AN ABSTRACT OF A DISSERTATION

DETECTION AND ANALYSIS OF AMPHETAMINE-TYPE STIMULANTS IN WASTEWATER

Tammy Hatfield Boles

Doctor of Philosophy in Environmental Science-Chemistry

This research focused on the detection of the amphetamine-type stimulants (ATSs) amphetamine and methamphetamine in wastewater treatment plant (WWTP) influent, effluent before UV disinfection, and effluent after UV disinfection and samples within the sewage collection system from sites suspected to house clandestine methamphetamine laboratories. Wastewater samples were collected four times over the spring, summer and fall seasons and filtered through glass fiber filters before being subjected to solid-phase extraction. Sewage samples within the collection system were collected over a time interval of four weeks using a Polar Organic Chemical Integrative Sampler (POCIS), a passive sampling device. The ATSs were extracted from the POCIS sorbent with methanol. All extracted samples were analyzed via high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). Methamphetamine was found in wastewater influent and in sewage from one of the suspected methamphetamine laboratories.

DETECTION AND ANALYSIS OF AMPHETAMINE-TYPE STIMULANTS IN WASTEWATER

A Dissertation

Presented to

the Faculty of the Graduate School

Tennessee Technological University

By

Tammy Hatfield Boles

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

Environmental Science-Chemistry

December 2009

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

CERTIFICATE OF APPROVAL OF DISSERTATION

DETECTION AND ANALYSIS OF AMPHETAMINE-TYPE STIMULANTS IN WASTEWATER

by

Tammy Hatfield Boles

Graduate Advisory Committee:

Martha J. M. Wells, Chairperson	Date
Eugene A. Kline	Date
Hong Zhang	Date
S. Bradford Cook	Date
G. Kim Stearman	Date

Approved for the Faculty:

Francis Otuonye
Associate Vice President for
Research and Graduate Studies

Date

DEDICATION

To Jeffrey, Kathleen, and Jarrod for their loving support.

ACKNOWLEDGEMENTS

I would like to thank the Methamphetamine Research: Environmental Impact and Detection, Department of Justice – COPS for their funding. I would also like to thank Tom Graham, John Buford, and personnel at the Cookeville Wastewater Treatment Plant for their willingness to aid this research by providing wastewater and sludge samples, and passive sampler placement.

I am indebted to Gene Mullins both for training me to use the LC-MS/MS as well as his untiring assistance in the lab, even late in the evening and on weekends. I extend my appreciation to Dr. Daniel Swartling for his provision of chemical structure drawings, as well as his personal support.

I thank my advisory committee, Dr. Eugene Kline, Dr. Hong Zhang, Dr. S. Bradford Cook, and Dr. G. Kim Stearman for their time and valuable input as to my research and dissertation.

I extend my deepest thanks to my research advisor, Dr. Martha Wells, for her unwavering support and direction. Her faith in my ability to complete this research and degree program never lagged, even when mine did.

Finally I want to acknowledge my family. My father and mother, Bill and Kathleen Hatfield, gave me unending support and continuously kept me in their prayers. My husband Jeffrey, daughter Kathleen, and son Jarrod allowed me the time away from them to attend classes and participate in research and buoyed my spirits with their pride in my work. I could not have done any of this without their love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Description
ACN	acetonitrile
AIDS	acquired immune deficiency syndrome
AM	amphetamine
API	atmospheric pressure ionization
ATS	amphetamine-type substance
CNS	central nervous system
D5-MA	D5-methamphetamine
DEA	Drug Enforcement Agency
DTR	drug target residue
EDC	endocrine disrupting chemical
Eff	effluent
ESI	electrospray ionization
H_2O	water
HCl	hydrochloric acid
HLB	hydrophilic-lipophilic balanced
HPLC	high performance liquid chromatography
Inf	influent
LC	liquid chromatography
MA	methamphetamine
MCX	maximum cation exchange
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxyethamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MeOH	methanol
mM	millimolar
MRM	multiple reaction monitoring
NH_4OAc	ammonium acetate
NH_4OH	ammonium hydroxide
pK_a	acid dissociation constant
POCIS	polar integrative chemical sampler
PPCP	pharmaceutical and personal care product
PTFE	polytetrafluoroethylene
SPE	solid-phase extraction
SPMD	semipermeable membrane device
SRM	single reaction monitoring
STP	sewage treatment plant
SW	sewage water
UNODC	United Nations Office on Drugs and Crime
UPLC	ultra performance liquid chromatography
UV	ultra-violet
WCX	weak cation exchange
WW	wastewater
WWTP	wastewater treatment plant

CHAPTER 1

METHAMPHETAMINE: AN AMPHETAMINE-TYPE STIMULANT

1.1 Introduction

Methamphetamine, also known as “meth,” “speed,” and “crank,” is a highly addictive, powerful nervous system stimulant. Methamphetamine increases wakefulness and physical activity, but its effects can also cause extreme nervousness, hyperactivity, convulsions, and even death. Chronic use leads to tolerance and further drug dependence. Because methamphetamine is so addictive, it is illegal to manufacture or use methamphetamine under Federal Law.

The chemical formula of methamphetamine is $C_{10}H_{15}N$, and its 2D chemical structure is shown in Figure 1, and properties of methamphetamine are shown in Table 1. Methamphetamine is a synthetic drug illegally sold in pill, capsule, powder, and chunk form. In its pure form, the hydrochloride salt of methamphetamine is yellow to colorless, although the street drug may be colored due to impurities. The drug can be created cheaply and easily in clandestine, “clan,” labs and can be smoked, snorted, inhaled, or injected. Methamphetamine exists as the d-methamphetamine and l-methamphetamine stereoisomers; d-methamphetamine is a potent CNS stimulant, but l-methamphetamine has little CNS activity.

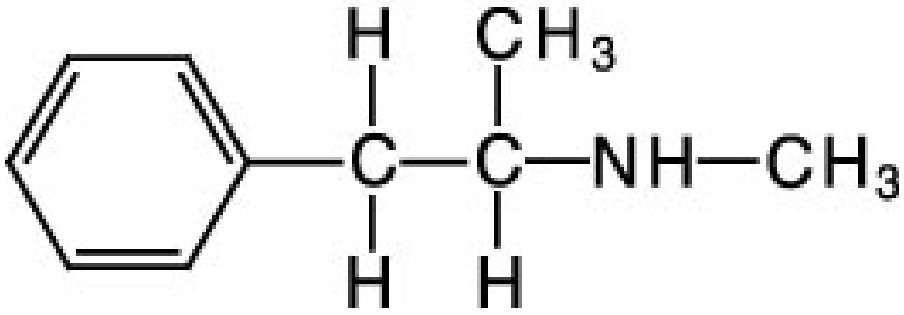

Figure 1-1. Structure of methamphetamine

Table 1-1. Properties of Methamphetamine

Molecular Weight		**149.23**
pKa		**10.38 ± 0.10**
log D @ pH 7		**-0.97**
log D @ pH 8		**-0.36**

According to the Koch Crime Institute (1) there are literally thousands of recipes and information about making methamphetamine on the Internet. Approximately one-third of the chemicals that can be used to make methamphetamine are extremely toxic, and many of them are reactive, flammable, and corrosive. A short list of the chemicals used in the preparation of methamphetamine includes muratic acid, lye, acetone, brake fluid, brake cleaner, iodine crystals, lithium metal or batteries, lighter fluid, pseudoephedrine, ethyl ether, anhydrous ammonia, sodium metal, red phosphorus, and ephedrine. The chemicals can be found in over-the-counter medicine, gasoline, drain cleaners, fertilizer, and matches.

Methamphetamine causes a euphoric effect similar to that of cocaine, but it lasts longer. Heart and breathing rates rise, blood pressure increases, and feelings of hunger and fatigue are reduced. In addition to the decrease in appetite, dry mouth occurs making swallowing difficult. Extremely high doses can cause the user to flush or become pale, become uncoordinated, and physically collapse. Injecting the drug creates such an increase in blood pressure that high fevers, strokes, and even heart failure can occur. Fatigue and feelings of depression plague users as the drug wears off, and to compensate, addicts increase the amount of methamphetamine taken. Long term use of methamphetamine can lead to malnutrition, skin disorders, and vitamin deficiencies. Users who take the drug intravenously are at risk for diseases such as AIDS and hepatitis C. In addition to devastating physical effects, mental illnesses can occur, sometimes resulting in suicide or other violent deaths (2). Methamphetamine is so highly addictive that statistics show that methamphetamine users live only an average of five years after taking the first dose of the drug (3).

1.2 Significance

Because methamphetamine is so cheap and easy to produce, clandestine labs have proliferated and have been found in all states of the United States. In 1993, Drug Enforcement Agency (DEA) officials estimated that 218 labs existed; in 2004 almost 15,000 labs were seized (4). In Tennessee alone, law enforcement agencies across the state have encountered approximately 7,600 clandestine methamphetamine labs from 1998 to the present (5). Toxic and combustible chemicals used in the manufacture of methamphetamine pose a danger to individuals who manufacture the drug. In 1996, three fatalities occurred from exposure to phosphine gas, which is a by-product in the ephedrine/hydriodic acid/red phosphorus method of methamphetamine manufacturing. Besides the dangers posed to drug manufacturers, law enforcement officials who respond to clandestine labs are exposed to the products. According to one report, injuries to responding law officers almost doubled from 2002-2003 (6).

Based on published reports from both the United States and Europe, measurable concentrations of amphetamine and methamphetamine, two of a class of drugs known as amphetamine-type stimulants (ATSs) have been found in wastewater, surface water, and some biosolids samples. Local news reports have chronicled several instances of clandestine methamphetamine laboratories, lending credence to the theory that ATSs would be found in wastewater from the local WWTP. However, no studies have been conducted to detect and quantify the concentration of ATSs in sewer pipes from suspected clandestine methamphetamine laboratories.

The focus of this research was on the detection and quantification of amphetamine and methamphetamine in wastewater. Firstly, grab and composite influent and effluent

water samples, both before and after ultra-violet (UV) were analyzed. Secondly, sewage samples were collected from areas suspected to house clandestine methamphetamine labs. Polar Organic Chemical Integrative Samplers (POCIS) were put into the sewer lines where pipes from the property entered the larger city sewer lines to determine contributions of methamphetamine from suspected clandestine laboratories to WWTP influent. The ability to pinpoint the location of methamphetamine in the sewer may at some time in the future be used as a tool in law enforcement.

1.3 References

[1] KCI, The Anti-Meth Site, http://www.kci.org/Meth_info/faq_Meth.htm.

[2] KCI, The Anti-Meth Site, http://www.kci.org/Meth_info/meth_facts2.htm.

[3] Tennessee District Attorney General Conference, http://www.methfreetn.org.

[4] "109th Congress Report, House of Representatives," The Library of Congress, http://thomas.loc.gov/cgi-bin/query/D?c109:2:./temp/~c109IoDPBm::

[5] "Cleanup of Methamphetamine Contaminated Properties," http://tennessee.gov/environment/dof/meth, June 1, 2009

[6] **Methamphetamine Use: Lessons Learned,** Hunt, D., Kuck, S., Truitt, L., Final Report, United States Department of Justice, 2006.R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, California, 7th ed., 2004.

CHAPTER 2

ANALYSIS OF AMPHETAMINE-TYPE STIMULANTS AS EMERGING POLLUTANTS

2.1 Introduction

The phrase "emerging pollutants" can be defined as substances that are not presently known to cause impairments in water systems but that have characteristics such as ability to bioaccumulate, persistence in the environment, and toxicity and potentially impact the integrity of water [1]. Neither monitoring nor reporting is required of these substances, but they may still be present in the urban water cycle. Much of the peer-reviewed literature has focused on pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs), but recent research has also included groups of illicit drugs such as cocaine, opioids, opioid pharmaceuticals, cannabis, and amphetamine-type stimulants (ATSs). Although drugs in all of these categories have been found in environmental samples, this review will focus on analytical determination of amphetamine and methamphetamine.

Amphetamine and methamphetamine enter our water supply by human excretion after legal or illegal consumption and via manufacturing in clandestine laboratories. Amphetamines and methamphetamines are sometimes legally prescribed for certain medical conditions such as attention deficit hyperactivity disorder and exogenous obesity; therefore, their presence in wastewater cannot be attributed solely to illegal consumption. However, according to the United Nations Office of Drug and Crime (UNODC), the

global problem with clandestine ATS markets is worsening, with estimates that between 230 and 640 metric tons of amphetamine-group substances (excludes ecstasy-group substances) were manufactured in 2007 [2].

Approximately 62% of methamphetamine [3] and 30-40% of amphetamine [4] consumed is excreted in the urine within 24 hours of an oral dose, and both amphetamine and methamphetamine are primarily excreted as the intact drug [5]. Once these drugs enter the wastewater treatment plant (WWTP) as influent, they can potentially enter surface or groundwater from inadequately treated WWTP effluent, wet-weather run-off, landfill seepage, contaminated streams and lakes, drainage from fields irrigated with effluent, and even from effluent used to recharge aquifers [6]. Jones-Lepp et al. [6] also noted that unlike non-polar pollutants of historic concern, these polar compounds are not readily sorbed to the subsoil, increasing the potential to enter surface and groundwater.

The purpose of this manuscript is to compile a review of state-of-the-art analytical methodology used for sampling, sample preparation, separation, and detection of ATSs in environmental samples. Reported occurrences of ATSs in the environment are noted, and future research needs that challenge applications of analytical techniques are discussed.

2.2 Analytical Methodology

2.2.1 Sample Types and Collection

Samples from surface waters (rivers and lakes) and WWTP influent and effluent were collected in Europe and the United States. The types of water sampled, the

geographic regions in which they were sampled, and the types of samples collected, for analyses of ATSs are summarized in Table 1. Sampling methods varied and included (1) grab samples, (2) 24-hour composite samples, and (3) passively collected samples. A grab sample is collected simultaneously and reflects a single data point in time. A 24-hour composite sample involves collecting discrete samples taken at specific intervals of time and combining them at the end of 24 hours into a single sample. The composite sample reflects an average concentration of the analyte in the water source over a 24-hour time period. Passive water sampling is based on the free flow of water molecules across a sampling medium. Semipermeable membrane devices (SPMDs) and polar organic chemical integrative samplers (POCIS) are the most common passive sampling devices currently in use, but because of the polar nature of methamphetamine and amphetamine, only POCIS was used in the water sampling reviewed in this article.

2.2.2 Sample Preparation

2.2.2.1 Solids removal and internal standards. After collection, Zuccato et al. [7] and Castiglioni et al. [11] filtered surface water and WWTP samples through Whatman GF/A 1.6 µm glass filters. Prior to solid-phase extraction (SPE), 50 mL of sample was spiked with 20 ng of either amphetamine-D_6 or methamphetamine-D_9 as an internal standard, and the pH was adjusted to 2.0 with 37% HCl. Alternatively, samples were acidified before filtration by Kasprzyk-Hordern et al. [8,9]. Surface water and WWTP samples were acidified to pH 2.0 with 37% HCl and vacuum filtered through

Table 2-1.
Geographic Regions and Waters Sampled for ATSs

Reference	Region	Water Type	Sample Type
Zuccato et al. [7]	Rivers Po, Lambro and Olona and Lakes Maggiore, Varese, and Lugano in northern Italy; River Arno in central Italy; River Thames in Oxfordshire and London, UK.	River samples downstream from largely populated areas. Lake samples in order to study medium (Varese, Lugano) and large (Maggiore) water basins	Two hour composite samples; pooled lake or river samples every 20 minutes
Kasprzyk-Hordern et al. [8]	Six sampling sites on the River Taff and four sample sites on the River Ely in South Wales, UK	River Taff samples from the source to where the river enters the Bristol Channel. WWTP[a] Cilfynydd is on the river. River Ely samples upstream and downstream of WWTP Coslech	Replicate grab samples
Kasprzyk-Hordern et al. [9]	River Taff samples collected upstream and downstream of WWTP Cilfynydd and WWTP influent and effluent. River Ely samples collected upstream and downstream of WWTP Coslech and WWTP influent and effluent.	River and WWTP influent and effluent	Replicate grab samples and 24-hour composite samples were collected at WWTP Coslech
Bartelt-Hunt et al. [10]	Surface waters upstream and downstream of WWTP outfalls and in WWTP effluent in Nebraska, USA	Surface waters and Omaha, NE WWTP effluent	POCIS samplers were deployed for seven days. At one site, POCIS was recovered after four weeks due to vandalism

Jones-Lepp et al. [6]	Three WWTPs located in Nevada, Utah, and South Carolina	WWTP effluent	POCIS samplers were deployed for 30 days
Castiglioni et al. [11]	WWTPs in Milan-Nosedo, Italy, and in Lugano, Switzerland	WWTP influent and effluent	Two 24-hour composite samples; pooled every 20 minutes
Huerta-Fontela et al. [12]	WWTPs in Catalonia, Spain, and samples from the Llobregat River, Spain	WWTP influent and effluent and surface waters	24-hour composite samples from WWTPs; grab samples from Llobregat River
Huerta-Fontela et al. [13]	WWTPs in Northeast Spain	WWTP influent and effluent	Grab samples initially, then 24-hour composite samples; pooled every hour
van Nuijs et al. [14]	WWTPs in Belgium	WWTP influent	24-hour composite samples
Bijlsma et al. [15]	WWTPs in Castellón, Spain	WWTP influent and effluent	24-hour composite samples
Postigo et al. [16]	El Prat STP[b] in Barcelona, Spain; STPs in Valencia, Benicasim, and Gandia	WWTP influent and effluent	24-hour composite samples
Chiaia et al. [17]	Seven WWTPs in the US	WWTP influent	24-hour composite samples

[a] WWTP – Wastewater Treatment Plant
[b] STP – Sewage Treatment Plant

Whatman GF/F 0.7 µm glass fiber filters. A 1 L sample was spiked with 200 ng phenacetin-ethoxy-1-^{13}C as the internal standard/surrogate standard in preparation for SPE [8,9]. Huerta-Fontela et al. [12,13] filtered samples through Whatman GF/A 1.6 µm glass microfiber filters and added amphetamine-D_8 and methamphetamine-D_9 as the internal standards. A 100 mL sample was used for SPE. Influent and effluent 24-hour composite samples collected by Postigo et al. [16] were vacuum-filtered through Whatman 1 µm glass fiber filters followed by 0.45 µm nylon membrane filters. The samples were spiked with amphetamine-D_5 and methamphetamine-D_{14}. Instead of using vacuum filtration, Chiaia et al. [17] centrifuged a 7 mL aliquot of the WWTP influent samples for 30 min at 7,100 rpm. The supernatant was transferred to a 6-mL vial and spiked with amphetamine-D_6 and methamphetamine-D_5.

2.2.2.2 Extraction procedures. In 12 articles cited for this review, SPE was used to extract amphetamine and methamphetamine from the sample. The sorbents used and conditioning and elution procedures for all articles reviewed are summarized in Table 2. SPE was not used in two articles: Jones-Lepp et al. [19] used accelerated solvent extraction (ASE) for the extraction of methamphetamine from biosolids while Chiaia et al. [17] used large-volume injection followed by LC-MS/MS to eliminate SPE entirely. Postigo et al. [16] described a fully online SPE method in which PLRP-s, a cross-linked styrene-divinylbenzene polymer, was used for extraction of ATSs.

