ENVIRONMENTAL IMPACTS OF ONE POT METHAMPHETAMINE CLANDESTINE LABORATORIES – CHARACTERIZATION AND DETECTION OF TRACE MATERIALS

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Abstract: The One Pot methamphetamine production method has become the primary method of choice in clandestine drug laboratories across the United States, due to its simplicity and the availability of required materials. While the method is simple, it also generates risk to innocent bystanders within the community from flammability and toxicity hazards. Previous studies have determined the adverse effects of methamphetamine consumption, but there has been little research exploring the health impact of being in close proximity to methamphetamine manufacturing. Despite investigative efforts, clandestine laboratories may not be discovered for an extended period of time, after which, numerous methamphetamine productions will have been completed. As a result, the probability of exposure to toxic substances involved with the One Pot method increase significantly. This study was undertaken to determine and quantify the characteristic products and byproducts of the One Pot methamphetamine method. In addition, studies were conducted to determine the feasibility of detection of methamphetamine clandestine laboratories through monitoring waste water effluents. Methamphetamine was produced by the One Pot method and the methamphetamine hydrochloride product was filtered out. All post-reaction liquids and solids were characterized. In collaboration with local authorities, simulated One Pot methamphetamine waste disposal was performed using a representative lift station. Waste water samples were collected post-distribution to determine a time course detection window and analyzed via solid phase extraction with liquid chromatography-tandem mass spectrometry. Methamphetamine, pseudoephedrine, and ephedrine were all detectable in the waste water. Also, an over-reduced product, characteristic of the One Pot synthesis, CMP [1-(1',4'-cyclohexadienyl)-2-methyl-aminopropane] was detected. As a means to determine the value of CMP as a unique identifier, urine samples that previously tested positive for methamphetamine were analyzed, and only one sample demonstrated positive results for the primary One Pot byproduct. This work identifies the components produced following One Pot methamphetamine production. Additionally, results demonstrate the possibility and potential for analyzing waste water to monitor and detect clandestine One Pot methamphetamine laboratories within communities.

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CHAPTER I

INTRODUCTION

Methamphetamine, perhaps the most widely known illicit substance, continues to be a drug of major concern. From its first synthesis in 1893 to becoming a Drug Enforcement Administration (DEA) Schedule II compound in 1971, methamphetamine has been used in many applications due to the drug's stimulant properties. During World War II, for example, soldiers were administered methamphetamine to reduce fatigue.[1] In addition to nonmedical use, doctors in the United States were prescribing methamphetamine, and other related amphetamines, to help treat certain conditions like hyperactivity and obesity, to name a few.[2] In fact, one prescribed form of methamphetamine still exists, that being the brand name Desoxyn. While an argument can be made for drug administration in the scenarios listed above, the true problem with methamphetamine arises in the illicit and abusive use of the drug. Around the 1980s, the illegal use of methamphetamine began to gain popularity.[2] From these scenarios, and with consumption levels well above previously reported therapeutic ranges, methamphetamine use became a widespread problem.

As mentioned above, methamphetamine is a powerful stimulant. According to the National Institute on Drug Abuse, it is a synthetic drug that can be used as a white powder or crystal glass. Additionally, this stimulant is typically smoked, but can be

administered via other possible routes.[3] As with many biology and molecular processes, exposure to foreign objects can alter normal function. Literature has shown that methamphetamine causes such alterations with dopamine and its molecular players, including dopamine receptor and dopamine transporter. Methamphetamine can increase dopamine release and function in the brain.[4] Historically, methamphetamine was used for medicinal purposes, but with the strong majority of this drug unavailable through legal action, it is more recently abused as an illicit substance. And instead of administering therapeutic levels, unmonitored methamphetamine use has the potential and in most cases, likelihood of reaching high or toxic drug concentrations. Before any interaction can take place, methamphetamine, as is the case with other drugs, must first enter the neuron. The dopamine transporter controls the active re-uptake of dopamine from the synapse, which is the part of a neuron that allows for signal transfer.[5] In addition to increasing dopamine release, methamphetamine also blocks re-uptake of dopamine, thus cancelling the function of the transporter. In this sense, the combination of extra dopamine release and dopamine not being able to be transferred back into the terminal creates an additive-like effect. The pleasure and reward system associated with dopamine is intensified when under the influence of methamphetamine. This intensity is commonly referred to as the euphoria or "high" that users will describe. The molecular characteristics listed above contribute greatly to the continued used and addiction commonly associated with methamphetamine.

Historical and prescription uses for methamphetamine have displayed minor prevalence and success, but illicit use has witnessed increasing popularity. Several hypotheses may explain this trend, but one likely cause is the formation of clandestine

laboratories. While the majority of these "laboratories" do not resemble a typical academic or research laboratory, they are considered such because they perform a chemical reaction, i.e. converting a starting material into methamphetamine. Clandestine, by definition, means hidden or operated in secrecy. Individuals, either looking to use or sell methamphetamine, can manufacture the drug within a residence or other dwelling of choice. Despite the incentive to produce methamphetamine individually, the process and its ingredients create a hazardous environment, which has the potential to become severely dangerous. The unsafe conditions are exposed to family, friends, and pets living within the immediate area, but also any first responders and law enforcement personnel who may enter the scene. A study conducted in 1996, reviewed the adverse medical effects in clandestine laboratory investigators. Findings from the retrospective study indicated that methamphetamine laboratories accounted for 81-97% of all law enforcement responses and if health issues were reported, symptoms primarily included headache and respiratory, mucous membrane, and skin irritation.[6] While safety measures within law enforcement communities have likely increased over the past 20 years, innocent bystanders within dangerous clandestine environments are unlikely to be equipped with the proper personal protective equipment. An additional note is that the law enforcement personnel are only exposed to potential dangers for a short, limited time period. Residents living within, on the other hand, may experience exposure for weeks, and possibly months. Due to the abundant health risks, research needs to be conducted to determine the amounts of contamination within typical clandestine laboratories.

In 2005, VanDyke et al. developed a study within an actual household to examine the clandestine scene over a 24-hour period.[7] To replicate an actual clandestine scene,

methamphetamine was manufactured in one room or region of the household. On day 1, two methamphetamine reactions were produced using a previously common procedure called the "Red Phosphorus" method, which will be discussed further in Chapter 2.[7] Although the technique is not the same as the more recent and popular "One Pot" method, the study still provides insight about possible contamination levels. On day 2, the researchers started collecting evidence, which included atmosphere and wipe samples. Based on the results, methamphetamine can be detected within the atmosphere for at least 24 hours after production.[7] Depending on the frequency of methamphetamine manufacturing, anyone dwelling nearby may have a constant exposure, or at the very least, consistent exposure to toxic methamphetamine fumes. The authors noted that the airborne methamphetamine particles were rather small and had the ability to be inhaled by anyone near the production site.[7] Another sample type collect was wipes or swabs. As the name suggests, a piece of material can be swiped across a hard surface, furniture, piece of clothing, etc. and then extracted to detect the presence of drugs collected on the wipe. Results for this type of sample collection demonstrated positive methamphetamine detection in every room of the house, and even on the clothing of the researchers.[7] The findings from this study confirm the presence of methamphetamine and related chemicals throughout the immediate area surrounding a manufacturing reaction. Additionally, the detection of toxic fumes and surface contamination in adjoining or adjacent rooms of the house, provided understanding that health risks associated with clandestine laboratories can spread to nearby areas. An important note, as discussed by the authors, is that there is high variability amongst methamphetamine laboratories, in terms of production and exposure.[7] Therefore, any controlled research results must be interpreted individually.

