a flame ionization detector and a 4-m × 2-mm (id) stainless steel column packed with 15% by weight methyl trifluoropropyl silicone (DCFS 1265, or QF-1, or OV-210, or SP-2401) stationary phase on 80/100 mesh Gas Chrom R or the equivalent. A newly packed column should be conditioned at 120° and 30 ml/min helium flow for at least 2 h (preferably overnight) before it is attached to the detector. For analysis the column is maintained isothermally at 105°; the injection port and detector are maintained at 250°; the carrier gas flow rate is set at 11 ml/min; fuel gas flows should be optimized for the gas chromatograph and detector in use. The experimental conditions may be changed as necessary for optimal resolution and sensitivity. The signal to noise ratio should be at least 10:1.

Preparation of Standard Solutions Prepare a standard solution of the organic compounds to be quantitated in hydrochloric acid (known to be free of interfering impurities) at approximate concentrations of 5 mg/kg, or within ±50% of the concentrations in the samples to be analyzed.

Place a stirring bar in a 1-L volumetric flask equipped with a ground glass stopper and tare the combination. Fill the flask with reagent-grade hydrochloric acid so that no air space is present when the flask is stoppered and determine the weight of the hydrochloric acid. Calculate the volume (V) in µl of each organic component to be added from the formula:

$$V = (C \times W)/(D \times 1000),$$

in which C is the desired concentration in mg/kg, W is weight of the hydrochloric acid in g, and D is density of the organic compound in mg/µl, and 1000 is a conversion factor with the units g/kg. Add the calculated amount of each component to the hydrochloric acid with a syringe (assure that the syringe tip is under the solution surface); stopper the flask and stir the solution for at least 2 h using a magnetic stirrer.

Calibration Treat the standard in the same way as described for the sample under *Procedure* (below). Determine a blank for each lot of reagent-grade hydrochloric acid and calculate a response factor (R) by dividing the concentration (C) in mg/kg for each component by the peak area (A) for that component (subtract any area obtained from the blank sample):

$$R = C/(A - \text{area of blank}).$$

Gaseous compounds present special problems in the preparation of standards. Therefore, to determine response factors for gaseous compounds use the following method, which will be referred to as the *Method of Multiple Extractions*. Dilute a sample of hydrochloric acid known to contain the gaseous compound of interest with an equal volume of water. Draw 20 ml of this solution into a 50-ml glass syringe; then draw 20 ml of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Withdraw 1 ml of the vapor through the septum and inject it into the chromatograph. Expel the vapor phase from the 50-ml syringe, draw in another 20 ml of air, repeat the extraction, and inject another 1-ml vapor sample into the chromatograph. Carry out the extraction and GC analysis on the same sample of acid a total of six times. For each impurity plot the area (A_n) determined for extraction *n* against the difference between A_n and the area determined for extraction ($n + 1$); that is, plot A_n against [$A_n - A_{n+1}$]. The slope of this line is the extraction efficiency (*E*) for that impurity into the air.

Inject into the chromatograph 1 ml of a 0.1% (by volume) standard gas sample of each impurity in air and determine the absolute factor (F_a) in g per peak area (*A*) by the following formula:

$$F_a = (M \times 4.0816 \times 10^{-8})/A,$$

in which *M* is the molecular weight of the compound.

The concentration (*C*) in mg/kg of the component in the original sample is calculated by the formula:

$$C = (A \times F_a \times 1.6949 \times 10^{6})/E,$$

in which *A* is the peak area corresponding to the compound (as above), F_a is the absolute factor, and *E* is extraction efficiency. The response factor is then calculated as:

$$R = C/A.$$

Procedure Dilute a 10-ml sample of hydrochloric acid to be analyzed with an equal volume of water. Draw this solution into a 50-ml glass syringe. Then draw 20 ml of air into the syringe, cap with a rubber septum, and place the syringe on a shaker for 5 min. Draw 1 ml of the vapor through the septum and inject it into the gas chromatograph. Approximate elution times in min for some specific organic compounds are as follows:

Methane and acetylene	1.70
Methyl chloride	2.21
Vinyl chloride	2.29
1,1,1-Trichlorofluoromethane	2.62
Ethyl chloride	2.90
Vinylidene chloride	3.20
Methylene chloride	3.64
Chloroform	4.49
1,1-Dichloroethane	4.53
Carbon tetrachloride	4.86
1,1,1-Trichloroethane	5.50
Benzene	6.00
Trichloroethylene	6.22
Ethylene dichloride	6.61
Propylene dichloride	8.41
Perchloroethylene	9.73

Alternate columns may be required to resolve some combinations of components. Methyl chloride and vinyl chloride are resolved by a 3.7-m × 3-mm (id) squalane column at 45° and a helium flow of 10 ml/min. Chloroform and 1,1-dichloroethane are resolved by a 4-m × 3-mm (id) DC 550R column at 110° and a helium flow of 12 ml/min.