Table 2-2.
Extraction Procedures

Drugs Detected	Sample Type	Reference	Sorbent	Conditioning	Elution	Recovery %
AM[a], MA[b]	Surface waters, composite samples	Zuccato et al. [7]	Oasis-MCX[c]	MeOH, MilliQ H_2O, pH 2 H_2O	MeOH, 2% NH_3 in MeOH	AM – 101 ± 4.5 (sw [f]) MA – 108 ± 6.9 (sw)
AM	Surface waters, grab samples	Kasprzyk-Hordern et al. [8]	Oasis-MCX	Not available	MeOH, 5% NH_4OH in MeOH	AM – 91 (sw)
AM	Surface waters and wastewater influent and effluent, composite and grab samples	Kasprzyk-Hordern et al. [9]	Gilson ASPEC XL4 and Oasis-MCX	Not available	MeOH, 5% NH_4OH in MeOH	Not available
AM, MA	Surface waters and wastewater effluent, passively collected samples	Bartelt-Hunt et al. [10]	POCIS, Oasis-HLB[d] sorbent	Not applicable	MeOH	Not available
MA	Wastewater influent and effluent, passively collected samples	Jones-Lepp et al. [6]	POCIS, Oasis-HLB sorbent	Not applicable	MeOH	Not available

AM, MA	Wastewater influent and effluent, composite samples	Castiglioni et al. [11]	Oasis-MCX	MeOH, MilliQ H_2O, pH 2 H_2O	MeOH, 2% NH_3 in MeOH	AM – 110 ± 4.5 (inf[g]) MA – 112 ± 6.5 (inf) AM – 103 ± 4.2 (eff[h]) MA – 97 ± 3.4 (eff)
AM, MA	Surface waters, wastewater influent and effluent, composite and grab samples	Huerta-Fontela et al. [12]	Zymark Rapid Trace SPE Workstation using Oasis-HLB	MeOH, MilliQ H_2O, 5% MeOH in H_2O	MeOH	AM – 75 ± 3.9 (sw) MA – 83 ± 2.1 (sw) AM – 70 ± 6.8 (ww) MA – 80 ± 4.3 (ww)
AM, MA	Wastewater influent and effluent, grab samples	Huerta-Fontela et al. [13]	Zymark Rapid Trace SPE Workstation using Oasis-HLB	MeOH, MilliQ H_2O, 5% MeOH in H_2O	MeOH	Not available
AM, MA	Wastewater influent and effluent, composite samples	Bijlsma et al. [15]	Oasis-MCX	MeOH, MilliQ H_2O, pH 2 H_2O	2% NH_3 in MeOH	AM – 113 (inf) MA – 116 (inf) AM – 102 (eff) MA – 94 (eff)

AM, MA	Wastewater influent and effluent, composite samples	Postigo et al. [16]	Online SPE, PLRP-s[e]	ACN, H_2O	ACN, H_2O	AM – 94 (inf) MA – 114 (inf)
AM, MA	Wastewater influent, composite samples	Chiaia et al. [17]	Not applicable	Not applicable	Not applicable	Not applicable
AM	Sewage sludge (biosolids)	Kaleta et al. [18]	Oasis-HLB	Acetone, H_2O, borate buffer (pH 10)	MeOH: HCOOH (20:80, v/v)	Not available
MA	Sewage sludge (biosolids)	Jones-Lepp et al. [19]	Not applicable	Not applicable	Not applicable	Not available

[a] AM – amphetamine
[b] MA – methamphetamine
[c] MCX – poly(divinylbenzene-co-N-vinylpyrrolidone) with a surface bonded sulfonic acid group
[d] HLB – poly(divinylbenzene-co-N-vinylpyrrolidone)
[e] PLRP-s – cross-linked styrene-divinylbenzene polymer
[f] sw – surface water
[g] inf – influent
[h] eff – effluent
[i] ww – wastewater, influent/effluent not specified

Five studies used Oasis Hydrophilic-Lipophilic Balance (HLB) sorbents [6, 10, 12, 13, 18] and five used Oasis MCX sorbents [7, 8, 9, 11, 15] for SPE. Oasis HLB is a reversed-phase sorbent that can be used for all compounds, and Oasis MCX is a mixed-mode cation-exchange reversed-phase sorbent for bases with pK_a of 2-10. Oasis HLB sorbents are made by polymerizing divinylbenzene (lipophilic) and N-vinylpyrrolidone (hydrophilic) monomers. They are capable of extracting acidic, basic, and neutral analytes, which may be polar or nonpolar. Oasis MCX sorbents are formed by introducing a sulfate functional group into the Oasis HLB sorbent to generate a benzenesulfonic acid moiety with a $pK_a < 1$.

van Nuijs et al. [14] compared the use of Oasis HLB and MCX sorbents for analysis of abused drugs in wastewater, and the results for amphetamine and methamphetamine are presented in Table 3. For Oasis HLB, 500 mg and 60 mg sorbent masses were tested at pH 7 and pH 3. The washing step was evaluated using Milli-Q water, Milli Q water at pH 2, hexane, or no washing step. Oasis MCX 500 mg and 60 mg sorbent masses were evaluated only at pH 2, but as with the HLB cartridges, the washing steps tested were the same. Recoveries using the Oasis HLB sorbent at pH 7 were low (13-63%) regardless of the sorbent size. However, at pH 3, both the 500 and 60 mg HLB sorbents gave recoveries of 91-106%. Recoveries were also excellent (98-105%) for the Oasis MCX 60 mg sorbent. Because the Oasis HLB sorbent at pH 3 and the MCX sorbent at pH 2 yielded similar recoveries for amphetamine and methamphetamine in the van Nuijs et al. [14] study, the deciding factor influencing the choice of sorbent may be

Table 2-3.
Extraction Recoveries (%) for Oasis HLB and Oasis MCX Cartridges with Different Sample pH, Sorbent Mass and Washing Step

	Oasis HLB						**Oasis MCX**				
Sample pH	**pH 7**				**pH 3**		**pH 2**				
Sorbent	500 mg/6 cm^3			60 mg/3 cm^3	500 mg/6 cm^3	60 mg/3 cm^3	500 mg/6 cm^3	60 mg/3 cm^3			
Washing Step	No wash	Milli-Q	Hexane	Milli-Q	Milli-Q	Milli-Q	Milli-Q	No wash	Milli-Q	Water pH 2	Hexane
AM	20	16	13	53	106	91	68	103	102	105	100
MA	32	18	23	63	99	93	84	101	99	98	103

Excerpted from Reference [14]: A.L.N. van Nuijs, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Analysis of drugs of abuse in wastewater by hydrophilic interaction liquid chromatography—tandem mass spectrometry, Anal. Bioanal. Chem. 395 (2009) 819, with kind permission of the corresponding author and Springer Science + Business Media.

(a) the selection of additional illicit drugs or pharmaceuticals to be analyzed from the same sample and (b) removal of background co-extracted interferences.

2.2.3 Recovery from Passive Samplers

Jones-Lepp et al. [6] and Bartelt-Hunt et al. [10] each used the POCIS passive sampling system to collect surface water and WWTP samples. Three pharmaceutical POCIS devices, each with a 41 cm^2 surface area of hydrophilic polyethersulfone membranes (0.1 μm pore size) enclosing 200 mg of Oasis HLB sorbent were deployed in stainless-steel canisters. Pharmaceutical POCIS devices are designed for most pharmaceuticals and contain only HLB sorbent, as opposed to the pesticide POCIS devices that contain three different sorbents and target pesticides as well as hormones and wastewater treatment chemicals. After 7-d and 28-d sampling periods, respectively, the POCIS apparatuses were rinsed with water to remove debris and then opened. The sorbents were washed with MeOH into silane-treated vials, and the analytes were eluted by passing MeOH through glass gravity-flow chromatography columns (1-cm inside diameter) fitted with silanized glass wool plugs and stopcocks. Extracts were filtered and concentrated before separation and detection.

2.2.4 Separation and Detection

Of the manuscripts reviewed, HPLC or UPLC was used for separation followed by tandem-mass spectrometry, with C_{18} being used as the column sorbent in 13 of 14 studies (Table 4). A Phenomenex Luna hydrophilic interactive liquid chromatography

(HILIC) column, rather than the ubiquitous C_{18} column, was used by van Nuijs et al. [14], who reported better ionization in MS detection and higher sensitivity as rationale for using the HILIC column. Multiple reaction monitoring (MRM) and selected reaction monitoring (SRM) were commonly utilized modes of spectrometry. Jones-Lepp et al. [6] used full-scan mode. Although atmospheric pressure ionization (API) was sometimes used, electrospray ionization (ESI) was most commonly reported, and the MS analyses were always performed in positive mode.

2.3 Occurrence

Occurrence reports of emerging pollutants including ATS-type stimulants were reviewed [20]. Wastewater treatment did not remove amphetamine or methamphetamine completely from the effluent in most cases (Table 5); however, in instances in which ATSs were found in the influent, they were greatly reduced in the effluent.

The efficiency in removing ATSs strongly depended on the wastewater technology used in the WWTPs [9], with two different types of treatment technology studied at two treatment plants in South Wales in the United Kingdom. At WWTP Cilfynydd, technology relied on trickling filter beds and resulted, on average, in less than 70% removal of the PPCPs studied. In contrast, WWTP Coslech reported a greater removal efficiency more than 85%, which was attributed to the use of a more efficient activated sludge treatment as opposed to the trickling filter beds. Overall, activated

Table 2-4.
Separation and Detection

Sample Type	Reference	Column	Mobile Phase	Detection	Mode
Surface waters, composite samples	Zuccato et al. [7]	Waters XTerra MS C18, 100 x 2.1 mm, 3.5 µm	Not available	Applied-Biosystems-Sciex API 3000 triple quad with turbo ion spray source; Perkin-Elmer LC Series 200	SRM
Surface waters, grab samples	Kasprzyk-Hordern et al. [8]	ACQUITY UPLC BEH C18, 1 x 100 mm, 1.7 µm	H_2O, MeOH, CH_3COOH	Waters ACQUITY UPLC, ESI	MRM
Surface waters and wastewater influent and effluent, composite and grab samples	Kasprzyk-Hordern et al. [9]	ACQUITY UPLC BEH C18, 1 x 100 mm, 1.7 µm	H_2O, MeOH, CH_3COOH	Waters ACQUITY UPLC, ESI	MRM
Surface waters and wastewater effluent, passively collected samples	Bartelt-Hunt et al. [10]	Thermo Betabasic-18, 250 x 2.1 mm, 5 µm	MeOH, 0.1% HCOOH (in H_2O)	Quattro Micro triple quadrupole; Waters 2695 HPLC, ESI	MRM
Wastewater influent and effluent, passively	Jones-Lepp et al. [6]	Restek Allure C18, 150 x 3.2 mm, 5 µm	H_2O, NH_3CH_3COOH, CH_3COOH, MeOH	ThermoQuest Finnigan LCQ, ESI	Full-scan

collected samples					
Wastewater influent and effluent, composite samples	Castiglioni et al. [11]	Waters XTerra MS C18, 100 x 2.1 mm, 3.5 µm	Not available	Applied Biosystems-Sciex API 3000 triple quad with turbo ion spray source; Perkin-Elmer LC Series 200	MRM
Surface waters, wastewater influent and effluent, composite and grab samples	Huerta-Fontela et al. [12]	ACQUITY UPLC BEH C18, 2.1 x 100 mm, 1.7 µm	ACN: 0.1% HCOOH, 30mM HCOOH: NH_3COOH	Waters ACQUITY UPLC, ESI	SRM
Wastewater influent and effluent, grab samples	Huerta-Fontela et al. [13]	ACQUITY UPLC BEH C18, 2.1 x 100 mm, 1.7 µm	ACN: 0.1% HCOOH, 30mM HCOOH: NH_3COOH	Waters ACQUITY UPLC, ESI	SRM
Wastewater influent, composite samples	van Nuijs et al. [14]	Phenomenex Luna HILIC, 150 x 3 mm, 3 µm	5 mM NH_3COOH, ACN	Agilent 6410 triple quad, ESI	MRM
Wastewater influent and effluent, composite samples	Bijlsma et al. [15]	ACQUITY UPLC BEH C18, 2.1 x 50 mm, 1.7 µm	MeOH, 5 mM NH_3COOH: 0.1% HCOOH	TQD triple quad, ESI	SRM

Wastewater influent and effluent, composite samples	Postigo et al. [16]	Merck Purospher Star RP-18e, 125 x 2.0 mm, 5 µm with guard column 4 x 4 mm, 5 µm	ACN: H_2O	Applied Biosystems-Sciex 4000QTRAP hybrid triple quad with turbo ion spray source	SRM
Wastewater influent, composite samples	Chiaia et al. [17]	Waters Atlantis T3 C18, 4.6 x 150 mm, 5 µm with Phenomenex C 18, 2.0 x 4.0 mm guard column	5% MeOH: 0.1% CH_3COOH, ACN	Waters Quattro Micro tandem MS, ESI	MRM
Sewage sludge (biosolids)	Kaleta et al. [18]	Schermbeck YMC-Pack Pro C18, 12 nm bore, 3 µm with YMC ProC18 10 x 4.0 mm guard column	50 mM HCOOH; MeOH	Agilent UV-vis diode array detector and Thermo Finnigan LCQ Deca XP plus IT, API	SRM
Sewage sludge (biosolids)	Jones-Lepp et al. [19]	Agilent Zorbax RX-C18, 2.1 x 100 mm, 3.5 µm	82% MeOH: 18% ACN: 0.1% HCOOH, 99% H_2O: 0.1% HCOOH	Thermo Finnigan LCQ, ESI	SRM

ESI – Electrospray Ionization
API – Atmospheric Pressure Ionization
SRM – Selected Reaction Monitoring
MRM – Multiple Reaction Monitoring

Table 2-5.
Environmental Occurrence of ATSs

Reference	Amphetamine	Methamphetamine
Zuccato et al. [7]	River Olona < 0.65 ng/L River Lambro < 0.65 ng/L River Po < 0.65 ng/L River Arno < 0.65 ng/L River Thames < 0.65 ng/L	River Olona 1.7 ng/L River Lambro 2.1 ng/L River Po < 0.41 ng/L River Arno < 0.41 ng/L River Thames < 0.41 ng/L
Kasprzyk-Hordern et al. [8]	River Taff varied from below detection to 1-14 ng/L River Ely varied from below detection to 1-21 ng/L	Not tested
Kasprzyk-Hordern et al. [9]	River Taff found in very high frequency. Concentrations were 1-11 ng/L, with a mean of 3 ng/L. Samples downstream of the WWTP Cilfynydd showed in 100% of samples ranging 2-13 ng/L with a mean of 7 ng/L. Also found 100% of the time in the influent and 14% of the time in effluent samples. Influent 255-3225 ng/L and effluent 3-11 ng/L	Not tested
Bartelt-Hunt et al. [10]	Not tested	Grand Island, NE: upstream 1.4 ng/L, downstream 6.6 ng/L Columbus, NE: upstream 1.3 ng/L, downstream 2.3 ng/L Lincoln, NE: upstream ND, downstream 25.2 ng/L Hastings, NE: downstream 62.6 ng/L Omaha, NE: effluent 350.1 ng/L

Jones-Lepp et al. [6]	Not tested	Site 1: 1.3 ng/L; Site 1-II: 0.8 ng/L; Site 2: ND; Site 3: ND
Castiglioni et al. [11]	Nosedo: influent 14.7 ± 10.6 ng/L, effluent <LOQ Lugano: influent <LOQ, effluent <LOQ	Nosedo: influent 16.2 ±7.1 ng/L, effluent 3.5 ± 2 ng/L Lugano: influent <LOQ, effluent <LOQ
Huerta-Fontela et al. [12]	WWTP influent 15 ng/L, effluent <LOQ	Not tested
Huerta-Fontela et al. [13]	42 WWTPs in NE Spain:22 samples influent 3-688 ng/L, 10 samples effluent 4-210 ng/L	42 WWTPS in NE Spain: 17 samples influent 3-277 ng/L, 12 samples effluent 3-90 ng/L
van Nuijs et al. [14]	11 WWTPs in Belgium: 12 samples influent 3-681 ng/L	11 WWTPs in Belgium: 12 samples influent <1-16 ng/L
Bijlsma et al. [15]	WWTP in Spain: 28 samples influent <0.5-1.40 µg/L, effluent <0.5-0.21 µg/L	WWTP in Spain: 28 samples influent below detection limit
Postigo et al. [16]	El Prat: influent 41.1 ± 9.1 ng/L, effluent 0.5 ± 0.1 ng/L Valencia: influent 20.4 ng/L, effluent 2.2 ng/L Benicasim: influent 35.5 ng/L, effluent 1.0 ng/L Gandia: influent 6.5 ng/L, effluent 3.3 ng/L	El Prat: influent 18.2 ± 5.8 ng/L, effluent 6.3 ± 0.6 ng/L Valencia: influent 7.8 ng/L, effluent 2.7 ng/L Benicasim: influent 3.7 ng/L, effluent 2.0 ng/L Gandia: influent 3.0 ng/L, effluent 1.5 ng/L
Chiaia et al. [17]	Plant 1: 220 ± 30 ng/L, Plant 2: 550 ± 80 ng/L, Plant 3: 80 ± 10 ng/L, Plant 4: 120 ± 20 ng/L, Plant 5: 250 ± 40 ng/L, Plant 6: 90 ± 10 ng/L, Plant 7: 130 ± 20 ng/L	Plant 1: 920 ± 70 ng/L, Plant 2: 2000 ± 200 ng/L, Plant 3: ND, Plant 4: 10 ± 1 ng/L, Plant 5: 920 ± 70 ng/L, Plant 6: 150 ± 10 ng/L, Plant 7: <LLOQ

sludge technology was found to be more effective in the removal of ATSs [9]. Huerta-Fontela et al. [13] reported 52 to 99% removal efficiency of amphetamine and 44 to 99% removal efficiency of methamphetamine. Bijlsma et al. [15] observed 85% removal efficiency for amphetamine and 99% removal efficiency for methamphetamine.

Although the UNDOC has reported an increase in the production of illegal amphetamine and methamphetamine from clandestine laboratories in different countries based on crime statistics, drug monitoring, and seizure rates, some consumption data are based on information supplied by drug consumers themselves. These estimation techniques create a high level of uncertainty [2]. Additionally, because data collection and analysis are time consuming, it is not always possible to detect changing patterns and to compare test results among local communities.

To provide more realistic data, Zuccato et al. [21] reported a sewage epidemiology approach to monitoring collective community use of abused drugs. They conducted studies to provide objective, quantitative, near-real-time profiles of illicit drug consumption by monitoring the drugs entering the sewage system in Milan (Italy), Lugano (Switzerland), and London (England) (Table 6). The results shown are back-calculated rates of consumption determined by multiplying the concentration of the drug by the influent wastewater flow rate, normalizing the data for the local population size, and taking into account metabolic excretion.

A similar study was performed at 42 WWTPs in Northeast Spain by Huerta-Fontela et al. [13]. Load per capita (mg/(day 1,000 inhabitants)) ranged from nondetectable to 427 (mg/(day 1,000 inhabitants)) for amphetamine and nondetectable to 78 (mg/(day 1,000 inhabitants)) for methamphetamine. Huerta-Fontela et al. [13] also

Table 2-6.
Amounts (mg/day/1,000 People) of Major Drug Target Residues (DTR) from ATSs Conveyed Daily in Urban Wastewater to STPs in Milan, Lugano, and London

DTR	Milan	Lugano	London
Amphetamine	2.7 ± 2.8	ND	24 ± 5
Methamphetamine	4.5 ± 1.6	ND	2.4 ± 0.3

ND – not detected
Excerpted from Reference [21] – Reproduced with permission from *Environmental Health Perspectives*

examined daily variations in ATS concentrations at a WWTP during a one-week period (Table 7). ATS concentrations varied widely during the week but showed a sharp increase over the weekend [13].Analyses of abused drugs and their human metabolites were also used by Postigo et al. [22] to estimate community levels of drug abuse. These compounds were determined to occur in the μg/L to ng/L range in surface water and sewage water.