Although prior work has been done to help fully understand clandestine laboratory contamination potentials, continued research is needed to characterize results from more recent methamphetamine manufacturing methods. As briefly mentioned above, the One Pot method has become the primary method of choice for quick and easy methamphetamine manufacture. Expanded details on this specific method will be further discussed in Chapter 2. While research within the literature pertain to older manufacturing techniques, very few projects have been conducted to understanding not only the potential contamination levels, but also the hazards produced by the One Pot method. Additionally, the health and environmental hazards created during methamphetamine manufacturing has produced a demand to proactively locate clandestine laboratories within the community. To achieve this goal, distance monitoring appears to be a practical technique to pursue. One potential pathway to detect the presence of clandestine laboratories is following reaction waste disposal. Disposal routes include, but are not limited to, private property, public areas, and the sewer system. "There are two significant issues relating to such dumps of materials; they might contain valuable evidence as to drug manufacture, and they might be a source of pollution."[8] Analysis of waste water for target drug compounds has shown continued success, and will be explained in Chapter 2. Therefore, if drug compounds associated with methamphetamine, specifically the One Pot manufacturing method, can be detected within sewage effluent, determination of the location or general area of clandestine laboratories may be possible.

A signature byproduct of the One Pot method has been identified as 1-(1',4'-cyclohexadienyl)-2-methyl-aminopropane, commonly referred to as CMP. Before this

study, the amount of byproduct produced in a One Pot method and its potential use as a clandestine laboratory identifier had not been documented. The goals of this study include the following:

1) characterize the One Pot methamphetamine method,
2) develop waste water analysis methodologies to detect One Pot waste in sewage effluent, and
3) determine the significance of detecting the primary One Pot byproduct, CMP, within environmental samples.

Each goal, as expected, has an associated hypothesis. Respectively, the hypotheses include:

1) the One Pot methamphetamine method produces signature impurities, within both the final product and waste materials, that can uniquely identify the manufacturing technique,
2) One Pot waste products and byproducts are detectable in sewage effluent, and
3) CMP, following consumption within a One Pot methamphetamine salt, can be detected within urine.

For the first study, as completely explained in Chapter 2, One Pot methamphetamine was produced using two solvent types, diethyl ether and camp fuel, as the reaction medium. Reactions were conducted at the Oklahoma State University – Forensic Toxicology and Trace Laboratory (OSU-FTTL), and methamphetamine products and waste materials were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Results establish potential yields found within One Pot methamphetamine laboratories, and discover the detectable presence of target compounds

within reaction waste. The second study, discussed in Chapter 3, was achieved by creating a simulated One Pot waste disposal experiment. The One Pot reaction wastes, from the two solvent types mentioned above, were deposited into a local waste water system, with coordination through local authorities. Sewage effluent samples were collected downstream over time. Waste water samples were then prepared for instrumental analysis through solid phase extraction (SPE). Results indicate a significant increase or spike in One Pot target compound concentrations following a simulated disposal. The findings from this experiment demonstrate the potential to further improve and implement waste water analysis as a means to track and locate One Pot clandestine laboratories. And lastly, the third study, as detailed in Chapter 4, was investigated by analyzing human urine samples that had previously tested positive for methamphetamine. The approach was developed to understand if the primary One Pot byproduct, CMP, was detectable in urine post-methamphetamine consumption. Findings confirm the possibility of detection, but further research is needed to completely understand CMP and its interaction within the human body.

Overall, the research conducted within the three following chapters provides both previously unknown knowledge and a demand for continued investigation into the One Pot methamphetamine method. For the first time, One Pot methamphetamine product and reaction waste yields have been reported. Although clandestine laboratory yields can be highly variable, the results established the primary compounds of interest that would be important to monitor in any detection campaign. Additionally, a waste water analysis method was developed and validated to detect the target compounds of the One Pot method. Finally, investigation was performed to begin the process of understanding

CMP within human subjects. Continued efforts and research in all three facets of this study, will continue to increase the awareness of the law enforcement and scientific communities in regards to the One Pot manufacturing method, with the end goal of being able to proactively remove the dangerous clandestine laboratories from the community and environment before more harm is done.

CHAPTER II

ONE POT METHAMPHETAMINE CHARACTERIZATION

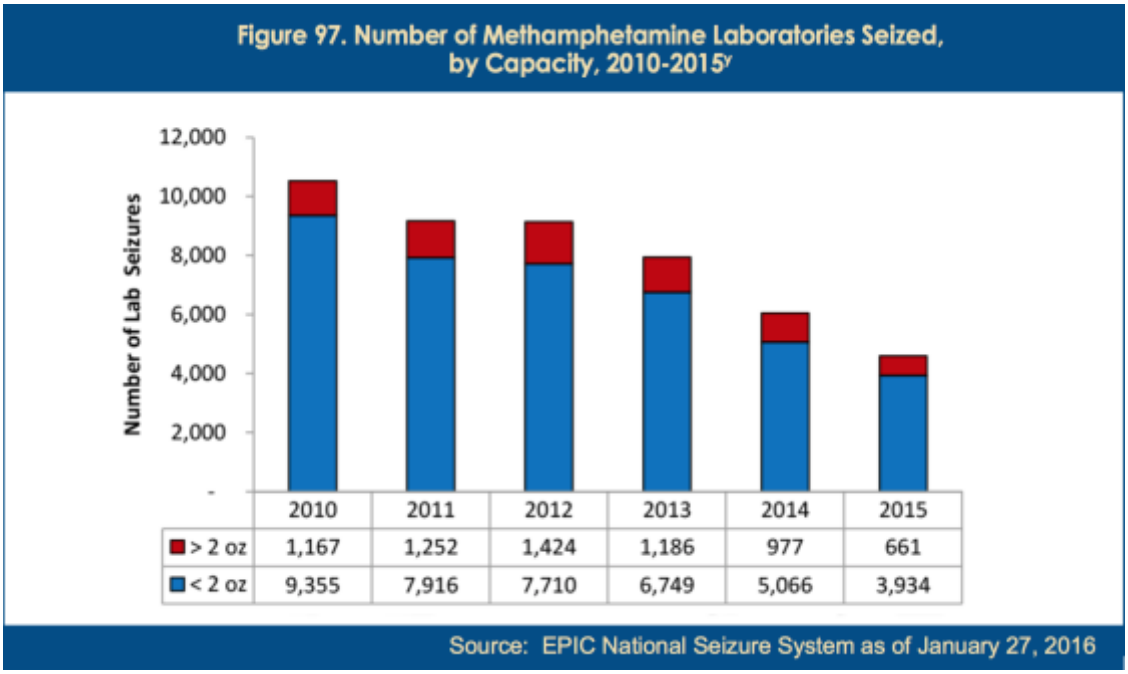
2.1 Introduction

Throughout the decades of methamphetamine manufacturing, there have been numerous methodologies to produce the illicit stimulant. Among these production techniques, include more popular methods such as the "Phenyl-2-Propanone", "Red Phosphorus", and "Birch Reduction" methods, to name a few. The aforementioned methodologies all result in the production of methamphetamine. However, each method utilizes varying ingredients, requires a different step-wise procedure, and produces signature byproducts. Through examination of the ingredients used, waste materials left behind, and trace detection of unique identifiers, law enforcement and the scientific community have had the ability to determine the method of production. Not only does this knowledge assist in understanding drug manufacturing trends within a certain population or region, understanding the production method can assist in tracking down the clandestine laboratory and/or the individual(s) producing methamphetamine. The Drug Enforcement Administration (DEA), with the assistance of several research projects, created a Methamphetamine Profiling Project (MPP) to track and monitor methamphetamine production within the country.[9] Byproducts, indicative of the manufacturing technique, have been identified by analyzing the final methamphetamine

product or salt. Although the majority of the salt is, in fact, methamphetamine, trace impurities can be detected with analytical instrumentation. Updated and complete knowledge of a methamphetamine production method can enhance identification within the community, and support research projects designed to improve detection capabilities.