Calculation Calculate the concentration (*C*) in mg/kg of each compound by multiplying its corresponding peak area (*A*) by the appropriate response factor (*R*) determined in the *Calibration* protocol.

$$C = R \times A.$$

Precision The relative standard deviation at 5 mg/kg should not exceed 15% for five analyses.

Solvent Extraction Method The solvent extraction technique is suitable for the determination of extractable organic compounds at 0.3-100 mg/kg, but is most appropriate for organic compounds with vapor pressures less than 10 mm Hg at 25°. The conditions for the gas chromatograph are the same as for the *Vapor Partitioning* method, except that the column temperature is 120° and the carrier-gas flow is 21 ml/min.

Preparation of Standards Prepare the *Standard Solution* as described under *Vapor Partitioning.*

Calibration Extract a sample of the *Standard Solution* as directed under *Procedure* (below) and inject it into the gas chromatograph. Determine a blank for each lot of reagent-grade hydrochloric acid and perchloroethylene by extracting the hydrochloric acid in the same way as the standard. Calculate a response factor (*R*) by dividing the concentration (*C*) in mg/kg for each component by the peak area (*A*) for that component (subtract any area obtained from the blank sample):

$$R = C/(A - \text{area of blank}).$$

Procedure Accurately transfer 90 ml of the hydrochloric acid sample and 10 ml of perchloroethylene (free of interfering impurities) into a narrow-mouth 4-oz bottle. Place the bottle in a mechanical shaker for 30 min. Separate the two phases (perchloroethylene on the bottom) and inject 3 µl of the perchloroethylene extract into the gas chromatograph. Approximate elution times in min for some chlorinated organic compounds are as follows:

Vinylidene chloride	2.94
Methylene chloride	3.27
Chloroform	3.83
Carbon tetrachloride	4.07
1,1,1-Trichloroethane	4.50
Trichloroethylene	4.97
Ethylene dichloride	5.26
Propylene dichloride	6.36
Perchloroethylene	6.95
1,1,1,2-Tetrachloroethane	10.12
1,1,2,2-Tetrachloroethane	13.70
Pentachloroethane	16.19

To determine perchloroethylene and higher-boiling impurities, substitute methylene chloride (free of interfering impurities) for perchloroethylene in the extraction step. For higher-boiling impurities such as monochlorobenzene and the three dichlorobenzenes use a 2.74-m × 2.1-mm (id) stainless-steel column packed with 10% carbowax 20M/2% KOH on 80/100 mesh chromasorb W (acid washed) at 150° and a nitrogen flow of 35 ml/ min.

Calculation Calculate the concentration (*C*) in mg/kg of each compound by multiplying the corresponding peak area (*A*) (subtract any area obtained from a blank sample) by the appropriate response factor (*R*) determined in the *Calibration* protocol:

$$C = R \times (A - \text{area of blank}).$$

Precision The relative standard deviation at 5 mg/kg should not exceed 15% for five analyses.

Fats and Related Substances, page 503

Insert the following new subsections in alphabetical order immediately following *Acid Value*, page 504.

COLOR (Lovibond)
(Based on AOCS Method Cc 13b-45)

Apparatus Lovibond tintometer or the equivalent. A set of color comparison glasses that conform to the AOCS-Tintometer Color Scale (available from the National Bureau of Standards); a minimum set consists of:

Red	0.1	0.2	0.3	0.4	0.5	0.6	0.8	0.9
	1.0	2.0	2.5	3.0	3.5	4.0	5.0	6.0
	7.0	7.6	8.0	9.0	10.0	11.0	12.0	16.0
	20.0							
Yellow	1.0	2.0	3.0	5.0	10.0	15.0	20.0	35.0
	50.0	70.0						

For making color comparisons use color tubes of clear colorless glass with a smooth, flat, polished bottom (length 154 mm; id 19 mm; od 22 mm), and marked to indicate liquid columns of 25.4 and 133.35 mm.

Procedure Add 0.1 g of diatomaceous earth to a 60-g sample, agitate for 2.5 min at room temperature (or 10°-15° above the melting point if the sample is not liquid) and filter. Adjust the temperature to 25°-35° (or not more than 10° above the melting point) and fill the color tube to the desired mark. Place the tube in the tintometer (in a dark booth or cabinet) and match the sample color as close as possible with a standard glass.