Table 2-7.

Drug Concentrations (ng/L) in Influent Samples from WWTP in NE Spain Sampled Over Seven Consecutive Days

Drug	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
Amphetamine	63 ± 4	35 ± 3	45 ± 7	24 ± 3	40 ± 5	72 ± 6	101 ± 10
Methamphetamine	<LOD	<LOD	<LOD	<LOD	1 ± 0.3	3 ± 1	12 ± 3

LOD: 0.4 ng/L

Excerpted from Reference [13] –Permission requested

2.4 References

[1] United States Environmental Protection Agency, Great Lakes National Program Office, http://www.great-lakes.net/humanhealth/about/words_e.html.

[2] United Nations Office of Drug and Crime; World Drug Report 2009, http://www.unodc.org/documents/wdr/WDR_2009/WDR2009_eng_web.pdf.

[3] RxList, The Internet Drug Index, http://www.rxlist.com/desoxyn-drug.htm.

[4] RxList, The Internet Drug Index, http://www.rxlist.com/adderall-xr-capsules-drug.htm.

[5] R.C. Baselt, Disposition of Toxic Drugs and Chemicals in Man, Biomedical Publications, California, 7th ed., 2004.

[6] T.L. Jones-Lepp, D.A. Alvarez, J.D. Petty, J.N. Huckins, Arch. Environ. Contam. Toxicol. 47 (2004) 427.

[7] E. Zuccato, S. Castiglioni, R. Bagnati, C. Chiabrando, P. Grassi, R. Fanelli, Water Res. 42 (2008) 961.

[8] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 42 (2008) 3498.

[9] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 43 (2009) 363.

[10] S.L. Bartelt-Hunt, D.D. Snow, T. Damon, J. Shockley, K. Hoagland, Environ. Pollut. 157 (2009) 786.

[11] S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelli, R. Bagnati, Anal. Chem. 78 (2006) 8421.

[12] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Anal. Chem. 79 (2007) 3821.

[13] M. Huerta-Fontela, M.T. Galceran, J. Martin-Alonso, F. Ventura, Sci. Total Environ. 397 (2008) 31.

[14] A.L.N. van Nuijs, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Anal. Bioanal. Chem. 395 (2009) 819.

[15] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, J. Chromatogr., A 1216 (2009) 3078.

[16] C. Postigo, M.J. Lopez de Alda, D. Barceló, Anal. Chem. 80 (2008) 3123.

[17] A.C. Chiaia, C. Banta-Green, J. Field, Environ. Sci. Technol. 42 (2008) 8841.

[18] A. Kaleta, M. Ferdig, W. Buchberger, J. Sep. Sci. 29 (2006) 1662.

[19] T.L. Jones-Lepp, R. Stevens, Anal. Bioanal. Chem. 387 (2007) 1173.

[20] M.J.M. Wells, A. Morse, K.Y. Bell, M.-L. Pellegrin, L.J. Fono, Water Environ. Res. 81 (2009) 2211.

[21] E. Zuccato, C. Chiabrando, S. Castiglioni, R. Bagnati, R. Fanelli, Environ. Health Perspect. 116 (2008) 1027.

[22] C. Postigo, M.J. Lopez de Alda, D. Barceló, Trends Anal. Chem. 27(11) (2008) 1053.

CHAPTER 3

OCCURRENCE OF AMPHETAMINE-TYPE STIMULANTS IN WASTEWATER

3.1 Introduction

Methamphetamine and amphetamine are two of a class of amphetamine-type stimulants (ATSs), and although sometimes legitimately prescribed for certain medical problems such as obesity and attention deficit hyperactivity disorder, the majority of use of ATSs is illicit [1]. According to the United Nations Office on Drugs and Crime, in the global market more people use ATSs than heroin and cocaine combined [2]. In addition to the sociological problems caused by drug abuse and addiction, excreted drugs become environmental contaminants by entering the urban water cycle through the sewer system. In recent years studies have reported finding ATSs in WWTP influent and sometimes effluent in South Wales, UK [3], Italy [4], Switzerland [4], Spain [5,6,7,8,] and Belgium [9]. In the United States, ATSs have been found in wastewater in Nebraska [10], Nevada [6], Utah [6], South Carolina [6], and other states [12]. Incomplete removal of ATSs during wastewater treatment allows the drugs to enter surface water, groundwater, and soil.

The Herald-Citizen, a local newspaper in Cookeville, TN, has reported the presence of clandestine methamphetamine laboratories as well as arrests of citizens in possession of methamphetamine. The first objective of this research was to determine

whether methamphetamine and amphetamine could be detected and quantified in wastewater influent in Cookeville, TN. The second objective was to determine whether amphetamine and methamphetamine, if found in the influent, would be removed during wastewater treatment.

3.2 Materials and Methods

3.2.1 Reagents

All reagents were Optima grade. For the HPLC mobile phase, acetonitrile, methanol, formic acid, and ammonium acetate (Fisher Scientific, Suwanee, GA, USA) were used. Water, methanol, formic acid, and ammonium hydroxide were also obtained from Fisher Scientific for use in solid-phase extraction procedures. Amphetamine, methamphetamine, methamphetamine-D5, ephedrine, ephedrine D-3, pseudoephedrine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamine (MDEA) were obtained from Cerilliant Corporation (Round Rock, TX, USA).

3.2.2 Materials

Oasis WCX and MCX SPE cartridges (6 cc, 150 mg sorbent) were purchased from Waters Corporation (Milford, MA, USA). Glass fiber filters, vacuum filter funnels and filter flasks, and a vacuum manifold were purchased from Fisher Scientific. Syringe

filters (0.2 µm PTFE) and 3-mL syringes were also purchased from Fisher Scientific. PTFE tubing was purchased from Supelco (Bellefonte, PA). Nitrogen gas (Airgas Mid America, Cookeville, TN, USA) was used for evaporation of extracts.

3.2.3 Sample Collection

Wastewater samples were collected from the Cookeville Wastewater Treatment Plant in Cookeville, TN, USA on February 27, March 12, August 26, and November 02, 2009, from Site 1 (influent), Site 2 (effluent before UV disinfection), and Site 3 (effluent after UV disinfection). Samples were collected as grab samples, although on August 26, composite samples were also collected. Composite samples were collected every hour for 24 hours and pooled to form the composite samples; however, composite samples were not collected for Site 2. Grab samples were collected by WWTP personnel following sampling protocol according to the Cookeville WWTP. In summary, five-gallon sample containers were lowered into rapidly flowing influent or effluent; containers were retrieved and sample was refrigerated for less than 6 years before being retrieved for analysis. Approximately 4L of influent, effluent before UV disinfection, and effluent after UV disinfection samples were collected during each sampling event. Figure 2 shows a scheme of the Cookeville WWTP and sampling locations. Table 1 shows physical (pH and temperature) and chemical (dissolved oxygen) parameters of wastewater on collection dates as measured by WWTP personnel.

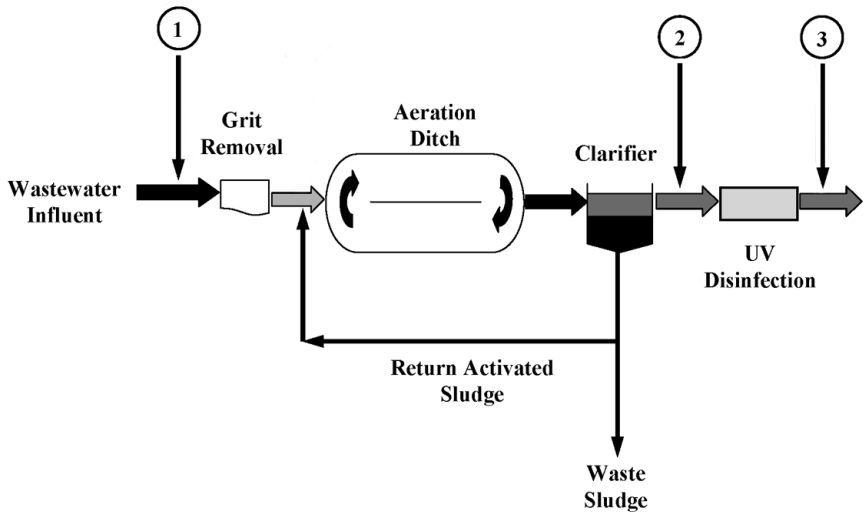

Figure 3-1. Cookeville WWTP scheme and sampling locations *(figure courtesy of Dr. Dennis B. George of the Center for Management, Utilization and Protection of Water Resources, Cookeville, TN)*

Table 3-1 Wastewater Physical and Chemical Parameters

	pH		Temperature (ºC)		Dissolved Oxygen (mg/L)	
Date	**Influent**	**Effluent**	**Influent**	**Effluent**	**Influent**	**Effluent**
02/27/09	7.1	7.1	15	NA	7.3	10.2
03/12/09	7.3	7.4	14	NA	6.1	9.1
08/26/09	7.3	7.4	23	NA	2.7	7.4
11/02/09	7.3	7.2	18	NA	5.9	8.2

NA – effluent temperature was not measured

3.2.4 Sample Filtration

Prior to solid phase extraction (SPE), all WWTP samples were filtered using a Fisherbrand 300 mL glass microanalysis filter holder assembly connected to a 1 L sidearm filtration flask and attached to a KNF (Trenton, NJ) lab filtration vacuum pump. Samples were filtered firstly through Fisherbrand G4 glass fiber filters (1.2 μm pore size, 42.5 mm) and secondly filtered through Millipore Glass Fiber Filters (0.7 μm pore size, 47 mm). Filters were baked at 180 ˚C for 2 hours before use. After filtration, samples were immediately extracted via SPE or refrigerated for up to 48 hours.

3.2.5 Solid-Phase Extraction

Two SPE extraction protocols were used for extraction of wastewater using Oasis WCX cartridges. In protocol #1, cartridges were washed with 3 mL MeOH and equilibrated with 3 mL of H_2O before sample loading. A second wash was performed with 4 mL of 5% NH_4OH in H_2O, and then extracts were eluted with 2% formic acid in MeOH. Using protocol #2 cartridges were washed with 3 mL MeOH, equilibrated with 4 mL of 5% NH_4OH. After the sample was loaded, a second wash was performed with 4 mL of 5% NH_4OH in H_2O, and then extracts were eluted with 2% formic acid in MeOH.

Oasis MCX SPE cartridges were also used for the extraction of wastewater. Cartridges were washed with 6 mL MeOH and conditioned with 3 mL of Optima H_2O and 3 mL of H_2O acidified to pH 2 with formic acid before samples were loaded at ~10

mL/min. Samples were eluted with 3 mL of MeOH and a 2% NH_4OH solution in MeOH [14].

Regardless of the SPE sorbent or extraction protocol used, a 100 mL volume of wastewater sample was extracted. Because of the large sample volume, two SPE cartridges were coupled together with a connector and the sample was extracted through both cartridges (effectively doubling the mass of sorbent). A stopcock was used to connect the lower SPE cartridge to the vacuum manifold in order to control flow and prohibit the SPE cartridges from becoming dry. To effect complete sample transfer, samples were connected from amber Boston round bottles via PTFE tubing to a connector in the top of the upper SPE cartridge. The sample was drawn through the cartridges by vacuum at a rate of ~10 mL/min. Figure 2 shows the extraction apparatus.

After extraction, eluates were evaporated to dryness under nitrogen and reconstituted in either 1 mL of 0.2% formic acid in 10 mM NH_4OAc:ACN:MeOH (75:12.5:12.5, v/v) or ACN/MeOH/H_2O/formic acid (5.5:17:77.25:0.25, v/v). Samples were mixed well for 30 seconds with a Thermo Scientific MaxiMix II Vortex Mixer and refrigerated overnight to allow the dried extract to become completely dissolved in the injection solvent before analysis by LC-MS/MS.

3.2.6 LC-MS/MS

A Varian 1200L HPLC triple quadrupole mass spectrometer equipped with a CombiPAL autosampler, ESI source, and a ProStar/Dynamax solvent delivery system with two PS-210 pumps was used for separation and detection of ATSs.

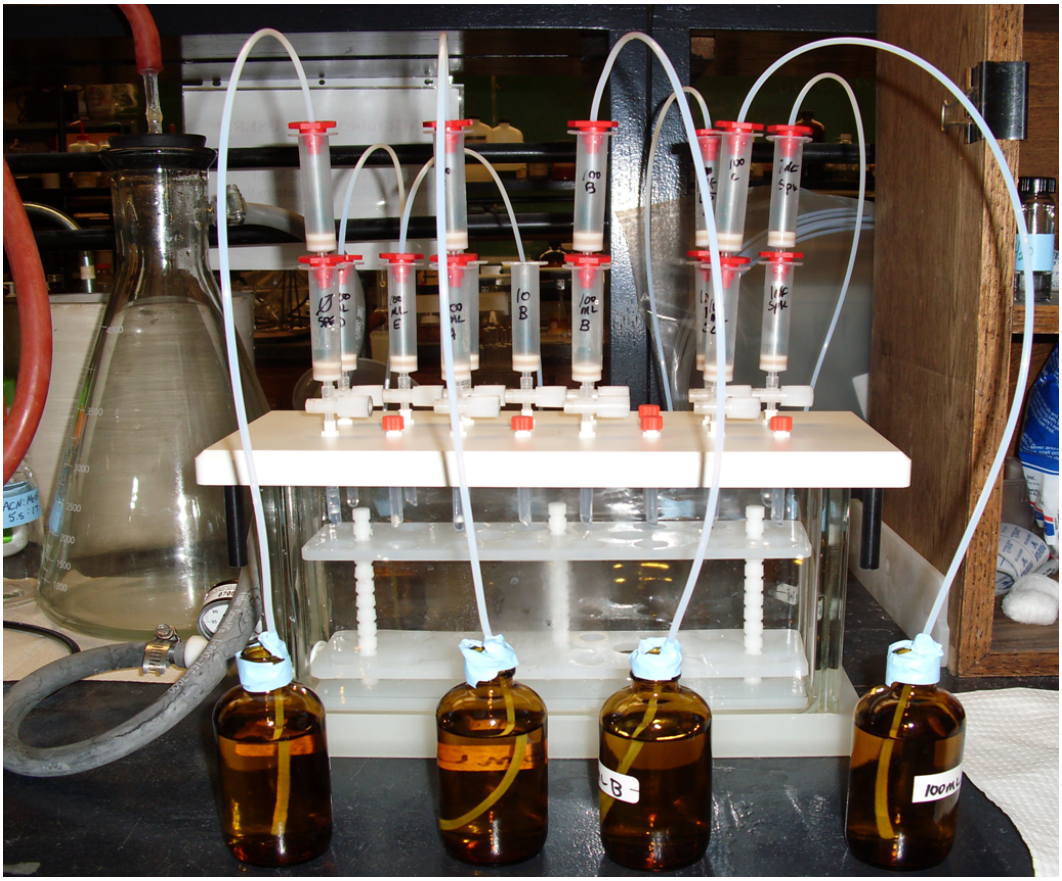

Figure 3-2. Wastewater samples loaded onto SPE cartridges on a vacuum manifold

3.2.6.1 HPLC conditions. Two different separation schemes were used and are shown in Table 2 as mobile phase 1 (MP #1) and in Table 3 as mobile phase 2 (MP #2).

3.2.6.2 MS parameters. LC-MS/MS with electrospray ionization in the positive ionization mode was used to detect amphetamine and methamphetamine in wastewater samples. MS parameters are shown in Table 4.

3.3 Results and Discussion

3.3.1 Solid-Phase Extraction

3.3.1.1 Oasis WCX cartridges. The pK_a of methamphetamine is equal to 10.38±0.10 making it suitable for SPE by Oasis WCX, which is intended for compounds with a $pK_a > 10$ [15]. According to the Waters Corporation, Oasis WCX is designed to provide superior sample preparation for strong bases. WCX is a mixed-mode, water-wettable, polymeric sorbent, stable from pH 0-14, able to confirm and quantify strongly basic compounds in biological fluids. Sorbents containing hydrophobic alkyl chains and cation- or anion-exchange sites on the same sorbent particle exhibit a mixed-mode mechanism. Mixed-mode sorbents have multiple retentive sites on an individual particle that is, incorporating different ligands on the same sorbent which exhibit different retention mechanisms. Mixed-mode sorbents exploit different functional groups on a single analyte or different functional groups on multiple analytes [16]. Figure 3 shows the interaction between methamphetamine and the WCX sorbent.

Table 3-2. Mobile Phase 1 HPLC Conditions

Column	Varian MonoChrom MS 5 µm, 50 x 2 mm			
Solvent A	0.2% formic acid:10mM NH_4OAc in water (v/v)			
Solvent B	acetonitrile/methanol (1:1, v/v)			
Gradient	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	75	25	0.25
	8:00	75	25	0.25
Injection Volume	40 µL			
Injection Solvent	acetonitrile/methanol/water/formic acid (5.5:17:77.25:0.25, v/v)			

Table 3-3. Mobile Phase 2 HPLC Conditions

Column	Varian MonoChrom MS 5 µm, 50 x 2 mm			
Solvent A	0.2% formic acid:10mM NH_4OAc in water (v/v)			
Solvent B	acetonitrile/methanol (1:1, v/v)			
Gradient	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	95	5	0.25
	2:00	95	5	0.25
	4:00	90	10	0.25
	6:00	90	10	0.25
	8.00	85	15	0.25
	10:00	85	15	0.25
	12:00	80	20	0.25
	14:00	80	20	0.25
	16:00	95	5	0.25
Injection Volume	40 µL			
Injection Solvent	0.2% formic acid in 10 mM NH_4OAc:ACN:MeOH (75:12.5:12.5, v/v)			

Table 3-4. MS Parameters

Ionization Mode	ESI positive
Collision Gas	2.3 mTorr Argon
API Drying Gas	30 psi at 380 ˚C
API Nebulizing Gas	57 psi
Scan Time	2.1 sec
SIM Width	0.7 amu
Needle	5000V
Shield	600V
Detector	1300V

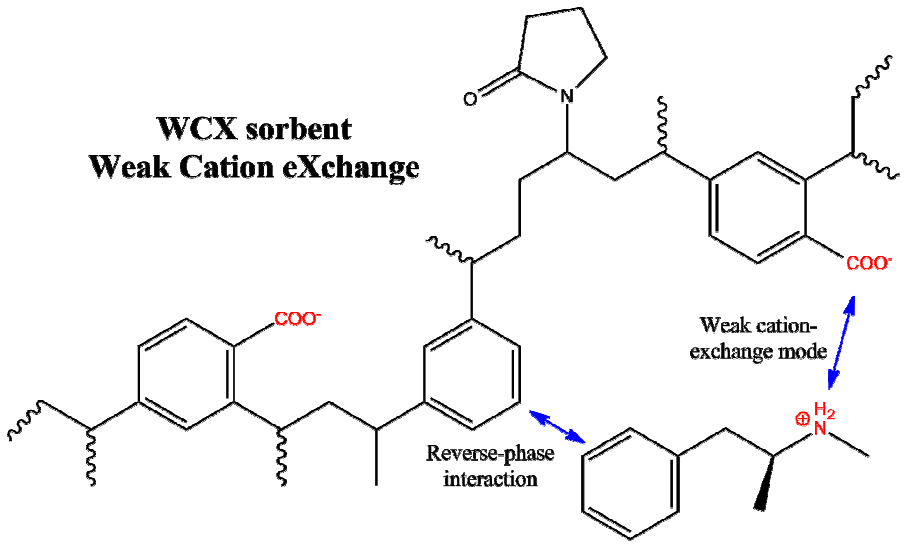

Figure 3-3. Oasis WCX - methamphetamine interaction (adapted from Waters [15])

Initially the extraction protocol suggested by Waters was followed with cartridges being washed with 3 mL MeOH and equilibrated with 3 mL of H_2O before sample loading. A second wash was performed with 4 mL of 5% NH_4OH in H_2O, and then extracts were eluted with 2% formic acid in MeOH (protocol #1). However, upon analysis by LC-MS/MS peak shape was poor and recovery indicated by peak area was low. In order to prepare the sorbent before the sample was loaded, protocol #2 was developed for extraction. Instead of a 3mL H_2O equilibration, 4 mL of a 5% NH_4OH solution in H_2O was used and the remaining extraction procedure was followed. Analysis of eluates showed improved peak shape and increased peak area.