As of recent, the "One Pot" method has emerged as the production technique of choice within clandestine methamphetamine laboratories. Due to the simplistic procedure and easy access to required ingredients, the One Pot method has become a widespread problem across the United States. As shown in Figure 1, the DEA released statistics in the 2016 National Threat Assessment regarding methamphetamine laboratories.[10]

Figure 1. Number of Methamphetamine Laboratories Seized from 2010-2015. Bar graph obtained from DEA 2016 National Threat Assessment.



According the bar graph, the number of seized clandestine laboratories have actually decreased. However, two adjoining factors indicate that One Pot methamphetamine is

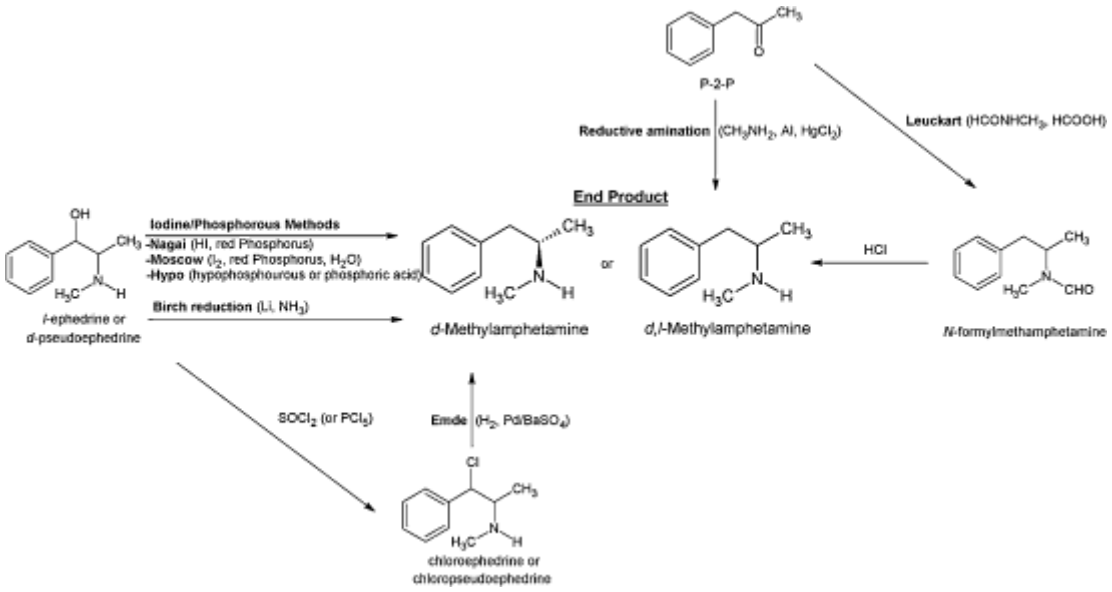
still prevalent throughout the country. The first is that the number of seizures each year does not account for clandestine laboratories left unfound. The second is that a strong majority, 86 percent, of laboratories seized each year had less than two ounces of final product present. Small quantities of methamphetamine, less than 2 ounces, are indicative of a One Pot laboratory. "Generally, these laboratories are small-scale, easy to conceal, and produce."[10] However, with all the risks associated with methamphetamine itself, the dangers of drug production create the opportunity for additive hazards. The popularity of One Pot laboratories within communities demonstrates a need to fully understand all products and byproducts created during the process. Until this project, little to no research has been done to fully characterize the One Pot methamphetamine method.

The purpose of this study was to investigate every aspect of the One Pot methamphetamine method post-reaction. Methamphetamine was manufactured in a safe, laboratory environment at the Oklahoma State University – Forensic Toxicology and Trace Laboratory (OSU-FTTL). The One Pot method developed by OSU-FTTL was adjusted from a One Pot method available through the Internet. Minor adjustments were performed in regards to increasing safety, but did not alter the overall reaction. Following One Pot syntheses, all components, including the final product, were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Methamphetamine, pseudoephedrine, ephedrine, and CMP [1-(1',4'-cyclohexadienyl)-2-methyl-aminopropane], a documented byproduct, were detected and quantified. Concentrations and percentages of target compounds present within the products and waste materials obtained provide awareness of the One Pot methamphetamine method, and insight regarding potential environmental contamination.

2.2 Review of the Literature

Methamphetamine in its useable form, typically a salt, will show little to no deviation in appearance regardless of the manufacturing method. Figure 2, below, demonstrates examples of the possible pathways.[11] As a side note, the goal of this section is not to discuss, in detail, all of the pathways illustrated, but review the discovery of unique identifiers for each.

Figure 2. Diagram of Various Routes to Produce Methamphetamine. Figure obtained from Stojanovska et al. 2013.



While visual observation of the final methamphetamine salt proves unsuccessful, laboratory analysis can reveal unique byproducts or impurities within the product that indicate the technique or precursors used to produce the drug. The importance of the ability to track the type manufacturing method used is that the information gained can be used in a variety of applications, within the law enforcement community for example. "Impurity profiling can provide information to help identify relationships between drug seizures, drug sources and trafficking routes."[12] Organizations within the United States, as well as the rest of the world, have created programs and/or research projects to

establish the ability to monitor methamphetamine byproducts. The following section will review literature pertaining to the identification of unique identifiers that link a methamphetamine drug sample to its corresponding manufacturing process. Several research projects have explored the impurities of the P2P, Red Phosphorous, and Birch Reduction methods. However, the One Pot method, which has shown continued popularity in clandestine laboratories, and potential signature byproducts, has yet to be fully investigated.

As mentioned above, there have been several methamphetamine production techniques implored throughout the 20th century. The first prominent technique will be referred to as the "Phenyl-2-Propanone" or P2P method, named after the precursor used to produce methamphetamine. The two major routes of manufacture are termed the "Reductive Amination" and "Leuckart" methods.[13] While these two specific methodologies have differing procedures, they both utilize phenyl-2-propanone as the precursor in the reaction. A 1980s report based on 190 methamphetamine laboratories seized by the DEA, established that the P2P method was implored in over 50 percent of the cases.[14] In a laboratory seizure, identification of the manufacturing method is typically not a challenge due to the presence of all the ingredients, and occasionally, a signature smell or odor. For example, a laboratory with precursor, methylamine, hydrochloric acid, formic acid, and mercury, as well as distinct cat urine odor, would be indicative of the P2P method.[15] While knowledge of laboratory specifics can provide confirmatory results of the manufacturing technique, the information is not always available. The final product of methamphetamine salt may be the only evidence seized or intercepted. However, trace components within the final product can indicate the method

of production. "As the synthesis proceeds, various impurities are accumulated: reactants, byproducts, and intermediates, as well as contaminants rising from within the reagents themselves."[16] A demand for identification of production route-specific impurities found in methamphetamine final products spurred several research projects.