COLD TEST
(Based on AOCS Method Cc 11-53)

Filter a sample (200-300 ml) and transfer to a clean, dry, 115-ml bottle. Fill the bottle completely and insert a cork stopper. Seal with paraffin and equilibrate at 25° in a water bath so that it is completely covered. Immerse the bottle in an ice and water bath so it is completely covered. Monitor the bath during the test and replenish the ice frequently to keep the bath at 0°.

After 5.5 h remove the bottle from the bath. The sample must be clear, fat crystals or cloudiness must be totally absent.

FATTY ACID COMPOSITION

Apparatus Use a suitable gas chromatograph (see page 475) equipped with a thermal conductivity detector, containing a 3.05-m × 6.4-mm (od) glass or aluminum column packed with preconditioned 10%, by weight, DEGS-PS on 100/120 mesh diatomaceous earth (Chromasorb WHP, or equivalent).

Operating Conditions The operating conditions may vary with the instrument used, but a suitable chromatogram may be obtained using the following conditions: *column temperature*, 215°; *inlet temperature* (injector), 300°; *detector*, 300°; *carrier gas flow*, 60 ml per min.

Standard Solutions Chromatograph a commercially available standard (such as NuCheck 17A*) containing a mixture of fatty-acid methyl esters, including the methyl esters of oleic acid ($C_{18:1}$) and erucic acid ($C_{22:1}$). The calculated concentration should compare to that claimed within $\pm 2\ \sigma$, where σ is the standard deviation calculated from at least 10 replicate determinations, preferably made over a period of several days.

Chromatograph a suitable number of samples of the standard to assure that the resolution factor *R* (see page 476) defining the efficiency of the separation between methyl stearate, eluting at approximately 5.6 min, and methyl oleate, eluting at approximately 6.5 min, is 0.9 or greater.

Sample Preparation Transfer approximately 10-15 g of sample (melted if necessary) to a 250-ml glass stoppered boiling flask and add 50 ml of sodium methoxide reagent prepared by dissolving 9 g of metallic sodium in 3 L of fresh reagent grade methanol (carry out this preparation in a hood, hydrogen gas is evolved). Place a stirring bar in the flask, attach a water cooled condenser, and reflux with stirring over low heat for 3-5 min or until the cloudiness disappears indicating that methylation is complete. Add 25 ml of 0.5% HCl saturated with NaCl through the condenser and continue stirring until the two solutions mix. Remove the sample and extract with hexane. Decant the hexane extract through reagent grade sodium sulfate in a filter paper assembly and evaporate the hexane with a stream of dry nitrogen on a steam bath.

Procedure Inject an appropriate volume (1 µl) of sample into the chromatograph. If an automated system is used, follow the manufacturer's instructions; if calculations are to be done manually, proceed as follows:

Calculate the percent of each component (C_N) by the formula:

$$C_N = [(A_N \times M_N)/T_s] \times 100,$$

in which A_N is the area of the peak corresponding to component C_N, M_N is the molecular weight of C_N, and T_S is the total obtained by summing over all ($A_N \times M_N$) calculated for all detected components [$T_s = \Sigma(A_N \times M_N)$].

MELTING RANGE I

Fats of animal and vegetable origin do not exhibit a sharp melting point. For the purpose of this test, melting range is defined as the range of temperature in which the sample becomes a perfectly clear liquid after first passing through a stage of gradual softening, during which it may become opalescent.

Apparatus Use any suitable commercial or other apparatus. For example, see page 519. Use melting-point capillary tubes—id, 1 mm; od, 2 mm; length, 50-80 mm; and open at both ends.

Procedure *Capillary Method* (Based on AOCS Method Cc 1-25). Melt the sample and filter it through filter paper; the sample must be absolutely dry. Dip three capillary tubes in the liquid sample so that the oil stands approximately 10 mm high in the tubes and fuse the end of the tube containing the sample without burning it. Place the tubes containing the liquid sample

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* NuCheck Prep. Inc., PO Box 172, Elysian, MN 56028.

in a beaker and equilibrate them at least 16 h at 4°-10° in a refrigerator. Determine the melting range using a temperature increase of 0.5° per min when within 10° of the anticipated melting point. The melting ranges of the three samples should be no more than 0.5° apart.

STABILITY (Active Oxygen Method)
(Based on AOCS Method Cd 12-57)

Fat stability is the time in h required for a sample of fat or oil to attain a peroxide value of 100. This period of time is determined by interpolation between two measurements and is assumed to be an index of resistance to rancidity.