3.3.1.2 Recovery from Oasis WCX solid-phase extraction sorbent. Methamphetamine-D5 (Figure 4) was used as the internal standard (IS) to correct for loss of methamphetamine and amphetamine during SPE. The IS was added to each sample before SPE in order to make a final concentration of 50 ng/mL IS. The addition of IS also allowed the determination of percent recovery of the SPE procedure. The IS was added to 100 mL of Optima H_2O, and the water sample was extracted by SPE. The eluate was analyzed by LC-MS/MS and the IS peak area for the extracted water was divided by the peak area of a 50 ng/mL D5-MA solution that had not undergone SPE. An arcsine transformation was performed on the means of percent recovery to more closely resemble a normal distribution (Table 5) [17].

3.3.1.3 Oasis MCX cartridges. Oasis MCX is a strong mixed-mode cation exchange, water-wettable, polymeric sorbent. MCX provides both ion exchange and reversed-phase modes of retention and is stable from pH 0-14 [15]. Oasis MCX Cartridges are intended for bases with pK_a = 2-10, and amphetamine has a pK_a =

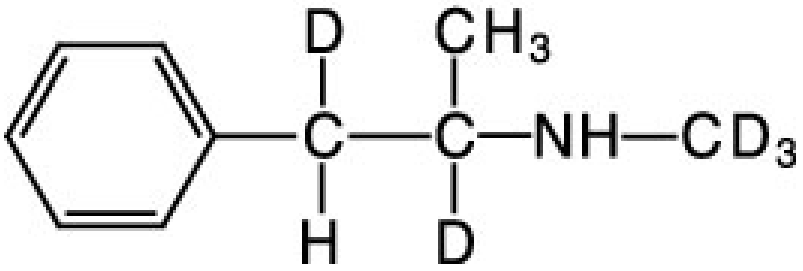

Figure 3-4. D-5 Methamphetamine Structure

Table 3-5. Solid-Phase Extraction Recoveries from Oasis WCX

Peak Area of Standard	Peak Area of SPE Sample	% Recovery
9.683×10^6	7.232×10^6	74.7%
9.683×10^6	7.616×10^6	78.6%
1.810×10^7	1.369×10^7	75.6%
9.515×10^6	8.101×10^6	85.1%
1.060×10^7	8.179×10^6	77.2%
	Average ± SD	78.4 ± 0.3%

9.94±0.10. Because methamphetamine and amphetamine both have pK_a values near 10, wastewater samples were also extracted using MCX cartridges to compare the two sorbent chemistries and determine if one has an advantage over the other. The interaction between the MCX sorbent and methamphetamine is shown in Figure 5.

Methamphetamine D-5 was added to wastewater samples before SPE to give a final concentration of 50 ng/mL. MCX cartridges were washed with 6 mL MeOH, conditioned with 3 mL of Optima H_2O and 3 mL of H_2O acidified to pH 2 with formic acid, and then 100 mL samples were loaded at ~10 mL/min. Samples were eluted with 3 mL of MeOH and a 2% NH_4OH solution in MeOH [14].

3.3.2 Optimization of LC Parameters

3.3.2.1 Injection solvent. After SPE and evaporation to dryness under N_2, eluates from SPE were reconstituted in an injection solvent. Initially, a published method [17] measuring the concentration of ATSs in urine was followed, and that method used an injection solvent of acetonitrile/methanol/water/formic acid (5.5:17:77.25:0.25, v/v). However, due to shifting retention times the injection solvent was changed to mimic the concentration and mixture of the mobile phase. The final injection solvent was 0.2% formic acid in 10 mM NH_4OAc:ACN:MeOH (75:12.5:12.5, v/v), which resulted in stable retention times.

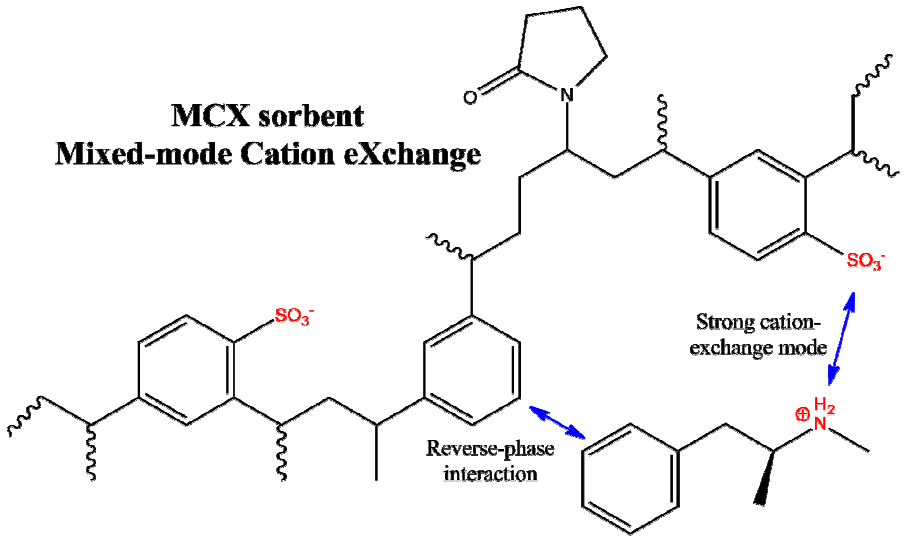

Figure 3-5. Oasis MCX – methamphetamine interaction (adapted from Waters [15])

3.3.2.2 LC gradient. Initial HPLC conditions featured an isocratic mixture of 0.2% formic acid:10mM NH_4OAc in water (v/v) (75%) (aqueous mobile phase) and acetonitrile/methanol (1:1, v/v) (25%) (organic mobile phase) with a total run time of 8 minutes (Table 2). Both amphetamine and methamphetamine elute during the 8 minutes, with amphetamine having a retention time of ~2.7 minutes and methamphetamine having a retention time of ~3.1 minutes. However, the 150 → 91 ion transition for methamphetamine showed an additional, unresolved peak (Figure 6). In order to resolve the two peaks, gradient elution was used, starting with 95% of the aqueous mobile phase and gradually increasing the organic mobile phase from 5% to a concentration of 20% over 14 minutes and then finally returning to the 95% A: 5% B mixture over the final 2 minutes for a total run time of 16 minutes (Table 3). Figure 7 shows the resolution between methamphetamine and the previously unresolved unknown peak.

3.3.3 Optimization of MS Parameters

3.3.3.1 Selection of ion transitions. The molecular weight of amphetamine is 135.21 and the $[M+1]^+$ molecular ion is located at a mass-to-charge (m/z) ratio of 136. Two intense product ions are observed at 91 and 119. The 119 ion is the result of the loss of the NH_2 group and the 91 ion is the result of the loss of $CH\text{-}CH_3\text{-}NH_2$, shown in Figure 8.

The molecular weight of methamphetamine is 149.23 and the $[M+1]^+$ molecular ion is located at a mass-to-charge (m/z) ratio of 150. Two intense product ions are

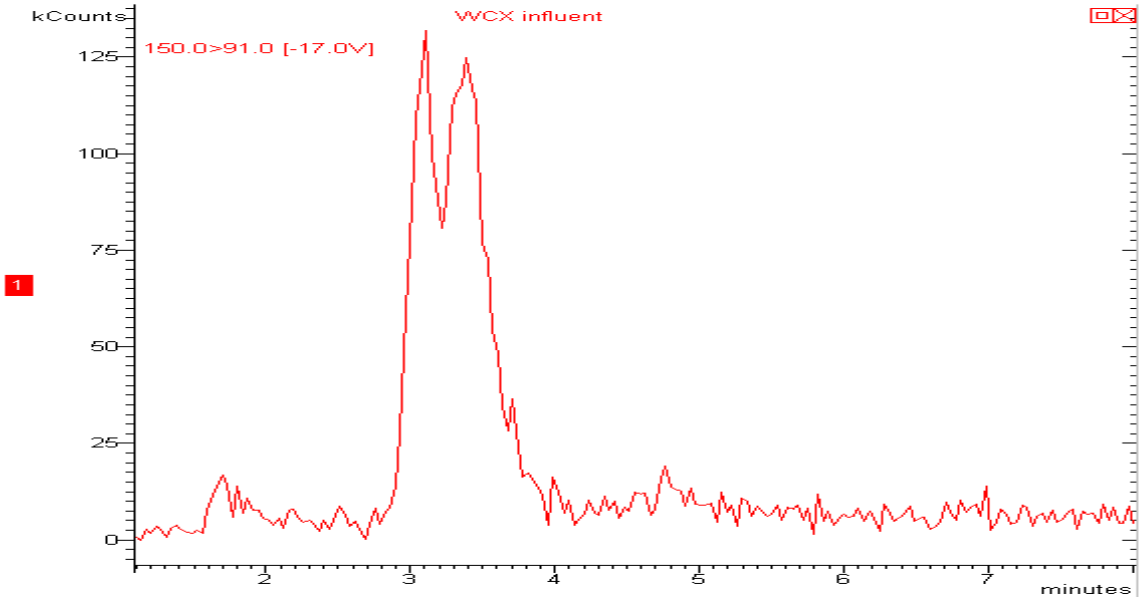

Figure 3-6. Methamphetamine 150 → 91 ion transition, isocratic mobile phase

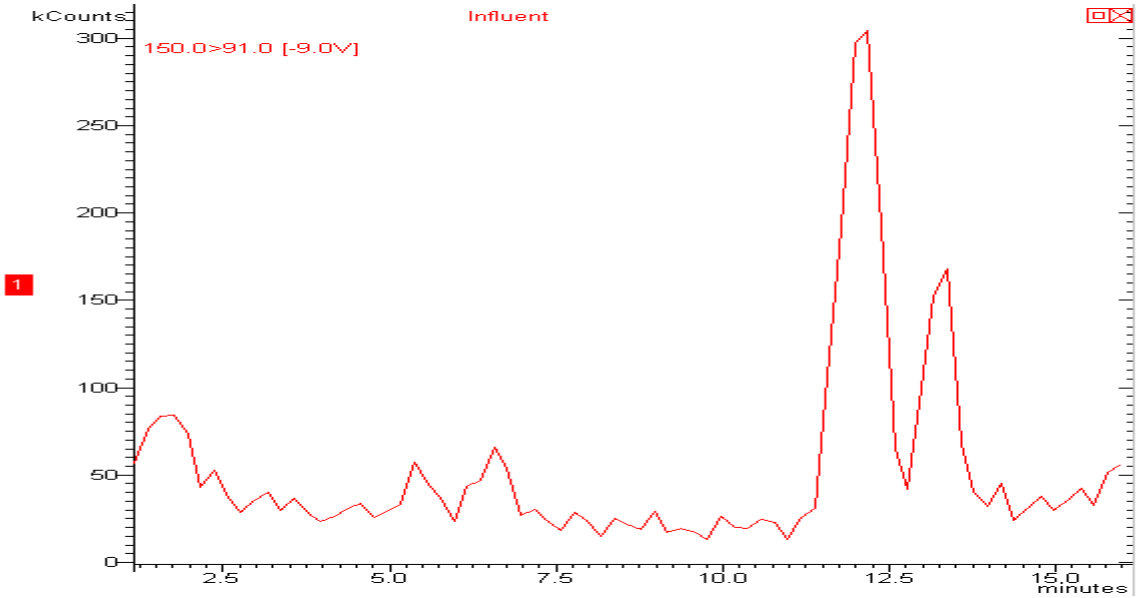

Figure 3-7. Methamphetamine 150 → 91 ion transition, gradient mobile phase

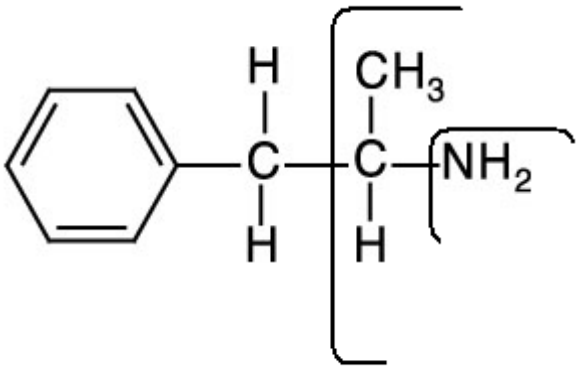

Figure 3-8. Amphetamine Ion Transitions

observed at 91 and 119. The 119 ion is the result of the loss of $NH\text{-}CH_3$, and the 91 ion is the result of the loss of $CH\text{-}CH_3\text{-}NH\text{-}CH_3$ as shown in Figure 9.

3.3.3.2 Optimization of capillary voltage and collision energy. Capillary voltages for amphetamine and methamphetamine were determined by infusing a 100 ng/mL standard of each compound into the MS by a syringe pump and using the Optimization utility to determine the optimum capillary voltage for amphetamine, methamphetamine, and D5-methamphetamine (Figures 10, 11, and 12). Once the capillary voltage was determined, an MS/MS breakdown curve was created to determine the optimum voltage of the collision cell that is based on the intensity of the ions. Figures 13, 14, and 15 show the MS/MS breakdown patterns for amphetamine, methamphetamine, and methamphetamine-D5 resulting from the optimization of the capillary voltage and collision energies. Table 6 summarizes the scan parameters determined by optimization and MS/MS breakdown.

3.3.4 Concentration of Amphetamine and Methamphetamine in Wastewater

Influent and effluent samples from the Cookeville Wastewater Treatment Plant were collected four times between March and November 2009. Table 7 summarizes the concentrations of amphetamine and methamphetamine in the influent, the effluent prior to UV disinfection, and the effluent after UV disinfection.