As early as 1977, research was being conducted to better understand the P2P method, and identify manufacturing impurities. Kram and Kruegel utilized gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy to identify several impurities of the P2P method, the Leuckart method in particular. The identified trace components included the following compounds: methyl benzyl ketone, amphetamine, N,N-dimethylamphetamine, dibenzylketone, α,α-dimethyldiphenethylamine, N-methylformamide, formic acid, methylamine, and N-formylamphetamine.[16] While not all trace components may be present in every sample, the knowledge of these possible contaminants within the methamphetamine salt can assist future investigations and research. In 1984, a separate research group continued the research above, but solely focused on the N-methylformamide impurity. Conclusions within this study stated that N-methylformamide was readily detected via GC-MS in methamphetamine samples from multiple P2P reactions.[17] An important note resulting from this project is that the authors experienced variable amounts of impurities between product samples. While the P2P reactions were performed in a controlled setting, the differing levels of impurities can be predicted to be present within the clandestine community as well. In contrast to P2P methamphetamine produced "in house", Dyrit and Dumlao conducted a study of impurities within seized methamphetamine using GC-MS and GC with a flame ionization detector (GC-FID). The research group reported four

previously unidentified impurities, those being p-bromotoluene, N-benzyl amphetamine, N-ethyl amphetamine, and N-ethyl methamphetamine.[18] Despite the methamphetamine samples coming from an unknown origin or manufacturing technique, the research group was also able to detect trace amounts of P2P, N,N-dimethylamphetamine, and N-formylamphetamine, which has been identified or is similar to contaminants already identified as originating from a P2P reaction.

A more recent study confirmed the aforementioned work, and provided a more decisive conclusion with regards to specific identifiers of the P2P manufacturing process. According to Kunalan et al., three compounds were likely to be unique identifiers of this manufacturing technique: α,α-dimethyldiphenethylamine, N-α,α-trimethyldiphenethylamine, and 1-phenyl-2-propanol.[19] The research group utilized GC-MS to obtain and compare the known methamphetamine impurities based on two varying P2P production methods. Another review pertaining to the P2P method concluded that other unique identifiers of this production type included amphetamine, 1,3-diphenyl-2-methaminopropane, and N-cyanomethyl-N-methyl-1-phenyl-2-propylamine.[11] While any of the compounds listed above may not be commonly found substances, the mere identification for future monitoring provided desired assistance for the detection and tracking of this type of manufactured methamphetamine.

Due to the popularity and widespread use of the P2P method, the DEA designated phenyl-2-propanone as a Schedule II compound in 1980. While some manufacturers sought to avoid this restriction by creating phenyl-2-propanone themselves, the majority of individuals and/or groups switched methodologies to using ephedrine and/or pseudoephedrine as the starting material.[15] As a reading note, any use of the word

"pseudoephedrine" implies that of pseudoephedrine and/or ephedrine, thus combining the two compounds into one name for simplicity. However, both compound names will be mentioned if necessary for listings in methodology, results, interpretations, etc. Incentives to switch to using pseudoephedrine as the methamphetamine precursor obviously included elusion from law enforcement surveillance, but also, elimination of precursor production steps. Pseudoephedrine, a common nasal decongestant, is available for purchase at most pharmacy stores. In fact, the amount an individual could purchase was seemingly unlimited until the mid 2000s. The Combat Methamphetamine Epidemic Act of 2005 was enacted, which limited the amount of pseudoephedrine an individual could purchase in a specific timeframe.[20] Therefore, from about 1980 through 2005, clandestine laboratories individuals, once learning of the pseudoephedrine pathway to methamphetamine, had unregulated access to the starting material. The readily available over-the-counter medication gave rise to the increasing number of pseudoephedrine-based laboratories throughout the country, as introduced in the previous section. Shortly following the implemented monitoring and limitations of pseudoephedrine purchases in 2005, clandestine laboratory seizures did experience a drop, but an obtainment method called "smurfing" continued methamphetamine manufacturing popularity.[21] Essentially, a clandestine laboratory can hire or persuade outside individuals to purchase the maximum allowable amount of pseudoephedrine and provide it to the laboratory. Once the limited timeframe has passed, the same individual(s) can return the store to buy another set of the maximum allowable amount and the cycle continues. Regardless of the various techniques to obtain the starting material, pseudoephedrine was being used to produce methamphetamine through a variety of different routes.

While the P2P method has a couple general routes of synthesis, the pseudoephedrine-based method contains many variations. Returning to Figure 2, the generally accepted names include the Nagai, Moscow, Hypo, and Birch Reduction methods.[11] A more recent addition to this list is the One Pot method which has also been termed the "Shake and Bake" method. However, for the purposes of this review, the One Pot method will be discussed separately in a following segment. Despite the many names, the various techniques can be sectioned into groups that will be referred to in this chapter as "Red Phosphorus" and "Birch Reduction" methods. Similar to the P2P method, several research projects have been conducted to identify the impurities or byproducts within the product methamphetamine salts of each of these two manufacturing groups.

For the Red Phosphorus method, named after one of the main ingredients, red phosphorus along with hydriodic acid or iodine are used to convert pseudoephedrine into methamphetamine. Around the 1990s, this manufacturing method was the most commonly used in the United States, in part, because it produced a more potent form of methamphetamine than the P2P method.[22] Methamphetamine has two isomers, d- and l-methamphetamine. The Red Phosphorus method produces strictly d-methamphetamine, rather than a mixture of the two isomers.[22] Although the P2P method product can be further manipulated to isolate the more potent form, d-methamphetamine, the extra time and effort does not make the endeavor desirable. Despite a more potent methamphetamine produced via the Red Phosphorus method, byproducts within the final product still exist.

One researcher reported that two impurities following the Red Phosphorus method are phenyl-2-propanone and naphthalene.[22] This result is potentially a very crucial finding, in that, the presence of phenyl-2-propanone within a methamphetamine salt sample, could indicate both the utilization of a P2P or Red Phosphorus method. The author also mentioned that pseudoephedrine was detectable if the reaction did not reach completion. In 1995, Windahl continued investigation into the Red Phosphorus method, and reviewed and confirmed the presence of previously identified impurities. The list of identifiers included phenyl-2-propanone, cis and trans-1,2-dimethyl-3-phenylaziridine, 1-benzyl-3-methylnapthlaene, and 1,3-dimethyl-2-phenylnapthalene.[23] The majority of these findings were conducted on a GC-MS system, and some groups utilized NMR as well. However, the contributors of this review, were able to discover two previously unidentified byproducts. These specific impurities include N-methyl-N-(α-methylphenethyl)amino-1-phenyl-2-propanone and a cis-cinnamoyl derivative of methamphetamine.[23] As stated previously, the impurities identified may be uncommon and irregular, but in attribution to the research above, identification via GC-MS database can help identify the methamphetamine manufacturing route.