Precautions All equipment must be scrupulously clean (for an acceptable cleaning procedure, see AOCS Official Method Cd 12-57). Do not use chromic acid or other acidic cleaning agents. All receptacles in the heater must be calibrated for temperature under the exact conditions of the test. During the test, the temperature must be monitored in a sample tube containing the recommended quantity of oil.

Apparatus Use a suitable heating block and aeration apparatus, such as shown in the Official and Tentative Methods of the AOCS or in JAOCS *33* (1956), pp. 628-630.

Percent Solids in 42% High Fructose Corn Syrup (0.05% Ash) Relative to the Refractive Index at Various Temperatures

	Refractive Index			
% Solids	20°	30°	45°	60°
0	1.33299	1.33194	1.32985	1.32725
2	1.33588	1.33479	1.33265	1.33002
4	1.33877	1.33765	1.33547	1.33279
6	1.34171	1.34055	1.33833	1.33561
8	1.34470	1.34350	1.34123	1.33848
10	1.34772	1.34650	1.34418	1.34140
12	1.35080	1.34954	1.34718	1.34436
14	1.35392	1.35262	1.35022	1.34737
16	1.35709	1.35575	1.35331	1.35042
18	1.36030	1.35893	1.35645	1.35353
20	1.36356	1.36216	1.35963	1.35668
22	1.36687	1.36544	1.36287	1.35988
24	1.37023	1.36876	1.36615	1.36314
26	1.37363	1.37214	1.36949	1.36644
28	1.37709	1.37556	1.37287	1.36980
30	1.38059	1.37903	1.37631	1.37320
32	1.38415	1.38256	1.37979	1.37666
34	1.38776	1.38613	1.38333	1.38017
36	1.39141	1.38976	1.38692	1.38373
38	1.39512	1.39344	1.39057	1.38735
40	1.39889	1.39717	1.39426	1.39102
42	1.40270	1.40096	1.39802	1.39475
44	1.40657	1.40480	1.40182	1.39853
46	1.41049	1.40869	1.40568	1.40237
48	1.41447	1.41264	1.40960	1.40627
50	1.41851	1.41665	1.41358	1.41022
52	1.42260	1.42071	1.41761	1.41423
54	1.42675	1.42483	1.42170	1.41830
56	1.43095	1.42901	1.42585	1.42243
58	1.43522	1.43325	1.43006	1.42662
60	1.43954	1.43755	1.43433	1.43087
62	1.44392	1.44191	1.43866	1.43519
64	1.44837	1.44633	1.44305	1.43956
66	1.45287	1.45081	1.44750	1.44400
68	1.45744	1.45535	1.45202	1.44851
70	1.46207	1.45996	1.45660	1.45308
72	1.46677	1.46463	1.46125	1.45771
74	1.47152	1.46936	1.46596	1.46241
76	1.47635	1.47416	1.47074	1.46718
78	1.48124	1.47903	1.47559	1.47202
80	1.48620	1.48397	1.48051	1.47693
82	1.49122	1.48897	1.48549	1.48191
84	1.49632	1.49405	1.49055	1.48696

Source: Wartman, A.M. et al. J. Chemical and Engineering Data *25*:277–282, 1980.

Sampling Remove samples from large containers or processing equipment with sampling devices only of stainless steel, aluminum, nickel, or glass. Solid fat samples should be taken at least 5 cm from the walls of large containers and 2.5 cm from the walls of small containers. If liquid oil is to be poured from a container, clean the spout or lip with an acetone-moistened cloth. Under no circumstances should samples be taken from containers equipped with plastic or enameled tops or paper or wax liners.

Procedure Unless already completely liquid, the sample should be melted at a temperature not more than 10° above its melting point. Pour 20 ml into each of two or more sample tubes assuring that the sample does not contact the tube where the stopper will later fit. Insert the aeration tube assembly so that the end of the air delivery tube is 5 cm below the surface of the sample. Place the sample tube in a container of vigorously boiling water for 5 min (During this time adjust the air flow rate from the manifold.). Remove the tube, wipe dry, and transfer immediately to the constant-temperature heater, maintained at 97.8° ± 0.2°, and connect the aeration tube to the manifold. Determine to the nearest h the time required for the sample to attain a peroxide value (see page 48) of 100 milliequivalents (meq) as follows: With 1-g samples determine when the peroxide value is approximately 75 meq and 125 meq, then perform the test on four 5-g samples determining the peroxide value in duplicate at the times corresponding to 75 and 125 meq. Make a second determination on two 5-g samples exactly 1 h after the first pair. Plot these values against aeration time; the AOM stability value in h is given where the line crosses 100 meq.