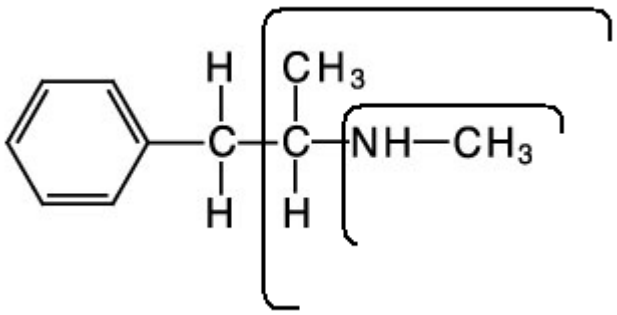

Figure 3-9. Methamphetamine Ion Transitions

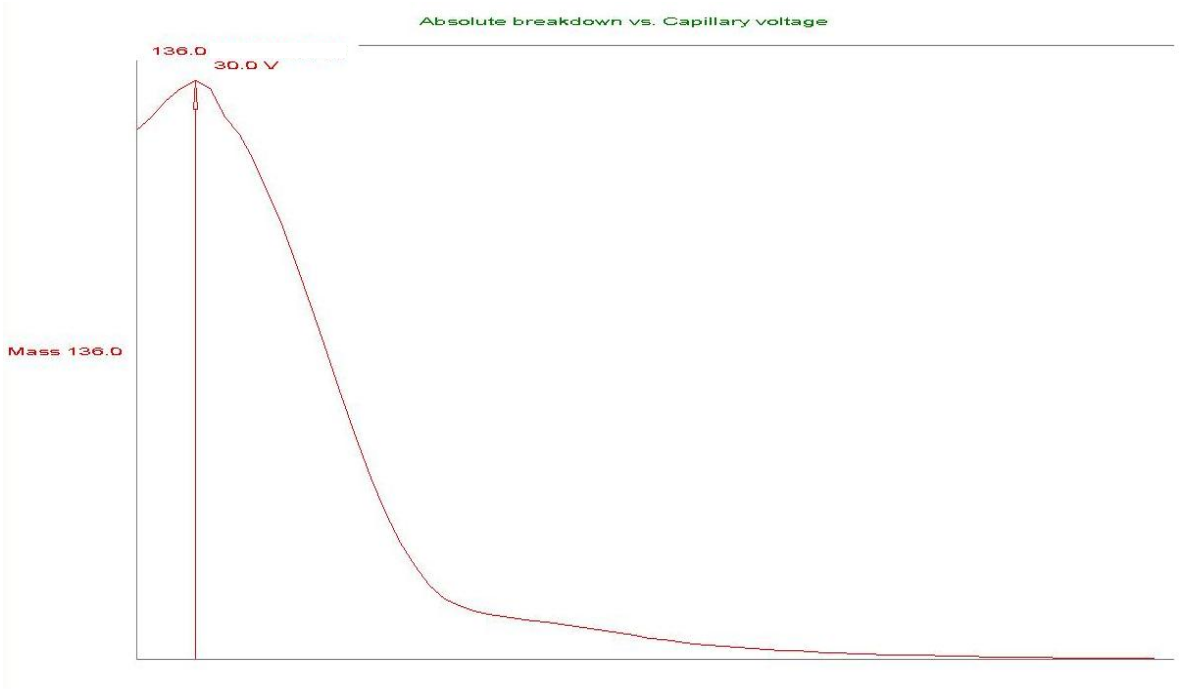

Figure 3-10. Capillary voltage optimization for amphetamine

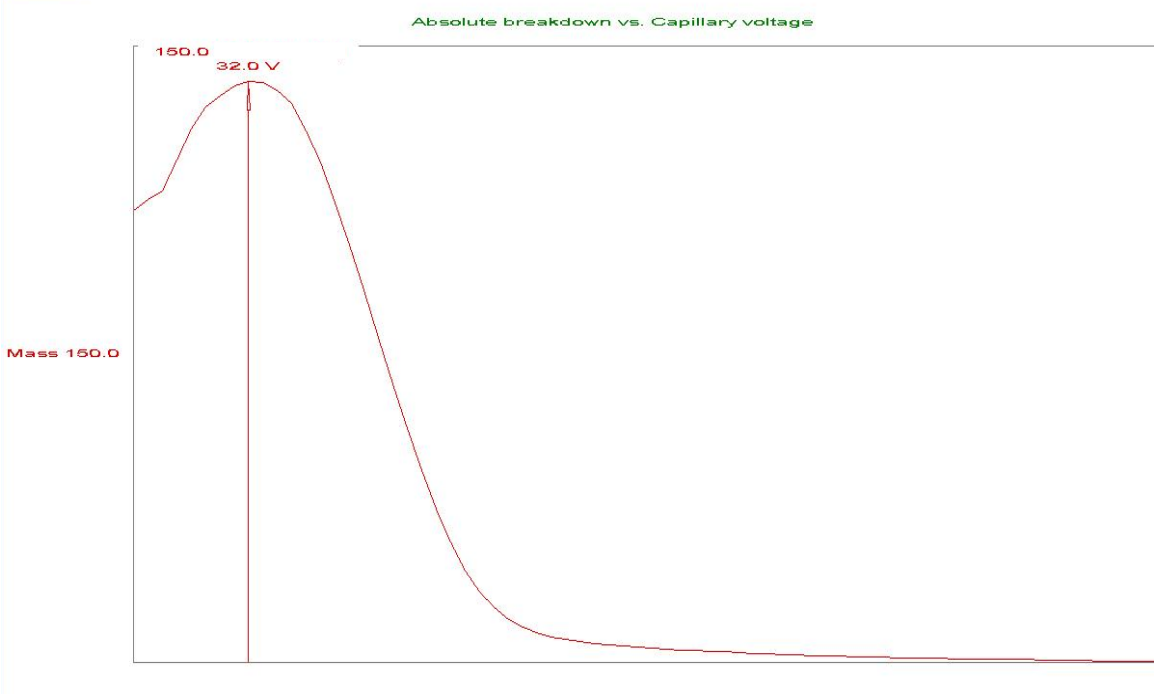

Figure 3-11. Capillary voltage optimization for methamphetamine

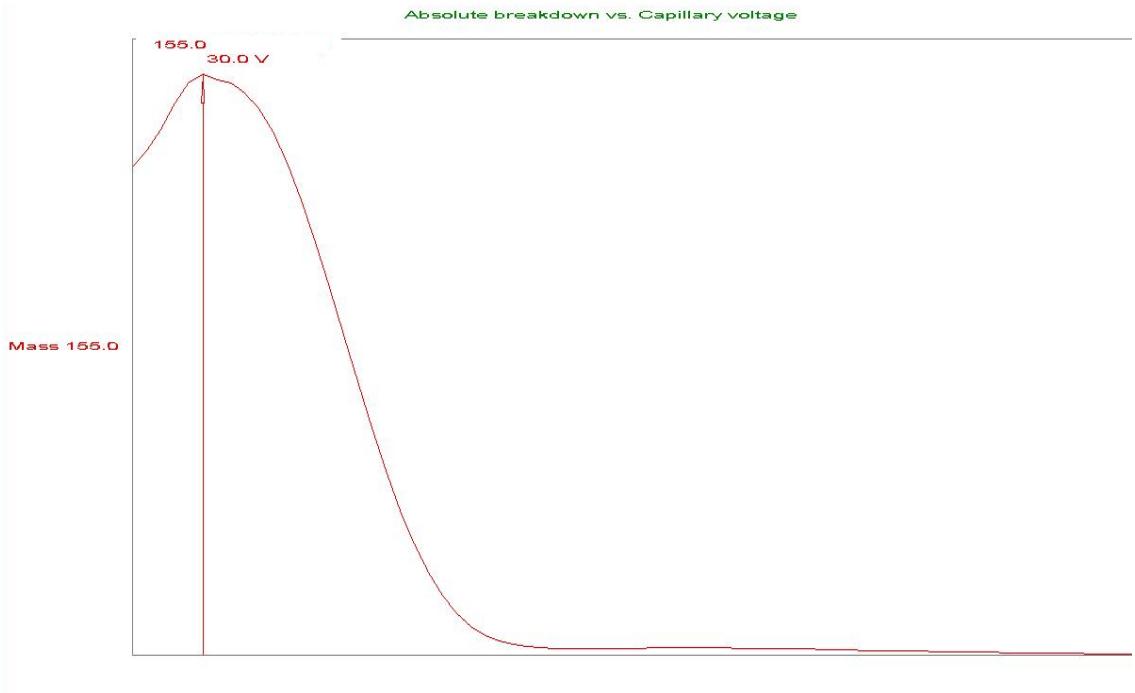

Figure 3-12. Capillary voltage optimization for D5-methamphetamine

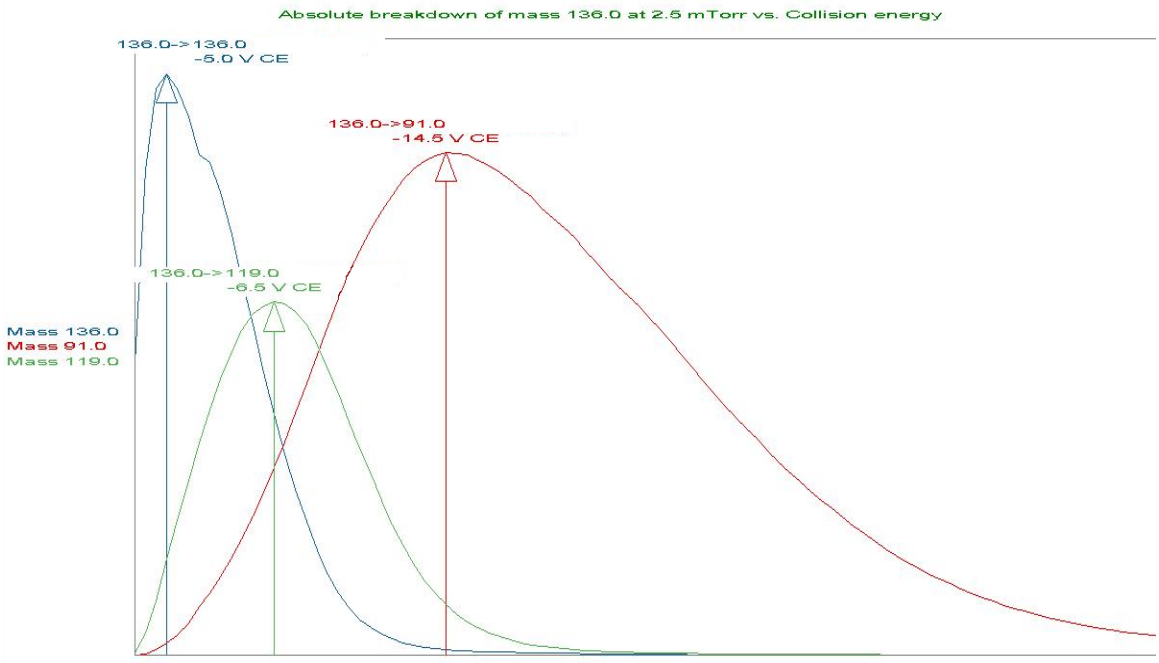

Figure 3-13. MS/MS breakdown for collision energies for amphetamine

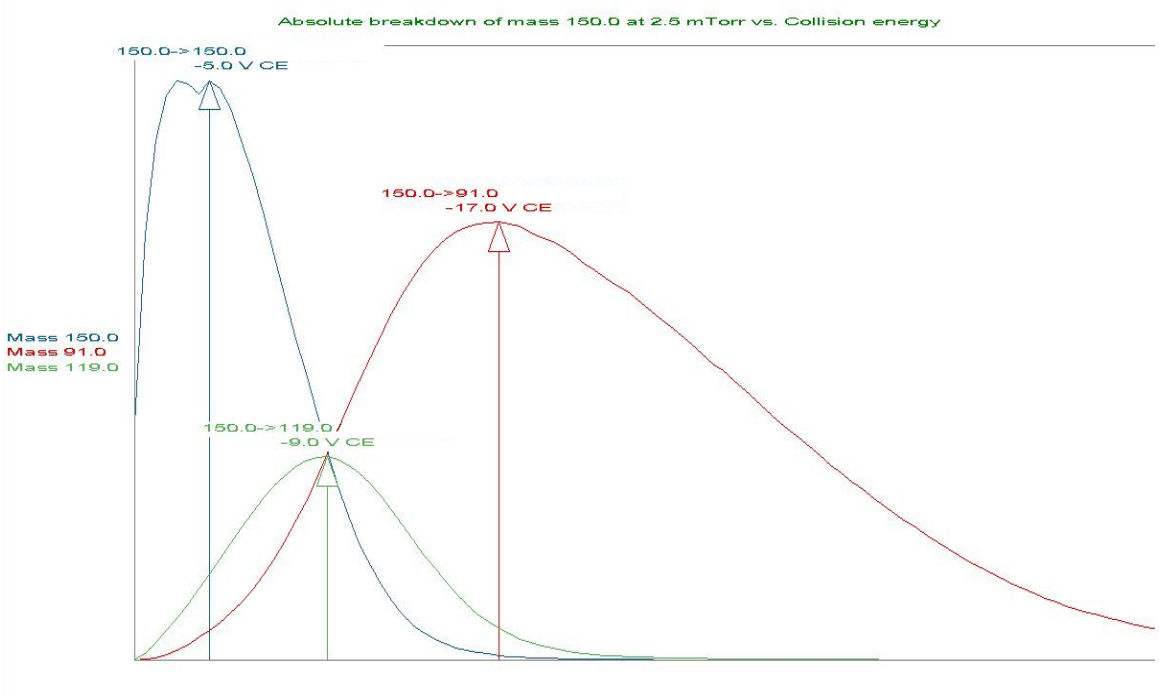

Figure 3-14. MS/MS breakdown for collision energies for methamphetamine

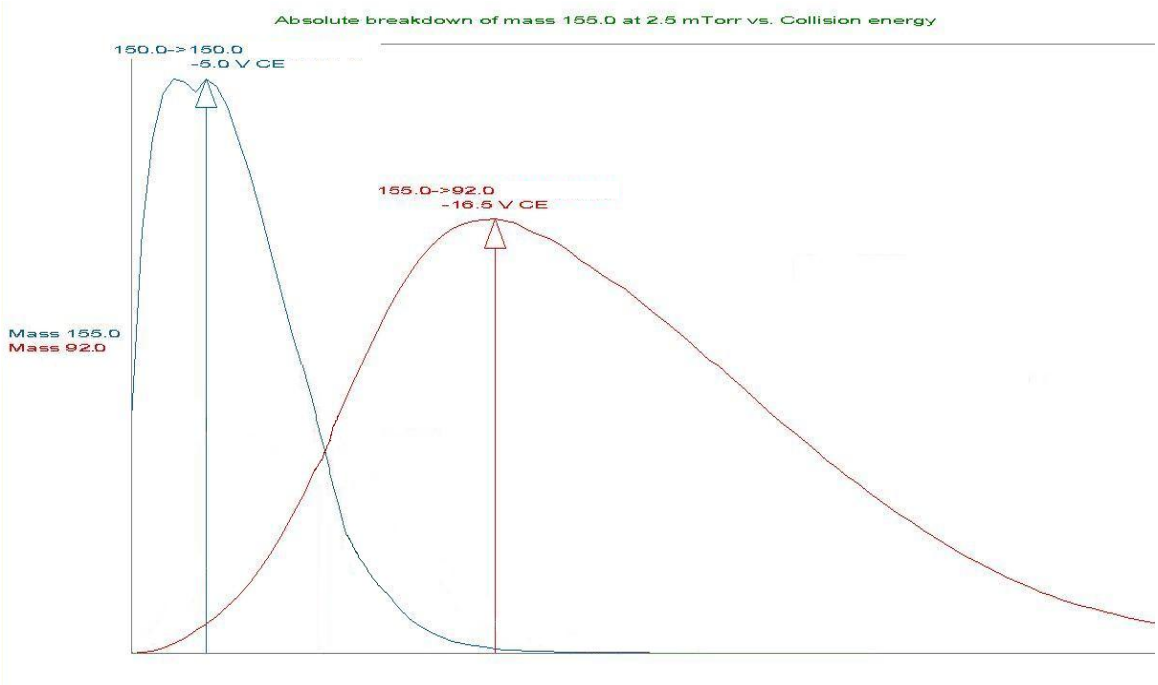

Figure 3-15. MS/MS breakdown for collision energies for D5-methamphetamine

Table 3-6. Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Capillary Voltage (V)	Collision Energy (V)
(±)-Amphetamine	136	91	30	6.5
	136	119	30	14.5
(±)-Methamphetamine	150	91	32	17.0
	150	119	32	9.0
(±)-Methamphetamine-D5	155	92	30	16.5

Table 3-7. Concentration of Amphetamine and Methamphetamine in WWTP Samples

Sample	Date Collected	Sample Type	Amphetamine (ng/L±SD)	Methamphetamine (ng/L±SD)
Influent	02/27/09	Grab	23.4 ± 3.99	37.2 ± 1.75
Effluent-pre UV	02/27/09	Grab	<LOD	6.44 ± 0.128
Effluent-post UV	02/27/09	Grab	8.74 ± 2.37	8.43 ± 0.280
Influent	03/12/09	Grab	24.6 ± 9.31	27.0 ± 0.526
Effluent-pre UV	03/12/09	Grab	<LOD	14.5 ± 0.101
Effluent-post UV	03/12/09	Grab	<LOD	14.8 ±0.175
Influent	08/26/09	Grab	54.1 ± 3.31	59.0 ± 4.34
Effluent-pre UV	08/26/09	Grab	<LOD	14.4 ± 0.692
Effluent-post UV	08/26/09	Grab	<LOD	13.9 ± 0.375
Influent	08/26/09	Composite	55.8 ± 13.4	60.3 ± 9.15
Effluent-post UV	08/26/09	Composite	<LOD	14.2 ± 0.709
Influent	11/02/09	Grab	86.4 ± 9.05	49.4 ± 2.31
Effluent-pre UV	11/02/09	Grab	<LOD	<LOD
Effluent-post UV	11/02/09	Grab	12.0 ± 0.276	10.8 ± 0.128

3.4 Conclusions

A method was developed for extraction of wastewater samples using Oasis WCX SPE cartridges and for the detection and quantitation of amphetamine and methamphetamine in wastewater influent and effluent. No other reports are known to exist in the literature for the use of Oasis WCX sorbents for the extraction and recovery of amphetamine and methamphetamine from wastewater.

Notably, amphetamine and methamphetamine were both found in the influent at all sampling dates. Amphetamine concentrations ranged from 23.4 – 86.4 ng/L in influent and 8.74 – 12.0 in effluent after UV disinfection. However, amphetamine was not detectable in any effluent samples collected prior to UV disinfection, possibly indicating that UV disinfection breaks down a metabolite back into the parent compound. Methamphetamine was detected in influent at concentrations ranging from 27.0 – 60.3 ng/L and in effluent, both prior to and after UV detection, at 6.44 – 14.8 ng/L. One sampling event was accomplished in each of the four seasons throughout the year, demonstrating that excretion of ATSs is a year-round problem. Future studies could focus on the daily load of ATSs into wastewater, which could also contribute to the estimation of community drug use based on the concentration of ATSs in the wastewater.

3.5 References

[1] T. Woodworth, DEA Congressional Testimony before the Committee on Education and the Workforce: Subcommittee on Early Childhood, Youth and Families, May 16, 2000, http://www.justice.gov/dea/pubs/cngrtest/ct051600.htm.

[2] United Nations Office of Drug and Crime; World Drug Report 2009, http://www.unodc.org/documents/wdr/WDR_2009/WDR2009_eng_web.pdf.

[3] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 43 (2009) 363.

[4] S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelli, R. Bagnati, Anal. Chem. 78 (2006) 8421.

[5] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Anal. Chem. 79 (2007) 3821.

[6] M. Huerta-Fontela, M.T. Galceran, J. Martin-Alonso, F. Ventura, Sci. Total Environ. 397 (2008) 31.

[7] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibáñez, F. Hernández, J. Chromatogr. A 1216 (2009) 3078.

[8] C. Postigo, M.J. Lopez de Alda, D. Barceló, Anal. Chem. 80 (2008) 3123.

[9] A.L.N. van Nuijs, I. Tarcomnicu, L. Bervoets, R. Blust, P.G. Jorens, H. Neels, A. Covaci, Anal. Bioanal. Chem. 395 (2009) 819.

[10] S.L. Bartelt-Hunt, D.D. Snow, T. Damon, J. Shockley, K. Hoagland, Environ. Pollut. 157 (2009) 786.

[11] T.L. Jones-Lepp, D.A. Alvarez, J.D. Petty, J.N. Huckins, Arch. Environ. Contam. Toxicol. 47 (2004) 427.

[12] A.C. Chiaia, C. Banta-Green, J. Field, Environ. Sci. Technol. 42 (2008) 8841.

[13] K. Kümmerer, Significance of antibiotics in the environment. Journal of Antimicrobial Chemotherapy 52 (2003) 5-7.

[14] E. Zuccato, S. Castiglioni, R. Bagnati, C. Chiabrando, P. Grassi, R. Fanelli, Water Res. 42 (2008) 961.

[15] Waters Corporation, Oasis Sample Extraction Products, (July 2008), http://www.waters.com/webassets/cms/library/docs/720001692en.pdf.

[16] M. J. M. Wells, Principles of Extraction and the Extraction of Semivolatile Organics from Liquids. In: *Sample Preparation Techniques in Analytical Chemistry,* S. Mitra, ed., John Wiley & Sons, Hoboken, NJ, (2003) 37-138.

[17] Sokal, R. R.; Rohlf, F. J. *Biometry*, 3rd ed.; W. H. Freeman and Company: New York, 1995; p 419.

[18] Z. Yang, S. Sadjadi, Varian LC/MS Application Note 10.r1, (December 2003), https://www.varianinc.com/media/sci/apps/lcms10.pdf

CHAPTER 4

USE OF A PASSIVE SAMPLING DEVICE TO DETECT AMPHETAMINE-TYPE STIMULANTS IN SEWER LINES

4.1 Introduction

During the past decade, increasing focus has been placed on substances such as pharmaceutical and personal care products (PPCPs) that are not regulated but enter the urban water cycle and have the potential to negatively affect water quality. More recently research has widened to include illicit drugs such as cocaine, opioids, and amphetamine type stimulants (ATSs). Like most PPCPs of concern, many illicit drugs are polar, hydrophilic compounds. Therefore, they are not sequestered in the environment by binding to organic fractions of sludges and suspended sediments, unlike many of the persistent bio-accumulable priority organic pollutants, such as PAHs, PCBs, and organochlorine pesticides [1]. Additionally, glucuronide and sulfate conjugates of many pharmaceuticals are released into the environment as detoxification end products of metabolism. Water treatment processes, microbial action, UV light or even time in the general environment can cause these metabolites to become deconjugated, increasing the environmental burden of the parent drug [1]. These factors make it difficult to estimate

accurately the total burden, and the biological availability, and to predict the fate and aquatic behavior of these compounds [2].

Wastewater and surface water samples for research are generally collected through traditional water sampling techniques, such as grab sampling or composite water sampling. A grab sample is collected simultaneously and reflects a single data point in time while a composite sample involves collecting discrete samples taken at specific intervals of time and combining them at the end of the sampling period into a single sample. The composite sample reflects an average concentration of the analyte in the water source over the sampling time period. However, grab samples, and even composite samples, only capture information at that moment or over a specified short sampling period. In addition, grab and composite sampling techniques may miss important events, such as high or low flow, precipitation, and variability in chemical loading. Passive sampling devises are used to monitor hydrophilic contaminants such as pesticides, PPCPs, and illicit drugs, in aqueous environments, and are designed to stay in the aqueous environment for several days, weeks, or even months. The Polar Organic Chemical Integrative Sampler (POCIS) combines the ability to integrate exposure over time and a range of hydrologic conditions with the ability to accumulate a detectable mass of a compound that may be present in a water sample at concentrations below the method detection level [3]. POCIS apparatuses have been employed for qualitative identification of numerous wastewater-related contaminants, although sampling rates have been determined for some compounds, including methamphetamine [4]. However, even though the membrane in POCIS disks is not as subject to biofouling as other membrane types [5], biofouling could still occur, causing uptake kinetics and

subsequently, laboratory-derived calibration data to be modified [6]. This study focused on the use of AQUASENSE-P, a POCIS device from Environmental Sampling Technologies (St. Joseph, MO) to monitor sewage for ATSs before the sewage reaches the Cookeville Wastewater Treatment Plant in Cookeville, TN. Because sewage is not as dilute as wastewater influent and is more likely to cause biofouling of the POCIS disk, the goal of this research was to provide a qualitative rather than quantitative assessment of the occurrence of amphetamine and/or methamphetamine using a passive sampling device.