A more recent study, conducted in 2006, further investigated the impurities of the Red Phosphorus method. Lee et al. discovered previously identified byproducts, such as phenyl-2-propanone, but also found N-formylmethamphetamine and N-acetylmethamphetamine.[24] These two compounds had only been identified as impurities of the P2P manufacturing method. The interpretations from this project confirm the importance to consider the possibility of impurities that are not definitively manufacturing route-specific. In 2007, a group analyzed the Red Phosphorus method

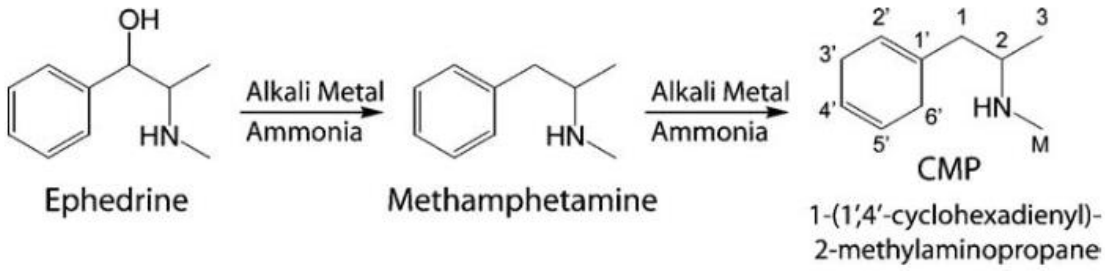
during their efforts to compare the various alkaloids of Ephedra, the plant from which pseudoephedrine and ephedrine naturally originate. The reactions pertaining to the Red Phosphorus method produced the following intermediates and byproducts according to GC-MS: cis- and trans-1,2-dimethyl-3-phenyl-aziridine, 1-phenyl-2-propanone, 1,3-dimethyl-2-phenylnaphthalene, and 1-benzyl-3-methylnaphthalene.[25] These findings confirm the presence of unique identifiers of the Red Phosphorus method to manufacture methamphetamine.

As mentioned above, one of the key ingredients to the Red Phosphorus method is iodine. However, the Comprehensive Methamphetamine Control Act in 1996 placed iodine on a list with compounds closely associated with methamphetamine production.[26] Increased surveillance and consequential reduction of iodine availability, forced clandestine laboratories to seek out another technique to produce methamphetamine. The second general manufacturing method utilizing pseudoephedrine is called the Birch Reduction method. Typically, this method utilizes the reaction of lithium and liquid ammonia, in combination with the pseudoephedrine precursor.[27] With increased use of this methodology, post-1996, another demand was created for identification of the Birch Reduction byproducts and impurities. In 1999, Bremer and Woolery produced a study focused on a Birch Reduction byproduct that had been tentatively identified. The authors, with assistance from Dal Cason[28], confirmed the identity of 1-(1',4'-cyclohexadienyl)-2-methylaminopropane, referred to as CMP, in the methamphetamine product of Birch Reduction reactions.[29] Unlike the previous manufacturing methods with many potential impurities, the focal byproduct of the Birch Reduction is CMP. According to the aforementioned study, "It is not likely that an operator would achieve a

yield of 80% or higher in a typical clandestine laboratory." [29] The authors continued to explain that a typical yield could be as low as 15-30% due to large amounts of CMP being produced when performing the Birch Reduction method. Following this study, the actual structure of CMP was only tentatively predicted in an anonymous publication.[30]

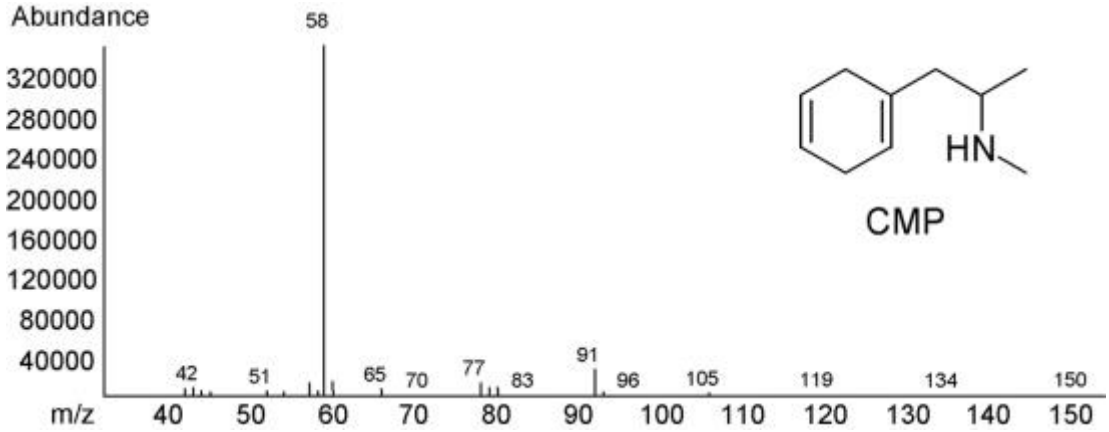
In 2005, a research group sought to confirm the structural identity of CMP. Person et al. determined the chemical structure utilizing GC-MS and NMR.[27] The structure of CMP is shown on the far right in Figure 3, below.

Figure 3. Reaction schematic of the production of CMP. Figure obtained from Person et al. 2005.



While confirming the structure previously hypothesized, this work increased the ability to detect CMP on multiple analytical platforms. In 2007, Barker and Antia, in their study introduced above, also identified CMP as a route-specific indicator of the Birch Reduction method.[25] The mass spectra of CMP from the study's findings is shown in Figure 4.

Figure 4. Mass Spectra of CMP. Figure obtained from Barker and Anita, 2007.



The ability to identify CMP based on comparison with GC-MS database profile greatly assisted the identification of methamphetamine being produced via Birch Reduction method.

Following these research projects, the unique identifiers for the Birch Reduction method had been primarily narrowed down to one compound, CMP. However, in 2012, a more comprehensive study was conducted to identify other byproducts of this methamphetamine production method. Kunalan et al. reported a finding of a previously undocumented impurity designated as "Unknown 3."[31] While the identity of this compound could not be determined, the results demonstrated the potential for a second unique identifier of the Birch Reduction method. In regards to CMP, the research group above successfully detected the known byproduct, but only when a pseudoephedrine salt was used as the starting material. If pseudoephedrine was used in freebase form, CMP was not detectable in the final product.[31] Although the findings of this specific study provided another compound to monitor when investigating a methamphetamine sample, they also provided an alert that the absence of CMP may not eliminate the Birch Reduction method as the manufacturing technique. In support of this claim, the DEA

performed a study about a method to isolate methamphetamine from any CMP impurity. Results demonstrated that treating the Birch Reduction method product salt with potassium permanganate and an aqueous base would result in sufficient separation of the methamphetamine and CMP.[32] Although the research was conducted to help create a cleaner sample for analysis, the methodologies could be implored in a clandestine setting as well to provide a more potent methamphetamine product. Consequently, this could eliminate CMP from being detected in seized methamphetamine from a Birch Reduction method.