Insert the following new section to precede *Hydrochloric Acid Table*, page 514.

High-Fructose Corn Syrup Solids

Using the table on page 83 of THIS SUPPLEMENT determine the percent solids in high fructose corn syrup as directed in the monograph.

Invert Sugar

Total Solids

Apparatus Use a suitable refractometer (see page 533) equipped with a jacket for water circulation or some other mechanism for maintaining the sample at 20.0° ± 0.1° or some other fixed temperature. Before proceeding with measurements assure that the prism has reached the equilibrium temperature.

Standardization In order to achieve the theoretical accuracy of ± 0.0001 it is necessary to calibrate the instrument daily by determining the refractive index of distilled water, which is 1.3330 at 20°, and 1.3325 at 25°.

Procedure Determine the refractive index after assuring that the sample and prism have reached the equilibrium temperature. Convert the refractive index to approximate percent solids (uncorrected for invert sugar) using the accompanying table. If the measurement is made at a temperature other than 20°, adjust the approximate percent solids using the correction table. Calculate the percent solids *(D)* using the formula:

$$D = (S + C) + (PI \times 0.022),$$

in which S is the approximate percent solids determined from the refractive index, C is the temperature correction if needed, and PI is the percent invert sugar as determined under *Assay*.

Conversion Factors for the Determination of Invert Sugar in the Presence of Sucrose

V(ml)	F_3[a]	F_1[b]
15	123.6	122.6
16	123.6	122.7
17	123.6	122.7
18	123.7	122.7
19	123.7	122.8
20	123.8	122.8
21	123.8	122.8
22	123.9	122.9
23	123.9	122.9
24	124.0	122.9
25	124.0	123.0
26	124.1	123.0
27	124.1	123.0
28	124.2	123.1
29	124.2	123.1
30	124.3	123.1
31	124.3	123.2
32	124.4	123.2
33	124.4	123.2
34	124.5	123.3
35	124.5	123.3
36	124.6	123.3
37	124.6	123.4
38	124.7	123.4
39	124.7	123.4
40	124.8	123.4
41	124.8	123.5
42	124.9	123.5
43	124.9	123.5
44	125.0	123.6
45	125.0	123.6
46	125.1	123.6
47	125.1	123.7
48	125.2	123.7
49	125.2	123.7
50	125.3	123.8

[a] Invert sugar only.

[b] Invert sugar containing 1 g sucrose per 100 ml.

Insert the following new test to precede the test entitled *Lead Limit Test*, page 518:

Lactose

Apparatus Use a suitable high-performance liquid chromatographic system (see page 476) equipped with a differential refractometer detector, a precolumn, an online 0.45-µm filter, and a 250-mm × 4.6-mm (id) stainless steel column.

Solid Phase Microparticle silica gel with siloxane bonded cyano-amino moieties (Whatman P-10 carbohydrate or equivalent) equilibrated and operated at room temperature.

Mobile Phase Acetonitrile–water (80:20) at a flow rate of 2 ml per min.

Reagents

Acetonitrile An appropriate grade for liquid chromatography.

Fructose Prepare a solution of fructose to be used as an internal standard by transferring 50 g of commercial grade β-D(−)fructose powder to a 500-ml volumetric flask; dissolve in and dilute to volume with water.

Standard Solution Transfer about 2 g of USP grade lactose (free of glucose), accurately weighed, to a 100-ml volumetric flask; add 10 ml of fructose internal standard solution and dilute to volume with water. Prepare fresh daily.

Water An appropriate grade for liquid chromatography.

System Suitability (see page 27, *First Supplement*)

Repeatability Allow the chromatographic system to equilibrate at a flow rate of 2 ml per min, then inject 25-µl aliquots of the *Standard Solution*. The chromatogram should show baseline resolution, and a retention time for water of 1-2 min, fructose 2-3 min, and lactose 5-6 min. The coefficient of variation for the relative peak heights (lactose peak height/fructose peak height) for 10 injections should be ≤0.6% when column equilibration is complete.

Linearity of Detector Response On a monthly basis (or when changes in the system are made) monitor the linearity of detector response by injecting standard lactose solutions containing 1.4, 1.8, 2.0, 2.2, and 2.6% lactose. Linear regression of the curve generated by plotting peak height versus concentration should give a correlation coefficient of at least 0.999.