4.2 POCIS Characteristics

An AQUASENSE-P disk consists of 200 mg of Oasis hydrophilic-lipophilic balance (HLB) sorbent (Figure 1) contained between two membrane disks made of hydrophilic polyethersulfone (PES) with a 0.1 μm pore size and a 41 cm^2 surface area. Upper and lower stainless steel support rings are used to seal the device and prevent loss of sorbent. Although deployment canisters can be used to house the disks, the small size of the sewer pipes prevents the use of canisters. An example of an individual POCIS disk is presented in Figure 2.

4.3 POCIS Deployment

On August 26, 2009 two POCIS disks each were deployed in three different sewer lines originating from three buildings suspected to house clandestine methamphetamine

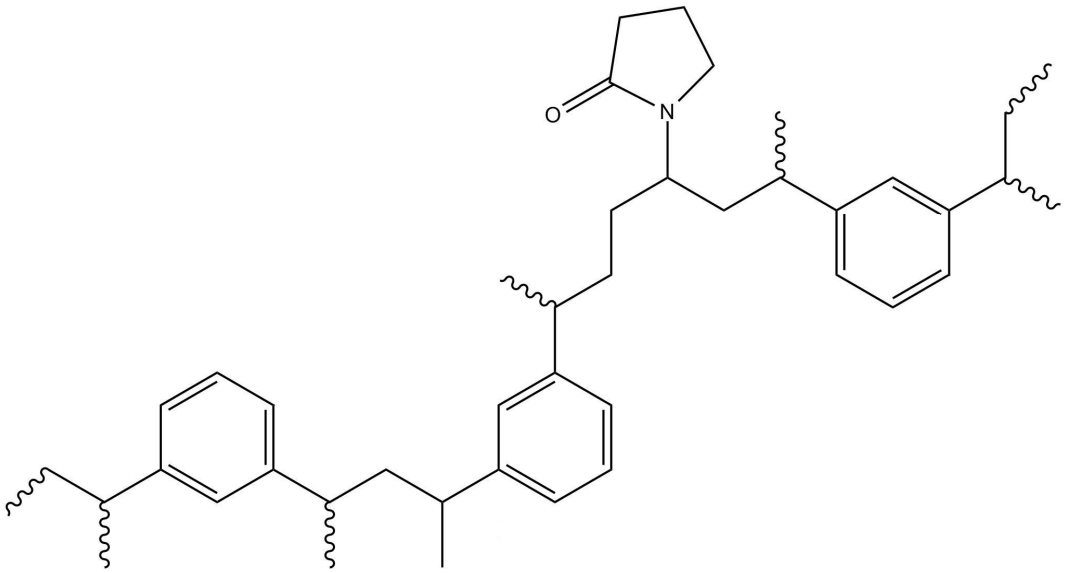

Figure 4-1. Oasis HLB sorbent structure

Figure 4-2. Individual POCIS disk housing HLB sorbent between PES membranes surrounded by stainless steel rings

laboratories. For privacy purposes the sites will be referred to as Site 1, Site 2, and Site 3. The two disks were held together with a plastic zip tie and were placed inside city-owned sewer lines at the junction between the private sewer line and the city-owned line. The samplers were retrieved on September 22 for a total sampling time of 27 days.

4.4 Analytical Methodology

4.4.1 POCIS Cleaning

After retrieval, the POCIS disks were stored in a metal canister and refrigerated for approximately one month before being analyzed. Disks from Site 1 and Site 2 were extremely dirty with an accumulation of slime on the PES membrane. One of the disks from Site 1 had a puncture through one side of the membrane. Disks from Site 3 were only slightly dirty. Working in a fume hood, each disk was gently swished in a pan of Optima grade water to remove loose debris while taking care not to puncture the membrane. A soft bristled brush was used to clean the metal rings and the nuts and bolts holding the two rings together to avoid contamination once the rings were separated and the sorbent was exposed. Rinse water was discarded and fresh water added after each set of POCIS disks was rinsed. After cleaning, the disks were placed on methanol-rinsed aluminum foil until extraction. Figure 3 illustrates the appearance of disks from Site 1 after cleanup.

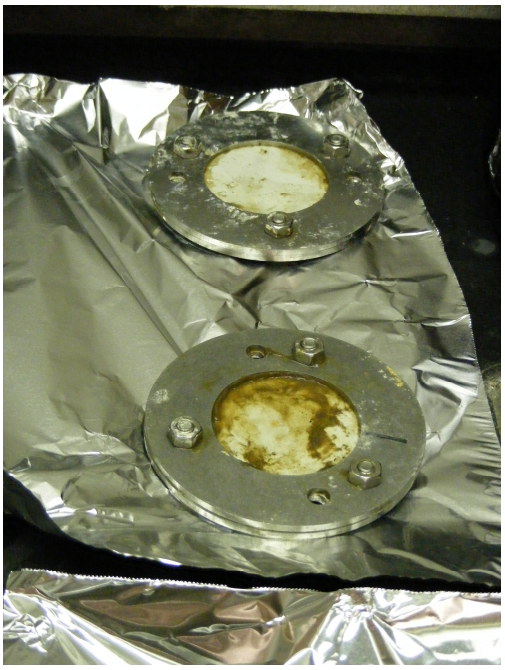

Figure 4-3. POCIS disks after deployment in sewer lines for one month and initial external cleaning.

4.4.2 Extraction

Extraction was performed in a fume hood and the work surface was lined with methanol rinsed aluminum foil. Unused solid-phase extraction cartridges were used as chromatography columns for extraction of the POCIS disks. Sorbent and plugs were removed from the cartridges, which were rinsed with methanol. A stopcock was fitted to the column and a 1-2 cm plug of silanized glass wood was seated at the bottom of the column. One column per POCIS disk was secured by a clamp to a ring stand, and a glass funnel was suspended above the column. The column was rinsed with methanol, the waste was discarded, and the stopcock was closed. The POCIS disks were held horizontally and the bolts were removed to allow separation of the two disks. Tweezers were used to separate the membranes and the sequestration medium was washed into the chromatography column through the funnel with methanol from a wash bottle. Residue was rinsed from the funnel with methanol. Another plug of glass wool was placed in the chromatography column on top of the sample to prevent it from washing up the sides of the column, and 40 mL of methanol was added to the column for extraction. A 100 mL glass beaker was placed under the column and the stopcock was opened to allow extract to drip at a slow and steady rate into the beaker (Figure 4). Extracts were quantitatively transferred to glass tubes and evaporated under nitrogen to a volume of 1-2 mL.

Fisherbrand G4 glass fiber filters (1.2 μm pore size) were cut into pieces approximately 5 mm^2. A glass fiber square was placed into a 6" Pasteur pipet with tweezers and gently seated into the pipet with a thin glass rod. A small amount of methanol was used to wet the filter paper and the sample was quantitatively transferred to

Figure 4-4. Chromatography apparatus for POCIS extraction

the filter pipet with methanol used as the transfer solvent. A glass tube was used for sample collection. After all of the sample was added to the filter pipet, the glass tube was rinsed several times with methanol and the rinses were added to the filter pipet. Because filtration was very slow, the sample was allowed to filter overnight, and the pipet was rinsed with ~0.5 mL of methanol as a final rinse.

Although two POCIS disks were used for sampling each site, the disks were extracted separately. After filtration, extracts from each site were combined and evaporated to dryness under nitrogen. In preparation for separation and analysis by LC-MS/MS, extracts were reconstituted with 1 mL of 0.2% formic acid in 10 mM NH_4OAc:ACN:MeOH (75:12.5:12.5, v/v). Samples were mixed well for 30 seconds with a Thermo Scientific MaxiMix II Vortex Mixer and refrigerated overnight to allow the dried extract to become completely dissolved in the injection solvent before analysis by LC-MS/MS.

4.4.3 LC-MS/MS Analysis

A Varian 1200L HPLC triple quadrupole mass spectrometer equipped with a CombiPAL autosampler, ESI source, and a ProStar/Dynamax solvent delivery system with two PS-210 pumps was used for separation and detection of ATSs. Separation was achieved with a Varian MonoChrom MS 5 µm, 50 x 2 mm HPLC column fitted with a MetaGuard MonoChrom 5 µm MS 2 mm guard column. Initial HPLC conditions featured an isocratic mixture of 0.2% formic acid:10mM NH_4OAc in water (v/v) (75%) (aqueous

mobile phase) and acetonitrile/methanol (1:1, v/v) (25%) (organic mobile phase) with a total run time of 8 minutes (Table 1).

However, the isocratic elution did not provide sufficient separation of analytes, and the 8 minute run time was too short to provide complete elution. The extract from Site 1 exhibited large unresolved peaks (Figure 5). Analysis of the extract from Site 2 produced similar results. However, Site 3 did not show any peaks greater than baseline and therefore, was not further analyzed. In order to effect better resolution and complete elution, the isocratic elution was changed to a gradient elution program, starting with 95% of the aqueous mobile phase and gradually increasing the organic mobile phase from 5% to a concentration of 20% over 14 minutes and then finally returning to the 95% A: 5% B mixture over the final 2 minutes for a total run time of 16 minutes (Table 2).

After several injections of Site 1 and 2 samples for method development, the amount of sample remaining was not sufficient for injection. However, because the goal of this research was to provide a qualitative assessment of amphetamine and/or methamphetamine using a passive sampling device, the samples were diluted with mobile phase 2 to allow for injection. The 16 minute run time did not allow for complete elution but the gradient program yielded better peak separation (Figure 6).

4.5 Results and Discussion

Analysis of a diluted Site 1 sample extract exhibited a peak matching the retention time and ion transitions (150 → 91 and 150 → 119) indicative for the presence of methamphetamine (Figure 6). However, sample retention times and ion transitions did

Table 4-1. Mobile Phase 1 HPLC Conditions

Column	Varian MonoChrom MS 5 µm, 50 x 2 mm			
Solvent A	0.2% formic acid:10mM NH_4OAc in water (v/v)			
Solvent B	acetonitrile/methanol (1:1, v/v)			
Gradient	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	75	25	0.25
	8:00	75	25	0.25
Injection Volume	40 µL			
Injection Solvent	acetonitrile/methanol/water/formic acid (5.5:17:77.25:0.25, v/v)			

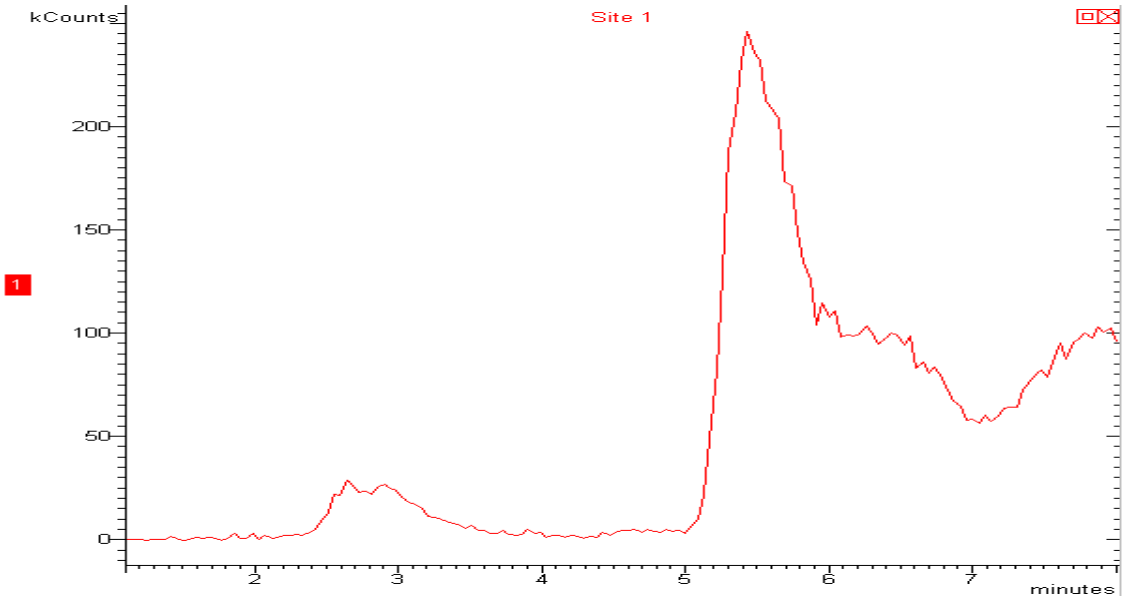

Figure 4-5. Total ion chromatogram of site 1 POCIS extracts

Table 4-2. Mobile Phase 2 HPLC Conditions

Column	Varian MonoChrom MS 5 µm, 50 x 2 mm			
Solvent A	0.2% formic acid:10mM NH_4OAc in water (v/v)			
Solvent B	acetonitrile/methanol (1:1, v/v)			
Gradient	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	95	5	0.25
	2:00	95	5	0.25
	4:00	90	10	0.25
	6:00	90	10	0.25
	8.00	85	15	0.25
	10:00	85	15	0.25
	12:00	80	20	0.25
	14:00	80	20	0.25
	16:00	95	5	0.25
Injection Volume	40 µL			
Injection Solvent	0.2% formic acid in 10 mM NH_4OAc:ACN:MeOH (75:12.5:12.5, v/v)			

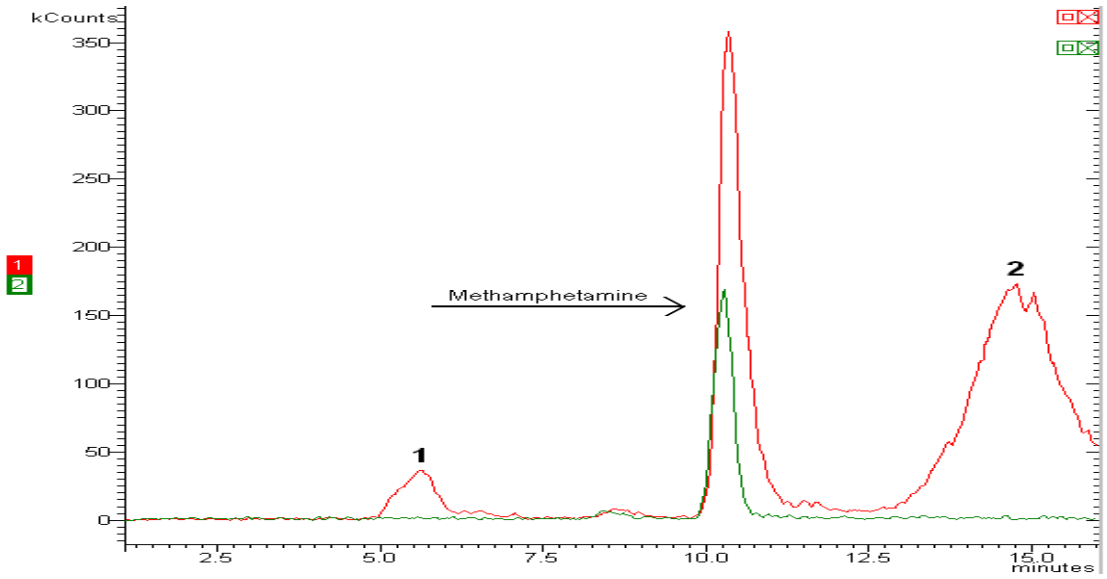

Figure 4-6. Total ion chromatogram for site 1, diluted sample, and 5 ng/mL methamphetamine standard. Peaks numbered 1 and 2 are unknown and from the site 1 sample.

not correspond to those for amphetamine (136 → 91 and 136 → 119). Data support the conclusion that methamphetamine is present in the sewer line from Site 1 and that it is present at concentrations greater than 5 ng/mL.

Analysis of a diluted sample extract from Site 2 exhibited multiple peaks, although none corresponded to the retention time of either amphetamine or methamphetamine (Figure 7). Therefore, neither amphetamine nor methamphetamine was qualitatively identified in the sewer line from Site 2.

Site 3 POCIS disks were only slightly dirty and the sorbent was not discolored and did not appear to have been wet. Analysis of Site 3 sorbent extracts did not show the presence of any peaks. The absence of peaks and the appearance of the disk and sorbent suggest minimal, if any, sewage flow across the disk. Table 3 summarizes deployment sites and summary of results.

4.6 Future Research

This research has demonstrated qualitative detection of methamphetamine in sewer lines using POCIS passive sampling technology. Future work could include monitoring of sites suspected of housing clandestine methamphetamine laboratories, either for law enforcement purposes or for studies to determine the load of illicit drugs being released into the Cookeville WWTP.

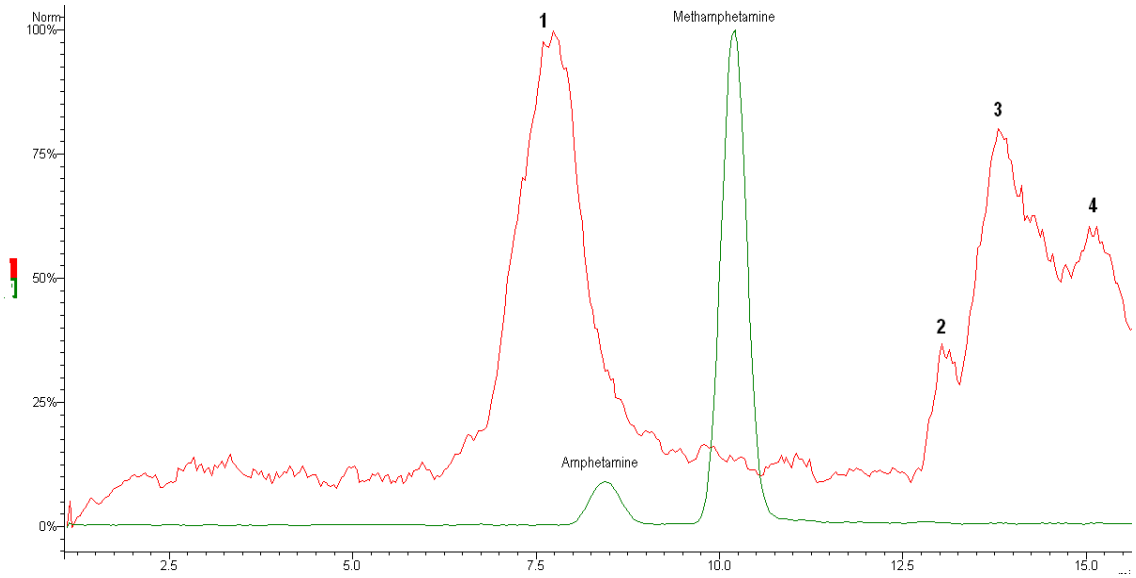

Figure 4-7. Total ion chromatogram for a diluted, extracted sample from site 2 shown with a mixed standard of amphetamine and methamphetamine. There are at least 4 unknown peaks in the site 2 sample, but none are amphetamine or methamphetamine. Chromatograms are normalized for clarity and are not to scale.

Table 4-3. POCIS Deployment Sites and Results

	Site #1	Site #2	Site #3
Methamphetamine Detected	**Yes**	**No**	**No**
Amphetamine Detected	**No**	**No**	**No**

4.7 References

[1] O.A.H. Jones, N. Voulvoulis, J.N. Lester, Environ Technol. 22 (2001) 1383.

[2] S.L. MacLeod, E.L. McClure, C.S. Wong, Environ. Toxicol. Chem. 26 (2007) 2517.

[3] D.A. Alvarez, P.E. Stackelberg, J.D. Petty, J.N. Huckins, E.T. Furlong, S.D. Zaugg, M.T. Meyer, Chemosphere, 61 (2005) 610.

[4] T.L. Jones-Lepp, D.A. Alvarez, J.D. Petty, J.N. Huckins, Arch. Environ. Contam. Toxicol. 47 (2004) 427.

[5] D.A. Alvarez, J.D. Petty, J.N. Huckins, T.L. Jones-Lepp, D.T. Getting, J.P. Goddard, S.E. Manahan, Environ. Toxicol. Chem. 23 (2004) 1640.