In continuing the trend of clandestine methamphetamine laboratories switching manufacturing methodologies following legislative action, the One Pot method became increasingly common. "Newer methods of manufacturing appear as restrictions are placed on common manufacturing ingredients."[33] The previous statement has been true for the products such as phenyl-2-propanone, iodine, and pseudoephedrine. The Combat Methamphetamine Epidemic Act of 2005, spurred the switch from the Birch Reduction method, to a simpler, modified version, being the One Pot method. A limit was set in regards to the amount of pseudoephedrine that could be purchased. With many of the Birch Reduction method procedures utilizing at least 30 grams of pseudoephedrine, clandestine laboratories began performing smaller quantity reactions, thus beginning the rise in the One Pot method popularity.[34]

The One Pot method is a modified Birch Reduction reaction. Essentially, ammonia, a key ingredient in the Birch Reduction method, is generated within the reaction of a One Pot. From there, the generated ammonia reacts with lithium and, in the same fashion as the Birch Reduction method, converts pseudoephedrine into

methamphetamine. A desirable characteristic of the One Pot method is that a smaller quantity of precursor material is required. The popularity of this manufacturing methodology is proven based on the DEA statistics provided in the previous section. According to 2015 statistics, 86% of seized laboratories within the United States had less than 2 ounces of methamphetamine.[10] To reiterate, small quantities of product within a clandestine environment are indicative of a One Pot laboratory. Due to the widespread use of the One Pot method, a couple research projects have been conducted to better understand the production yield. In 2006, Heegel et al. performed One Pot reactions with several sources of pseudoephedrine. Results indicated that many forms of pseudoephedrine can successfully be converted to methamphetamine, and without pre-extraction, unlike the Birch Reduction method.[33] An important note is that the quality of methamphetamine varied among sources of starting material, which suggests clandestine samples may exhibit similar variability. CMP, one of the signature byproducts of the Birch Reduction method, was detected in some of the One Pot reaction products. However, the GC-MS results from this study were only semi-quantitative. Since the One Pot method is a mere adjustment and down-scale of the Birch Reduction method, it is unsurprising to find the same byproduct.

A more recent project, performed in 2016 in conjunction with this chapter's study, investigated the impurities found within the One Pot method via GC-MS. According to results, three major byproducts were detected following One Pot syntheses: CMP, 1,2-dimethyl-3-phenylaziridine, and an unknown compound.[35] The first two compounds listed have been previously identified in the literature for the Birch Reduction and Red Phosphorus methods, respectively. The unknown compound, found to be structurally

similar to pseudoephedrine, produced results comparable to that of impurity of Unknown 3 from the Kunalan et al. in 2012.[35] The results from this study confirm the presence CMP within One Pot reactions. On the other hand, the quantitative amounts of CMP produced in a One Pot reaction have yet to be explored.

In summary, the majority of methamphetamine manufacturing routes have been studied analytically. Impurities in the methamphetamine salts have been investigated as a means to provide identifiable information about the production method. While some impurities carry over, or are shared, amongst various manufacturing techniques, the ability to detect the unique byproducts can assist in several ways. Windhal et al. describes the avenues as such, "(i) it can reveal information on the synthetic methods used to the produce the drug, (ii) it may link samples to a common source dealer or illicit laboratory, (iii) their identification is essential so that they do not interfere with the analytical techniques used for drug analysis and (iv) the toxicity of these impurities may have potential harmful effects on methamphetamine users."[23] In continuation of the fourth comment, the impurities can also affect individuals in close proximity to clandestine production of methamphetamine. Due to the lack of literature regarding the quantities of One Pot byproducts, CMP in particular, the current study was conducted to better understand and provide an example of clandestine methamphetamine yield using this method. In addition, all waste products of the One Pot reaction were analyzed and quantitated for the target compounds. Again, the overall goal was to determine the extent of byproduct formation and provide interpretation into the amount of possible contamination of innocent bystanders and the environment.

2.3 Methodology

To create One Pot methamphetamine and associated waste materials, a laboratory-safe production method was performed at OSU-FTTL. All reactions took place within glass reaction vessels rated for 100 pounds per square inch (psi), placed into a laboratory fume hood with overpressure relief valves set to depressurize the cook at 90 psi. The ingredients procured for the OSU-FTTL proprietary One Pot method include an organic solvent, ammonium nitrate, sodium hydroxide, lithium, water, a mixture of pseudoephedrine-HCl and ephedrine-HCl, and hydrochloric acid. For the reaction solvent, ether (diethyl ether) and camp fuel (light petroleum distillate) were used in side-by-side syntheses as shown in Figure 5.

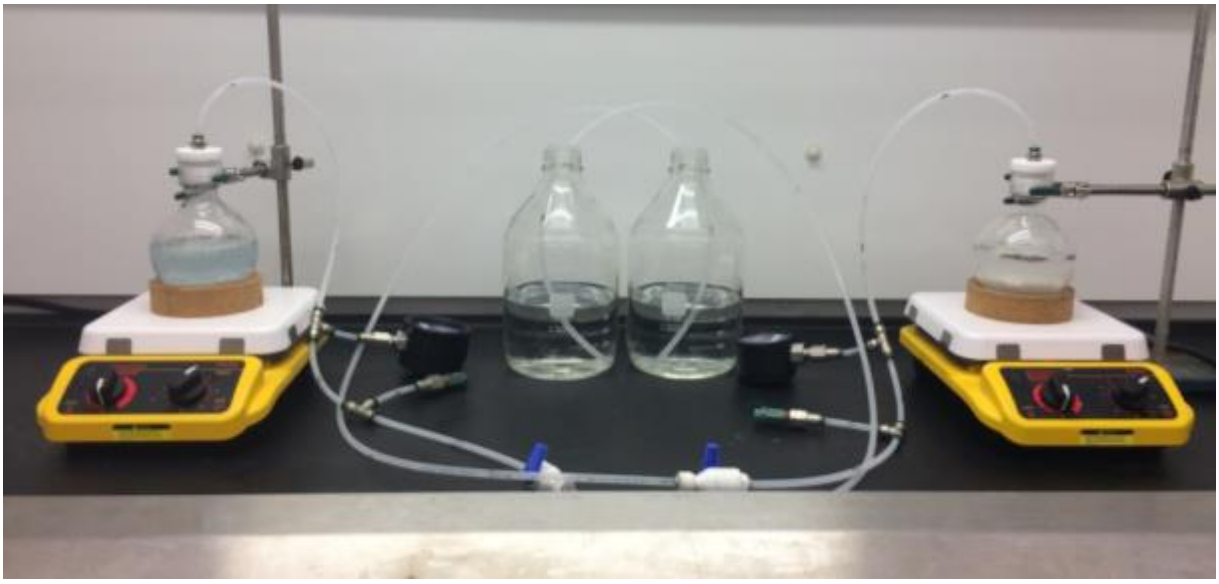


Figure 5. OSU-FTTL One Pot Reaction Setup. The side-by-side reactions had identical setups, with the exception of solvent type.

Ammonium nitrate was obtained from instant cold compress packs (GoGoods.com, Inc., Columbia, MD). The camp fuel (Light Petroleum Distillate, CAS Number 68410-97-9) was obtained from a local hardware store as Coleman® Camp Fuel (Model: 5103B253 Coleman®, Wichita KS). Ground pseudoephedrine/ephedrine tablets

were obtained from a government source. Sodium hydroxide beads, hydrochloric acid (37%), and ACS grade diethyl ether (CAS Number 60-29-7) were purchased from VWR Analytical (VWR, Sugar Land, TX). Lithium ribbon and 99+% hydrogen chloride (HCl) gas were purchased from Sigma (Sigma-Aldrich Corp, St. Louis, MO).