Sample Preparation Prepare the sample as directed in the individual monograph. Analysis must be performed within 24 h.

Procedure Inject triplicate 25-µl aliquots of sample and standard solutions. If more than one sample is to be analyzed inject the standard solution after every third sample. Calculate results using average standard response factors bracketing every 3 samples (see page 73 of THIS SUPPLEMENT).

Calculation Calculate the percent lactose (L) on the dry basis using the formula:

$$L = (R_L/R_F) \times (W_L/W_S) \times (100 - M_L/100 - M_S) \times P,$$

in which R_L and R_F are the response factors for lactose and fructose; W_S and W_L are the weights, in g, of the sample and lactose standard in their respective solutions; M_S and M_L are the percentages of moisture in the sample and lactose standard; P is the purity, in percent, of the lactose standard. Determine the moisture content as directed under *Loss on Drying*, page 518.

Insert the following new test to precede the table entitled *Sulfuric Acid Table*, page 546.

Sulfur (by Oxidative Microcoulometry)

(Based on ASTM Designation D3120)

NOTE: All reagents used in this test should be reagent grade; water should be of high purity, and gases must be high-purity grade.

Apparatus The Dohrmann Microcoulometric Titrating System (MCTS-30), or its equivalent, shown in Figure 43, is to be used unless otherwise specified in an individual monograph. It consists of a constant rate injector (A), a pyrolysis furnace (B), a quartz pyrolysis tube (C), a granular tin scrubber (D), a titration cell (E), and a microcoulometer with a digital readout (F).

Granular-Tin Scrubber Place 5 g of 20/30 mesh granular reagent grade tin between quartz-wool plugs in an elongated 18/9-12/5 standard-taper adaptor which connects the pyrolysis tube and the titration cell.

Microcoulometer Must have variable attenuation, gain control, and be capable of measuring the potential of the sensing-reference electrode pair, and comparing this potential with a bias potential, amplifying the potential difference, and applying the amplified difference to the working-auxiliary electrode pair so as to generate a titrant. Also the microcoulometer output voltage signal must be proportional to the generating current.

Pyrolysis Furnace The sample should be pyrolyzed in an electric furnace having at least two separate and independently controlled temperature zones, the first being an inlet section that can maintain a temperature sufficient to volatilize all the organic sample. The second zone shall be a pyrolysis section that can maintain a temperature sufficient to pyrolyze the organic matrix and oxidize all the organically bound sulfur. A third outlet temperature zone is optional.

Pyrolysis Tube Must be fabricated from quartz and constructed in such a way that a sample, which is vaporized completely in the inlet section, is swept into the pyrolysis zone by an inert gas where it mixes with oxygen and is burned. The inlet end of the tube shall hold a septum for syringe entry of the sample and side arms for the introduction of oxygen and inert

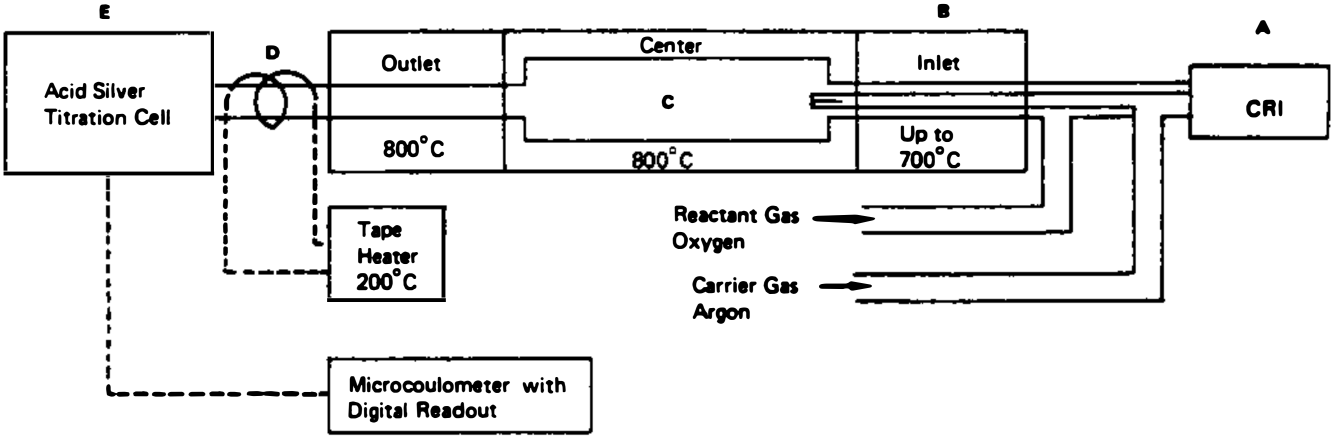


FIGURE 43 Microcoulometric Titrating System for the Determination of Sulfur in Hexanes.

gases. The center, or pyrolysis section, should be of sufficient volume to ensure complete pyrolysis of the sample.