[6] G.A. Mills, B. Vrana, I. Allan, D.A. Alvarez, J.N. Huckins, R. Greenwood, Anal. Bioanal. Chem. 387 (2007) 1153.

CHAPTER 5

RELATED UNKNOWN COMPOUNDS

5.1 Introduction

The objective of this research was to analyze wastewater for methamphetamine and amphetamine, two amphetamine-type stimulants (ATSs) that are excreted in urine with a portion of both being excreted as the intact drug. Methamphetamine is not currently prescribed by physicians, and based on several local news reports of arrests made for methamphetamine possession and manufacturing, the hypothesis of this research was that methamphetamine would be found in wastewater influent. Although amphetamine is also an illicit drug, it is currently also legally and regularly prescribed for attention deficit disorder. Because of the large numbers of prescriptions for legal amphetamine this research also hypothesized that amphetamine would be detected in local wastewater.

5.2 Unknown Compounds #1 and #2

Because the goal of the research was to detect and quantify amphetamine and methamphetamine, LC-MS/MS was used for selected reaction monitoring (SRM) after sample preparation by solid-phase extraction (SPE). The molecular weight of amphetamine is 135.21 and the $[M+1]^+$ molecular ion is located at a mass-to-charge (m/z)

ratio of 136. Two intense product ions are observed at 91 and 119. The molecular weight of methamphetamine is 149.23 and the $[M+1]^+$ molecular ion is located at a mass-to-charge (m/z) ratio of 150, and two intense product ions are also observed at 91 and 119.

Analysis of wastewater resulted in the detection of 14 unknown compounds. U1 was observed in wastewater influent in both the 136 → 91 and the 150 → 91 ion transitions and in wastewater effluent (both prior to and after UV disinfection) in the 150 → 91 ion transition. U2 was observed in the 150 → 91 in influent and in pre- and post UV effluent. Figures 1 and 2 show overlaid chromatograms obtained by isocratic elution for laboratory standards and influent samples for the 136 → 91 and 150 → 91 transitions, respectively.

In order to further investigate and separate the unknown peaks, the isocratic elution was changed to a gradient elution and the run time was increased from 8 minutes to 16 minutes. In both methods, a Varian MonoChrom MS 5 µm, 50 x 2 mm column equipped with a MetaGuard MonoChrom 5 µm MS 2 mm guard column was used for separation. The mobile phases of Solvent A 0.2% formic acid:10mM NH_4OAc in water (v/v) and Solvent B acetonitrile/methanol (1:1, v/v) were unchanged, but Table 1 shows the initial and final gradient conditions.

The gradient elution and increased run time provided a greater degree of separation for the large U1 peak, resulting in at least two additional unknown peaks, U3 and U4. Figure 3 shows the 136 → 91 transition of an influent sample and an

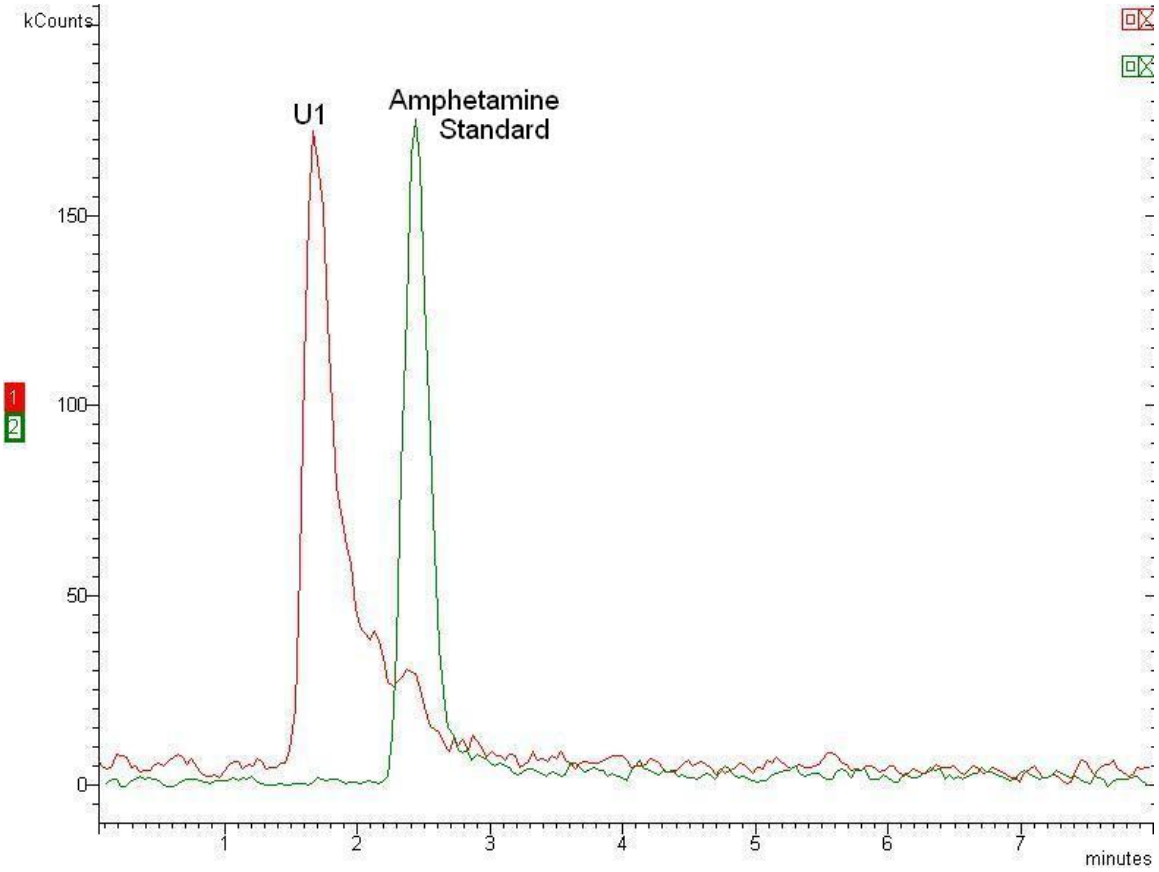

Figure 5-1. 136 → 91 ion transition for amphetamine standard and wastewater influent sample indicating the presence of at least one unknown compound (U1).

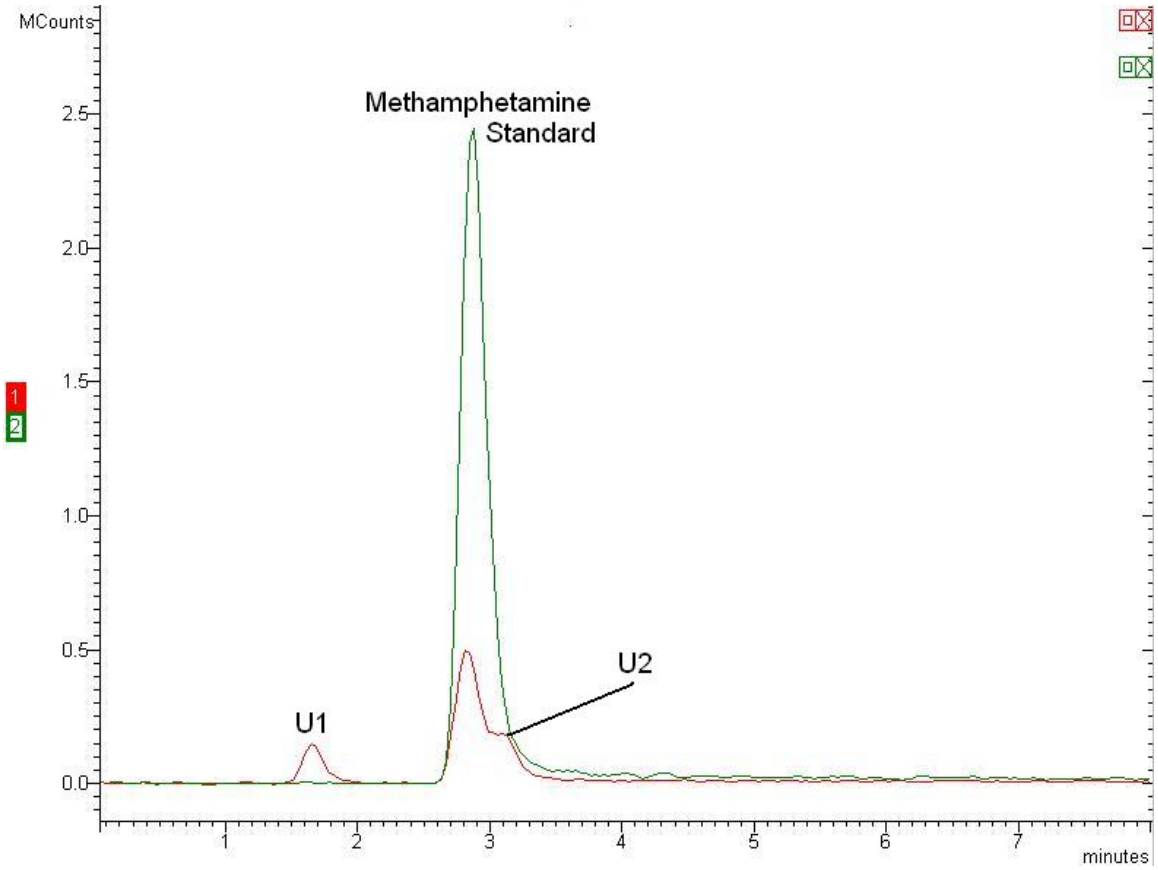

Figure 5-2. 150 → 91 ion transition for methamphetamine standard and wastewater influent sample indicating two unknown compounds (U1 and U2).

Table 5-1. Initial (Isocratic) and Final (Gradient) Elution for Improved Peak Resolution

Isocratic Elution	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	75	25	0.25
	8:00	75	25	0.25

Gradient Elution	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	95	5	0.25
	2:00	95	5	0.25
	4:00	90	10	0.25
	6:00	90	10	0.25
	8.00	85	15	0.25
	10:00	85	15	0.25
	12:00	80	20	0.25
	14:00	80	20	0.25
	16:00	95	5	0.25

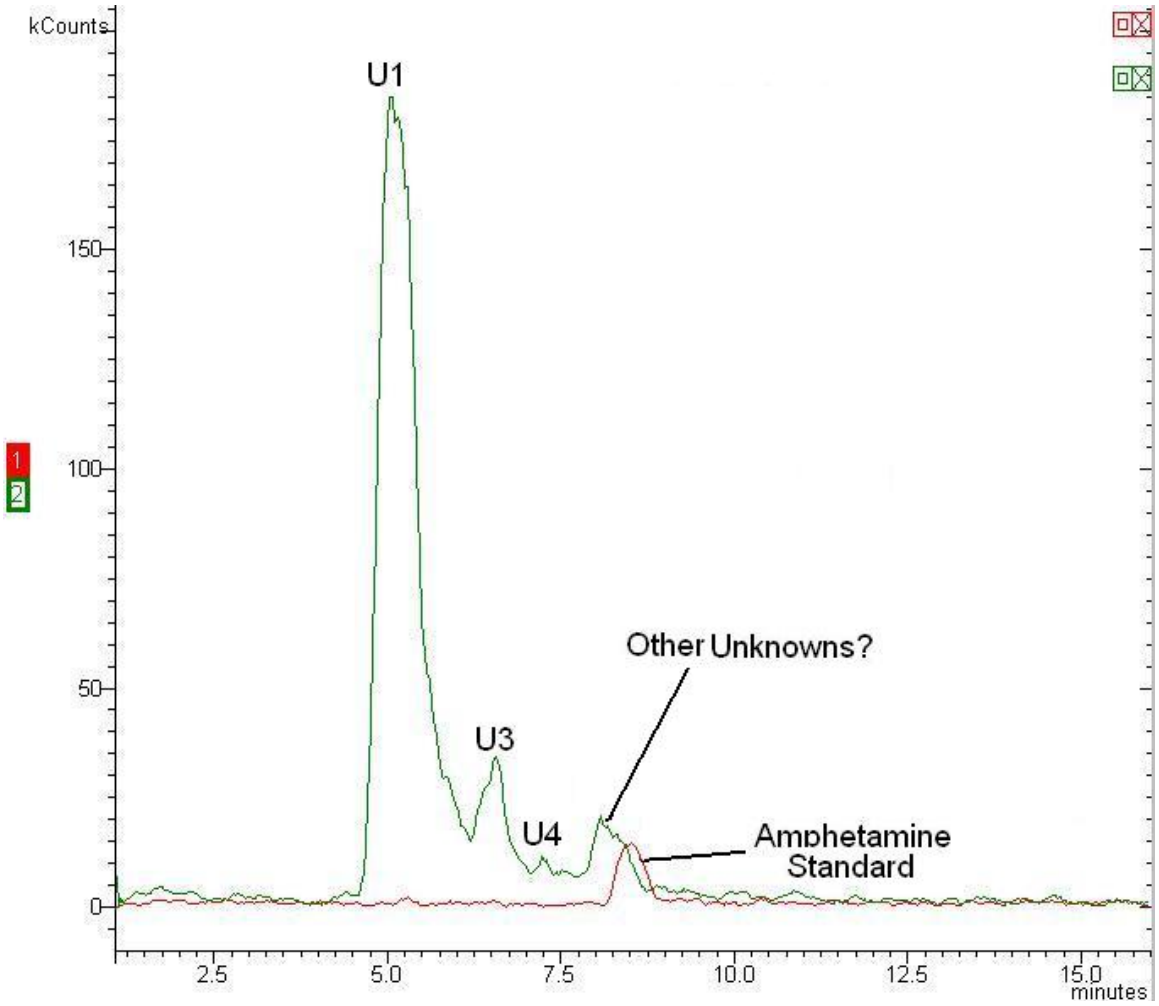

Figure 5-3. Separation of unknown peaks (U1, U3, and U4) in wastewater influent compared to an amphetamine standard.

amphetamine standard. The amphetamine peak identity was confirmed with the internal standard.

Gradient elution and increased run time provided near baseline resolution for methamphetamine and U2 in the 150 → 91 transition (Figure 4) for wastewater influent and methamphetamine standard. The identity of the peak methamphetamine peak was confirmed with the internal standard.

Standards of the related drugs ephedrine, pseudoephedrine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-Methylenedioxyethylamphetamine (MDEA) were analyzed by LC-MS/MS. Those compounds were selected for analysis because (1) ephedrine and pseudoephedrine can be used as starting products to produce methamphetamine, (2) pseudoephedrine is available as a restricted access over-the-counter medication without a prescription in some states and is found in the formulation of several prescription medications, and (3) MDA (the Love Drug), MDMA (Ecstasy), and MDEA (Love) are drugs of abuse with structures similar to methamphetamine (Figure 5). After analysis, retention times were compared for U2 and U3 and the compounds listed above. However, none of those compounds eluted with the same retention time as the unknown (Figure 6).

Investigation of unknown compounds in the wastewater should include metabolites of ATSs. The primary metabolites for amphetamine and methamphetamine are 4-hydroxyamphetamine and 4-hydroxymethamphetamine, which are Phase I metabolites. Phase II metabolites can result from glucuronidation or sulfation of the hydroxyl group.

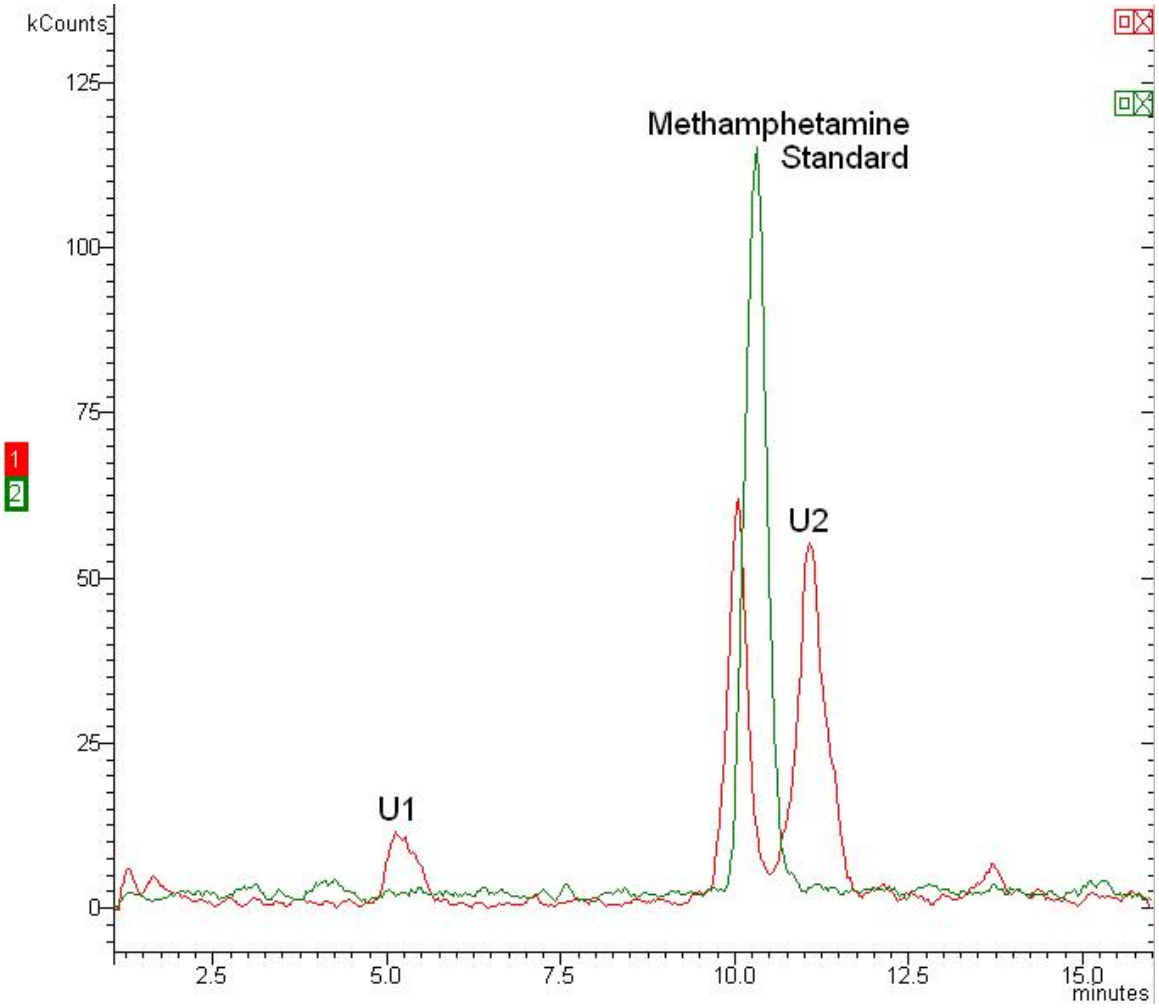

Figure 5-4. Near baseline separation of methamphetamine and U2 in wastewater influent compared to a methamphetamine standard.