For each synthesis, one gram of ground pseudoephedrine-HCl/ephedrine-HCl tablets was added to 250 milliliters of solvent, along with a proprietary ratio of water, ammonium nitrate, sodium hydroxide, and lithium ribbon pieces. All ingredients were added to a 400-milliliter pressurized, glass reaction flask, thus initiating the start of the One Pot reaction. An off-gassing apparatus, consisting of valve tubing rated for 150 psi, was inserted into the lid of the reaction vessel and included a pressure gauge (SSI Technology, Inc., Janesville, WI), an emergency pressure release valve set for 90 psi, and a manual valve. Valve tubing coming from the manual valve was inserted into a receptacle filled with water. The off-gassing apparatus setup is shown in Figure 6, below. Immediately following the start of the reaction, the manual valve was left open to the fume hood atmosphere. After 30 seconds, the manual valve was closed, sealing the system for two hours.

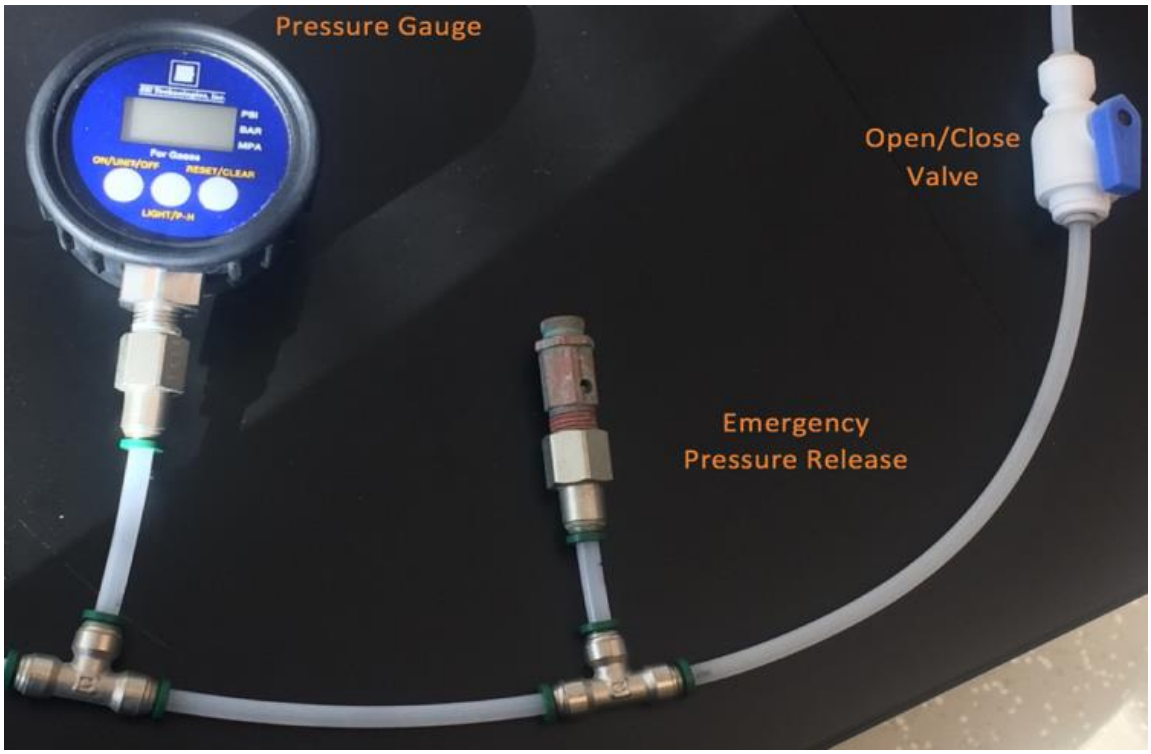


Figure 6. Off-gassing apparatus used during the One Pot methamphetamine syntheses. From left to right, the setup consisted of a digital pressure gauge, an emergency pressure release valve, and a manual valve.

Post-reaction, the head space overpressure was slowly released into a receptacle filled with water via slight opening of the manual valve closed during the reaction. Once the reaction vessel headspace was fully opened to atmosphere and depressurized, the off-gassing apparatus was removed, the round-bottom flask lid was unscrewed and removed. Lithium was removed from the reaction vessel via forceps and placed into a safe disposal or sampling container. The vessel lid was loosely placed on top of the reaction vessel to allow overnight ventilation. Following the allotted time period, the solvent within the reaction vessel was filtered using coffee filters (Farmer Bros Co., Ft. Worth, TX) that had been pre-moistened with un-reacted solvent of the respective synthesis. After filtration, the coffee filter was dunked into water to react any trace lithium present. The solvent filtered, deemed "pre-salt" solvent, was poured into a separate, clean bottle. The

remaining solid waste, or *sludge*, at the bottom of the reaction vessel was dissolved in 250 mL of water, poured into a separate, clean bottle, and stored for subsequent analysis. After collection of the sludge, the pre-salt solvent was purged with hydrogen chloride gas from a lecture bottle to convert methamphetamine, any remaining precursor, as well as formed byproducts, into hydrochloride salts. An example of a product salt can be seen in Figure 7. Once precipitation visually ceased, the product salt was recovered with another coffee filter, dried and weighed. The remaining solvent was poured into a separate, clean bottle and labeled "post-salt solvent."

Figure 7. Product Salt. An example of a product salt sample from a One Pot synthesis.

Six identical reactions were performed, differing only in selection of solvent: three syntheses used laboratory-grade diethyl ether and three used camp fuel. On each

synthesis day, an ether reaction was performed alongside a camp fuel reaction to help reduce variability between the two cooks. Diethyl ether syntheses are designated E1, E2 and E3, and camp fuel syntheses are designated C1, C2 and C3. All chemicals were weighed prior to reaction to a measured precision of less than 0.1 g difference per weighed ingredient between the two reaction types. Following the 2-hour reaction explained above, the lithium pieces from the first synthesis of each solvent type, E1 and C1, were removed from the reaction vessel, dissolved in 100 mL of deionized water, and saved for potential future analysis. For the remaining reactions, E2-E3 and C2-C3, the lithium was removed and quickly immersed into mineral oil for safe disposal. Sludge, post-salt solvent, and product salt samples were collected for analysis in the manner described in the previous subsection. Following a dilution of every sample to eliminate instrument saturation, all characterizations samples were placed into injection vials for LC-MS/MS analysis.

Shimadzu UFLC pumps paired with an Applied Biosystems 4000 Q Trap MS/MS was used for the LC-MS/MS analysis, shown in Figure 8.

Figure 8. LC-MS/MS Instrumentation Setup at OSU-FTTL. Shimadzu UFLC pumps on the left, and Applied Biosystems 4000 Q Trap MS/MS on the right.