Sampling Syringe A microlitre syringe of 10-µl capacity capable of accurately delivering 1 to 10 µl of sample into the pyrolysis tube. Three-inch × 24-gauge needles are recommended to reach the inlet zone of the pyroloysis furnace.

Titration Cell Must contain a sensor-reference pair of electrodes to detect changes in triiodide ion concentration, a generator anode-cathode pair of electrodes to maintain constant triiodide ion concentration, and an inlet for a gaseous sample from the pyrolysis tube. The sensor electrode shall be platinum foil and the reference electrode platinum wire in saturated triiodide half-cell. The generator anode and cathode half-cell shall also be platinum. The titration cell shall be placed on a suitable magnetic stirrer.

Preparation of Apparatus Carefully insert the quartz pyrolysis tube into the furnace, attach the tin scrubber, and connect the reactant and carrier-gas lines. Add the *Cell Electrolyte Solution* (see below) to the titration cell and flush the cell several times. Maintain an electrolyte level of 3.8 cm (1.5 in.) above the platinum electrodes. Place the titration cell on a magnetic stirrer and connect the cell inlet to the tin scrubber outlet. Position the platinum foil electrodes (mounted on the movable cell head) so that the gas-inlet flow is parallel to the electrodes with the generator anode adjacent to the generator cathode. Assemble and connect the coulometer in accordance with the manufacturer's instructions. Double-wrap the adaptor containing the tin scrubber with heating tape and turn the heating tape on. Adjust the flow of the gases, the pyrolysis furnace temperature, the titration cell, and the coulometer to the desired operating conditions. Typical operating conditions are as follows:

Reactant gas flow (oxygen), cm³/min	200
Carrier-gas flow (Ar, He), cm³/min	40
Furnace temperature, °C	
Inlet zone	700 (maximum)
Pyrolysis zone	800-1,000
Outlet zone	800 (maximum)
Tin-Scrubber temperature, °C	200
Titration cell	Stirrer speed set to produce slight vortex
Coulometer	
Bias voltage, mV	160
Gain	50
Constant Rate Injector, µl/s	0.25

The tin scrubber must be conditioned to sulfur, nitrogen, and chlorine before quantitative analysis can be achieved. A solution containing 10 mg/kg butyl sulfide, 100 mg/kg pyridine, and 200 mg/kg chlorobenzene in isooctane has proven an effective conditioning agent. With a fresh scrubber installed and heated, two 30-µl samples of this conditioning agent injected at a flow rate of 0.5 µl/s produces a steadily increasing response, with final conditioning indicated by a constant reading from the offset during the second injection.

Reagents

Argon or Helium (Argon preferred) High-purity grade; two-stage gas regulators must be used.

Cell Electrolyte Solution Dissolve 0.5 g of potassium iodide and 0.6 g of sodium azide in 500 ml of high-purity water, add 5 ml of glacial acetic acid and dilute to 1 L. Store in a dark bottle or in a dark place and prepare fresh at least every 3 months.

Oxygen High-purity grade.

Iodine Resublimed, 20 mesh or less.

Sulfur Standard (approximately 100 mg/kg) Weigh accurately 0.1569 g of *n*-butyl sulfide, into a tared 500-ml volumetric flask. Dilute to the mark with isooctane and reweigh. Calculate the sulfur concentration (S), in percent, by the formula:

$$S = W_b/W_s \times 2.192 \times 105,$$

in which W_b is the weight of *n*-butyl sulfide and W_s is the weight of the solution.

Calibration Prepare a calibration standard (approximately 5 mg/kg) by pipetting 5 ml of *Sulfur Standard* into a 10-ml volumetric flask and diluting to volume with isooctane. Fill and clamp the syringe onto the constant rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter in case of long-term drift in the automatic baseline zero circuitry. Switch S_1 automatically starts the stepper-motor syringe drive and initiates the analysis cycle. At 2.5 min (after setting switch S, set the digital meter with the scan potentiometer to correspond to the sulfur content of the known standard to the nearest 0.01 mg/kg. At the 3-min point, the number displayed on the meter stops, the plunger drive block is retracted to its original position, as preset by switch S_2, and a baseline re-equilibration period equal to the injection period must be allowed before a new sample may be injected. Repeat the *Calibration* step a total of at least four times.