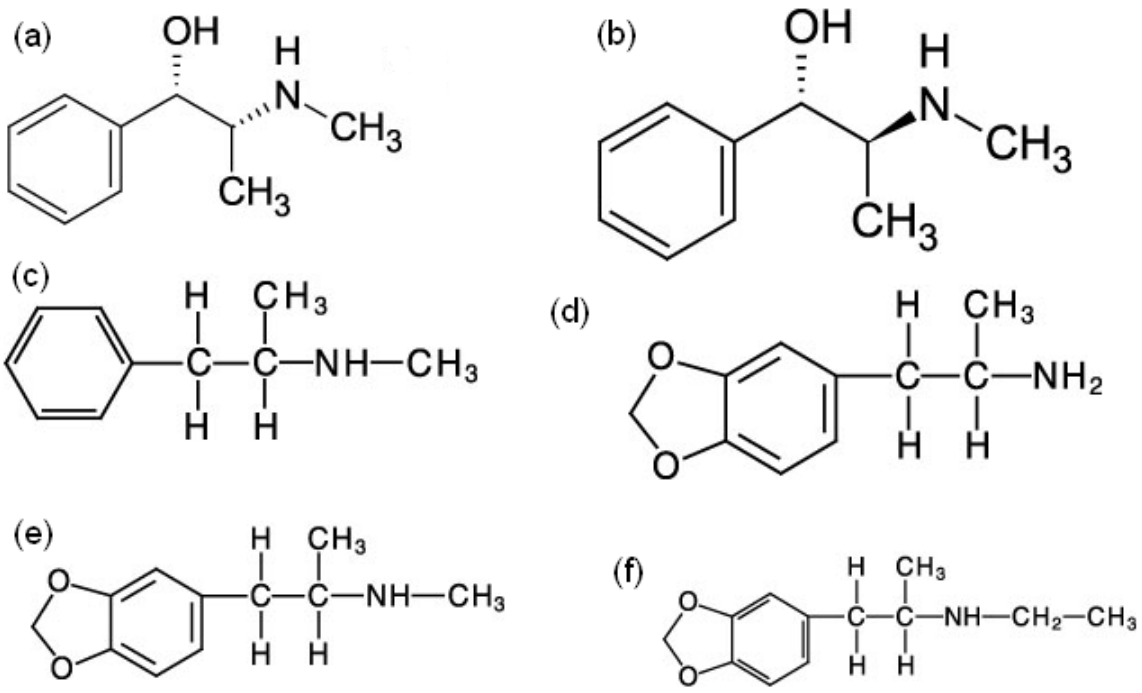

Figure 5-5. Structures for (a) ephedrine, (b) pseudoephedrine, (c) methamphetamine, (d) MDA, (e) MDMA, and (f) MDEA.

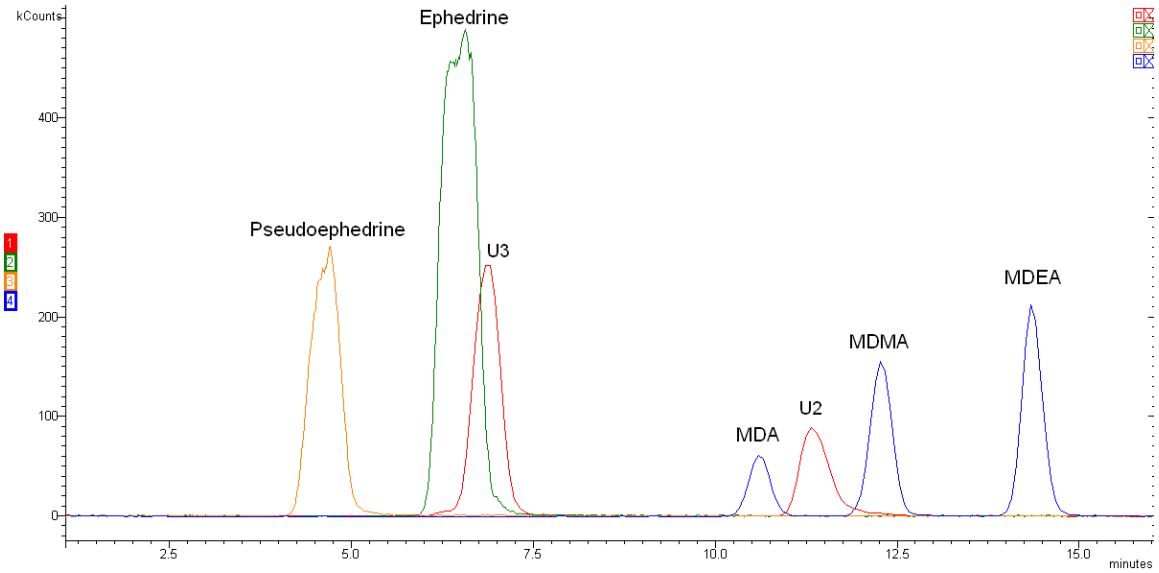

Figure 5-6. Chromatograms of wastewater influent overlaid with those of standards to compare retention times of ephedrine, pseudoephedrine, MDA, MDMA, and MDEA with unknown peaks U2 and U3.

5.3 Full Scan Mass Spectrometry

In order to more fully investigate unknown compounds and to determine what other compounds of interest may be in the wastewater influent, a full scan was performed from mass 80 to 500 utilizing the established 16 minute gradient. Several compounds were eluted, including the initial U1, U2, U3, and U4 compounds already identified as unknowns in the SRM analysis of wastewater influent. Other compounds were also found, for a total of at least 14 unknown compounds in the wastewater influent (Figure 7).

5.4 Future Research

Although this research focused on only two ATSs, several unknown compounds related to amphetamine and methamphetamine were found to be in wastewater influent and in some cases, in the effluent. Identification of the compounds would provide future opportunities for research. In addition, if the compounds were determined to be other ATSs or other drugs of abuse, quantitation and detection would provide interesting information regarding community drug use.

Another area for future research is the development of a screening method for illicit drugs and/or PPCPs in wastewater, which could involve the use of passive sampling devices to provide real-time information about what potentially harmful substances are entering the WWTP. Identification of compounds found in wastewater

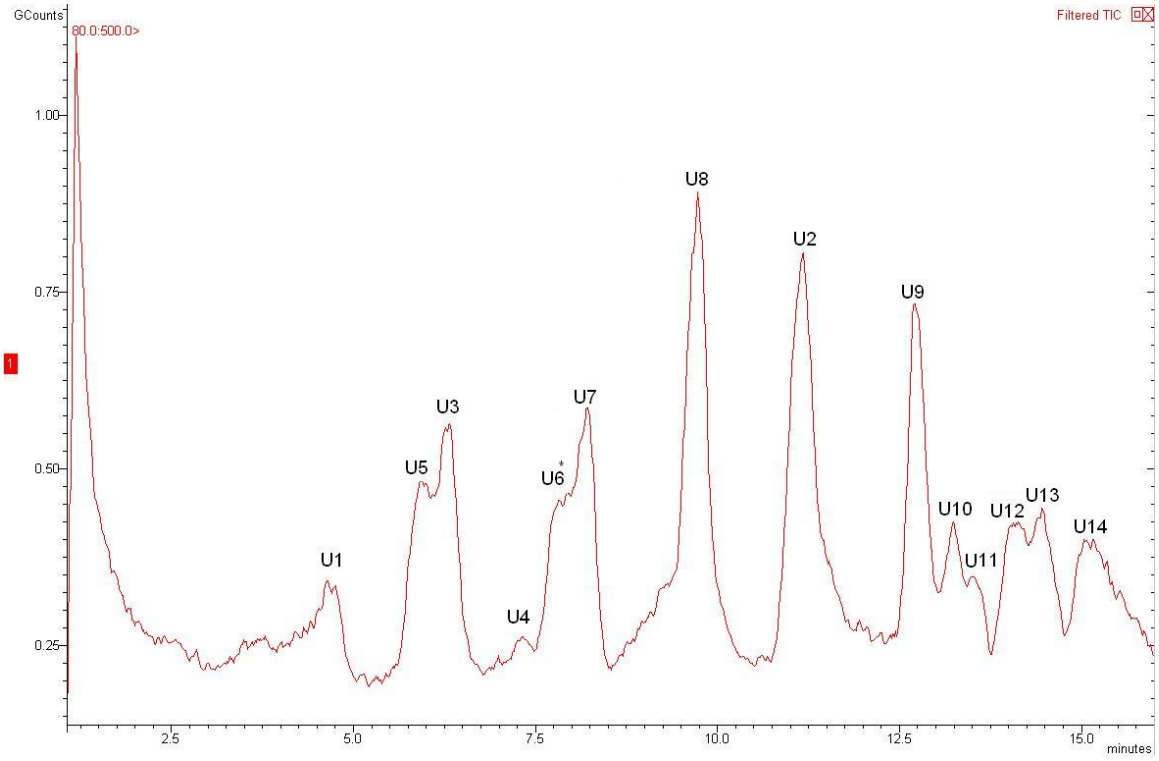

Figure 5-7. Full scan from mass 80-500. Several unknown compounds which may be of research interest were found.

effluent may lead to better water treatment methods that would alleviate the problem of potentially harmful chemicals entering surface water and groundwater.

CHAPTER 6

FUTURE RESEARCH

6.1 Amphetamine-Type Stimulants in Wastewater

Further work must be done in the area of wastewater treatment to determine which method(s) of treatment best and most completely removes ATSs from wastewater. Results of this research show that methamphetamine is not completely removed during water treatment and is released into surface water, although in low ng/L concentrations. The question that remains is if a substance is found, is it a threat to human or aquatic life? Zuccato et al. [1] stated that even if environmental concentrations are low, risks for human health and the environment cannot be excluded. ATSs and other illicit drugs have potent pharmacological activities, and their presence as complex mixtures in surface waters - together with residues of many therapeutic drugs – may lead to unforeseen pharmacological interactions causing toxic effects to aquatic organisms [1].

6.2 ATS "Hotspots"

This research provides the first report of the use of passive sampling devices to qualitatively identify methamphetamine in sewer pipes from buildings suspected to house clandestine methamphetamine laboratories. In the future, passive sampling devices could be placed in sewer lines from sites where ATS or other illicit drug use or manufacture is

suspected. This could potentially affect search and seizure laws, allowing searches of properties if drugs are detected. Calibration and validation of passive sampling devices would be required, even under such harsh conditions as would be encountered in sewers. In addition, areas contributing significant amounts of drugs could be identified and water cleanup could be initiated before the sewage reaches the WWTP.

6.3 Estimation of Community Drug Use

Zuccato et al. [2] and Huerta-Fontela et al. [3] conducted studies to provide objective, quantitative data to estimate community drug use based on sewage monitoring. Their methods of estimation could be used as models for other community drug use studies.

6.4 ATSs in Rural Areas

In areas where city sewer services are not provided, ATSs are excreted or disposed of in septic tank systems. Because septic tanks require bacteria to work properly, there is the potential for those bacteria to be killed because of exposure to ATSs. The raw sewage could then be released into the soil and have the potential to enter groundwater sources. There are also potential health and liability problems if property containing a contaminated septic tank or soil is sold. In addition, sludge from WWTP is often applied to agricultural land as fertilizer. Jones-Lepp et al. [4] reported the presence of methamphetamine in sludge, which could provide another path for drugs to enter the

groundwater. Drugs or other compounds from wastewater that enter the sludge could again find their way into the groundwater.

6.5 ATS Metabolites

Future studies should be performed to identify Phase I and Phase II metabolites of ATSs and to determine their fate in wastewater treatment. Although there are published reports of the detection of metabolites in urine, no studies have been conducted to detect and quantify the metabolites in wastewater.

6.6 References

[1] E. Zuccato, S. Castiglioni, R. Bagnati, C. Chiabrando, P. Grassi, R. Fanelli, Water Res. 42 (2008) 961.

[2] E. Zuccato, C. Chiabrando, S. Castiglioni, R. Bagnati, R. Fanelli, Environ. Health Perspect. 116 (2008) 1027.

[3] M. Huerta-Fontela, M.T. Galceran, J. Martin-Alonso, F. Ventura, Sci. Total Environ. 397 (2008) 31.

[4] T.L. Jones-Lepp, R. Stevens, Anal. Bioanal. Chem. 387 (2007) 1173.

APPENDIX

METHOD VALIDATION

Each filtered wastewater sample (influent, effluent pre-UV, and effluent post-UV) was analyzed in triplicate, and methamphetamine D-5 as internal standard was added prior to solid-phase extraction (SPE). Additionally, each sample was spiked with a known amount (10 ng/mL) of amphetamine and methamphetamine prior to SPE.

External standards of amphetamine and methamphetamine at concentrations of 0, 5, 10, and 20 ng/mL were analyzed each time wastewater was analyzed. External standards also contained internal standard at a concentration of 50 ng/mL. External calibration curves are shown in Figure 1 for amphetamine and Figure 2 for methamphetamine.

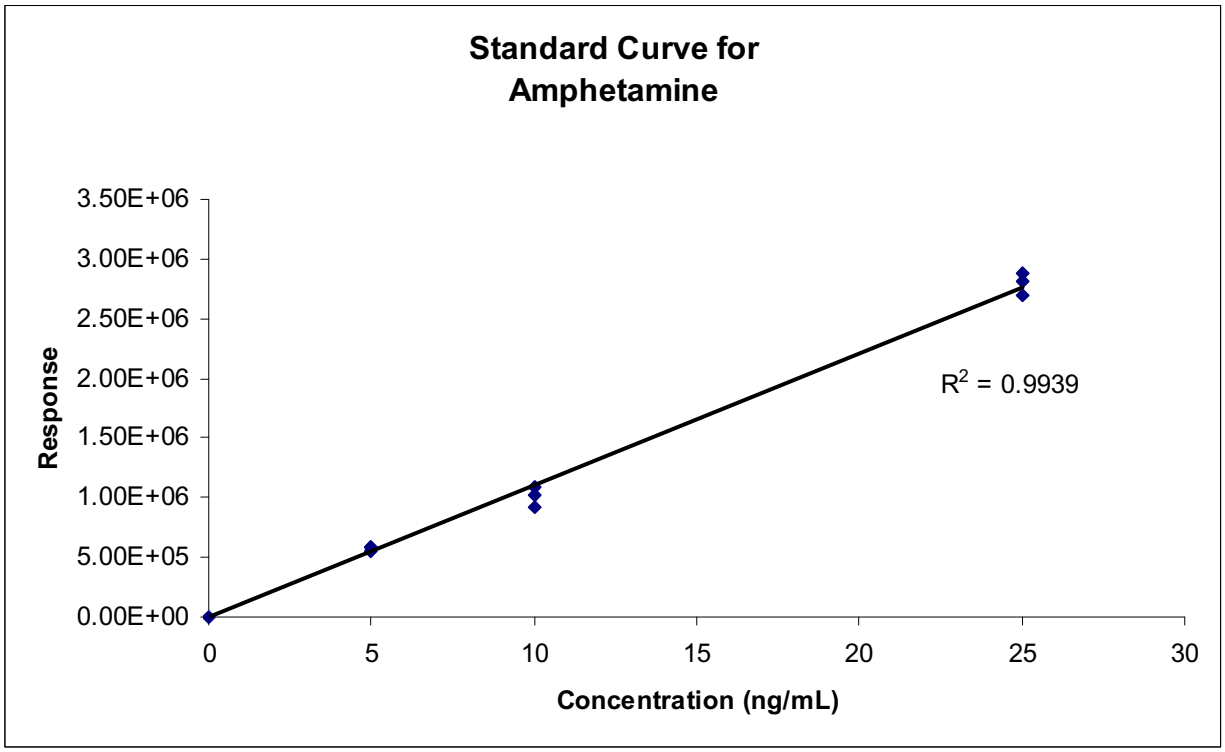

Figure 1. Standard curve for amphetamine

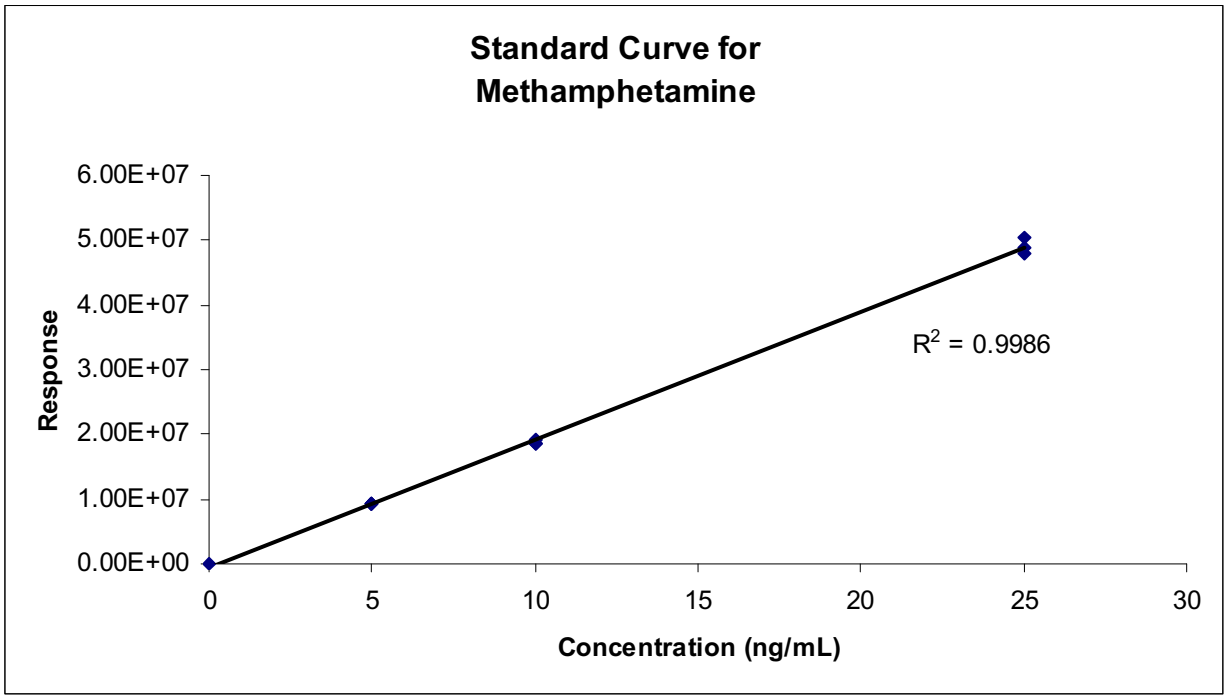

Figure 2. Standard curve for methamphetamine

BIOSOLIDS

Biosolids samples were obtained from the Cookeville Wastewater Treatment Plant. Two samples were obtained from each of two clarifying tanks, one sample from the holding tank, and one dewatered sample just before lime was added. All samples except the dewatered sample were placed into a Büchner funnel with Fisherbrand G6 glass fiber filters (1.6 μm pore size) and subjected to vacuum filtration to remove water. After water was removed, sludge samples, including the previously dewatered sample, were spread onto watch glasses and allowed to dry for 24-48 hours. Samples were pulverized to a homogeneous powder using a Spex CertiPrep ball mill mixer at a frequency of 23/s.

A proprietary EPA extraction procedure was performed using the Dionex ASE 200 accelerated solvent extraction system. A 1.0 g aliquot of the pre-dried homogenized biosolids was weighed and analyzed. Sample extracts were evaporated to dryness under nitrogen, reconstituted with injection solvent (1 mL of 0.2% formic acid in 10 mM NH_4OAc:ACN:MeOH (75:12.5:12.5, v/v) and internal standard, mixed thoroughly, and refrigerated overnight. Analysis was performed by LC-MS/MS.

VITA

Tammy Hatfield Boles was born in Chattanooga, TN, and attended elementary and middle school in Jasper, TN. In 1979, she moved to Savannah, TN, where she graduated from Hardin County High School in 1985. She entered Tennessee Technological University (TTU) in the fall of 1985 and remained there until 1988 when she transferred to the University of South Carolina (USC) in Columbia, SC. She received a Bachelor of Science in Chemistry in the fall of 1989 and entered the graduate school at USC in the spring of 1990. She received her Master of Science in Chemistry in the summer of 1992. In the spring of 2000, she was accepted into the graduate school at TTU and is a candidate for the Doctor of Philosophy Degree in Environmental Science – Chemistry.