Procedure Rinse the syringe several times with sample; then fill it, clamp it onto the constant-rate injector, push the sliding carriage forward to penetrate the septum with the needle, and zero the meter. Turn on switch S_1 to start the stepper-motor syringe drive automatically and initiate the analysis cycle. After the 3-min hold point, the number displayed on the meter corresponds to the sulfur content of the injected sample.

7/ Solutions and Indicators

Insert the following new section to precede the section entitled *Bromine*, under *Volumetric Solutions*, page 564.

Barium Hydroxide, 0.2 N [17.14 g $Ba(OH)_2$ per 1000 ml] Dissolve about 36 g of barium hydroxide, $Ba(OH)_2 \cdot 8H_2O$, in 1 L of recently boiled and cooled water and quickly filter the solution. Keep this solution in bottles with well-fitted rubber stoppers with a soda-lime tube attached to each bottle to protect the solution from carbon dioxide in the air. Standardize as follows: Transfer quantitatively about 60 ml of 0.1 *N* hydrochloric acid, accurately measured, to a flask; add 2 drops of phenolphthalein TS; and slowly titrate with the barium hydroxide solution, with constant stirring, until a permanent pink color is produced. Calculate the normality of the barium hydroxide solution and, if desired, adjust to exactly 0.2 *N* with freshly boiled and cooled water.

NOTE: Solutions of alkali hydroxides absorb carbon dioxide when exposed to air. It is preferable to connect the buret used for titrations with barium hydroxide solution directly to the storage bottle and to provide the bottle with a soda-lime tube so that air entering must pass through this tube, which will absorb carbon dioxide. Standard solutions of barium hydroxide should be restandardized frequently.

Sodium Thiosulfate, page 567:

Replace the last sentence on page 567 which begins, "Swirl to dissolve the sample, . . ." with the following:

Swirl to dissolve the sample, remove the stopper, and quickly add 2 g of sodium bicarbonate, 3 g of potassium iodide, and 5 ml of hydrochloric acid.

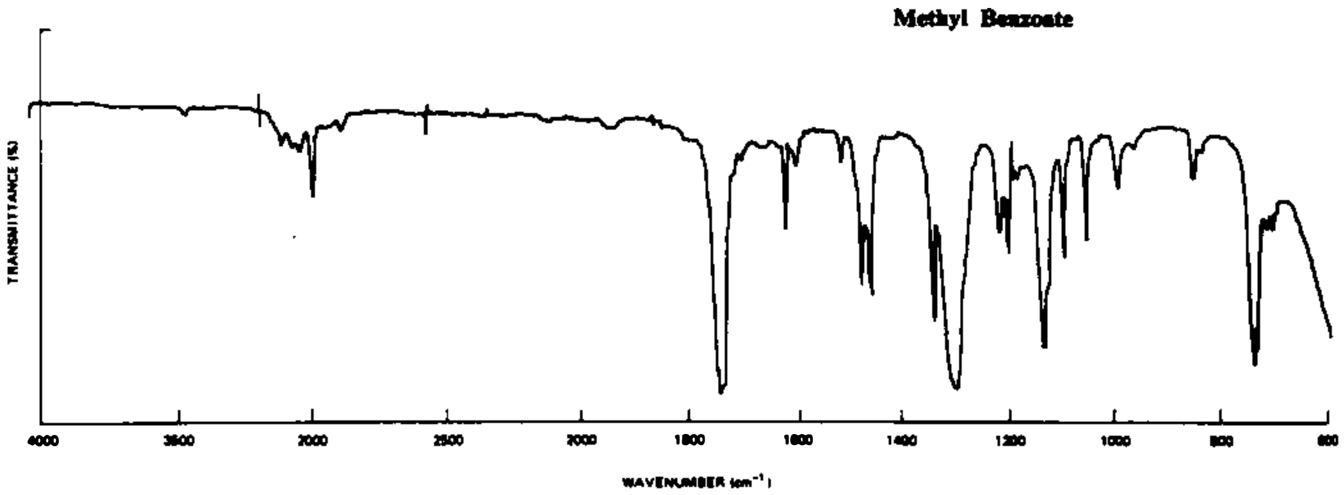
8/ *General Information*

No Change.

9/ *Infrared Spectra*

Methyl Benzoate, page 665

Replace the infrared spectrum of methyl benzoate with the spectrum shown below.



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Page citations refer to the First (1-34) and Second (35-90) Supplements to FCC III. An asterisk (*) indicates a new listing.