For liquid chromatography, separation was achieved with a Restek Raptor Biphenyl 2.7 µm column (50 x 2.1 mm) with a Restek Raptor Biphenyl 2.7µm guard cartridge (5 x 3.0 mm) (Restek Corporation, Bellefonte, PA). Mobile Phase A consisted of 2mM ammonium formate and 0.1% formic acid in LC-MS grade water, while Mobile Phase B consisted of 2mM ammonium formate and 0.1% formic acid in LC-MS grade methanol. Ammonium formate was purchased from Alfa Aesar (Alfa Aesar, Ward Hill, MA). Formic Acid was purchased from EDM (EDM Millipore Corp, Billerica, MA). Methanol was purchased from JT Baker (Avantor Performance Materials Inc., Center Valley, PA). Nanopure water was obtained using a Barnstead Nanopure Diamond laboratory water system (Thermo Scientific, Waltham, MA). The LC method had a total flow rate of 0.700 ml/min. Mobile Phase B concentration was held at 7.2% for 3.5 minutes, increased

to 35% for 1 minute, lowered to 7.2% for 0.25 minutes, increased to 100% for 0.5 minutes, and then lowered to 7.2% for 1.75 minutes, for a total run time of 7 minutes. All changes in mobile phase B concentrations were set to immediately occur and end with no ramp. Injections were set at 20 µL and the oven temperature was set to 30 °C.

For mass spectrometry, Table 1, below, shows the ion transitions and LC-MS/MS instrument parameters for the compounds of interest. Amphetamine, Amphetamine-d_6, Methamphetamine, Methamphetamine-d_5, 1S,2S(+)-Pseudoephedrine, and 1S,2R(+)-Ephedrine-HCl standards were all purchased at a concentration of 1 mg/mL from Cerilliant (Cerilliant Corp, Round Rock, TX). Pseudoephedrine-d_3 HCl and 1S,2R(+)-Ephedrine-d_3 HCl standards were also bought from Cerilliant at a concentration of 100 µg/mL. One gram of CMP-HCl standard was purchased from Cayman (Cayman Chemical, Ann Arbor, MI). For simplicity, the naming of all analytes will be as follows: methamphetamine, pseudoephedrine, ephedrine, amphetamine, and CMP. Additionally, there are four deuterated internal standards, methamphetamine-d_5, pseudoephedrine-d_3, ephedrine-d_3, and amphetamine-d_6.

Table 1. Mass Spectrometry Parameters. Target analytes Methamphetamine, Pseudoephedrine, Ephedrine, Amphetamine, and CMP were identified using two mass ion fragments each. Internal standards include Methamphetamine-d5, Pseudoephedrine-d3, Ephedrine-d3, and Amphetamine-d6.

Compound	Q1 Mass (Da)	Q3 Mass (Da)	DP (volts)	CE (volts)	CXP (volts)
Methamphetamine	150.100	91.000	56.000	25.000	14.000
	150.100	119.000	56.000	15.000	4.000
Methamphetamine-d_5	155.000	91.100	60.000	20.000	4.000

Pseudoephedrine	166.180	148.024	41.000	15.000	6.000
	166.180	90.961	41.000	43.000	12.000
Pseudoephedrine-d_3	169.200	151.040	26.000	21.000	26.000
Ephedrine	166.108	117.085	41.000	27.000	18.000
	166.108	114.796	41.000	35.000	18.000
Ephedrine-d_3	168.980	116.999	31.000	29.000	6.000
Amphetamine	136.200	119.000	36.000	13.000	18.000
	136.200	91.000	36.000	25.000	14.000
Amphetamine-d_6	142.100	125.100	41.000	13.000	6.000
CMP	152.163	79.114	41.000	27.000	12.000
	152.163	77.071	41.000	45.000	0.000

Trueness of the compound identity was confirmed through comparing the areas of the two MRM transitions, resulting in an identification or ID ratio, also known as an MRM ratio. Every Q1 Mass and Q3 Mass pairing generated a chromatographic peak. MRM ratios for each compound, with the exception of internal standards, were calculated by dividing the peak area of the second pairing by the peak area of the first pairing. To build an acceptable ID ratio range, the ratios observed for every calibrator were averaged. For results to be accepted, the ID ratio must be within 30% of the ID ratio average using two significant figures for the percentage value.

To generate a quantitative value for each compound, a calibration curve containing all target compounds was utilized. The linear range for all non-internal standard compounds in the LC-MS/MS method mentioned above contained the following calibrator points: 100, 50, 25, 5, 1, and 0.5 ng/mL of each drug compound. To calculate the concentration of all calibrators, quality controls, and research samples, MultiQuant software (SCIEX, Foster City, CA) was utilized, which is specifically designed for LC-MS/MS result analysis. All other values and statistical comparisons were obtained by utilizing Microsoft Excel (Microsoft Corporation, Redmond, WA).

2.4 Findings

LC-MS/MS analysis was performed using Shimadzu UFLC pumps paired with an Applied Biosystems 4000 Q Trap MS/MS. As mentioned in the Methodology section above, the sample types collected from the characterization syntheses included product salt, sludge, and post-salt solvent samples.

For product salt samples, methamphetamine, CMP, pseudoephedrine, and ephedrine were all detected. The LC-MS/MS quantification results are shown in Table 2. All values are listed in ng/mL and an asterisk designation refers to a result that met identification criteria, but did not quantitate above the limit of quantitation (LOQ).

Table 2. LC-MS/MS quantification results for the product salts. All concentrations given in ng/mL. Concentration below the LOQ of 0.5 ng/mL are designated with an asterisk.

Compound	E1	E2	E3	C1	C2	C3
Methamphetamine	21.8	56.3	35.9	23.1	35.6	25.8
CMP	1.62	2.19	2.28	0.22*	0.24*	0.16*
Pseudoephedrine	8.60	8.39	12.0	14.7	10.4	14.2
Ephedrine	36.9	44.2	52.1	59.8	33.4	49.7

The percentage of methamphetamine, CMP, pseudoephedrine, and ephedrine found in the product salts were calculated to compare the amounts of product and impurities present in each product salt; these percentages are summarized in Table 3.

Table 3. Percent of methamphetamine, CMP, pseudoephedrine, and ephedrine found in each product salt with LC-MS/MS. All values listed are percentages based on 100% for complete drug composition within the sludge sample.

Compound	E1	E2	E3	C1	C2	C3
Methamphetamine	31.6	50.7	35.1	23.6	44.7	28.7
CMP	2.35	1.97	2.23	0.23	0.30	0.18
Pseudoephedrine	12.5	7.56	11.7	15.0	13.1	15.8
Ephedrine	53.6	39.8	50.9	61.2	41.9	55.3

Statistical analysis of a two-population t-test, comparing average compound concentrations in product salt samples of ether and camp fuel One Pot syntheses, produced the corresponding results listed in Table 4. Based on the p-values, the mean concentrations of all drug compounds between ether and camp fuel syntheses are not statistically different. CMP was not statistically compared because the average value of camp fuel samples was below LOQ.

Table 4. Statistical Comparison of Ether and Camp Fuel Product Salt Samples. A two-population t-test was performed to compare the mean average concentration of ether and camp fuel for methamphetamine, psuedoephedrine, and ephedrine. A generated p-value less than 0.05 translates to a statistical difference between the two solvent types. A p-value less than 0.01 or less than 0.001, indicate a high statistical difference or extreme statistical difference, respectively.

Product Salts (ng/mL)	Ether	Camp Fuel	p-value
Methamphetamine	37.96	28.16	0.412
Pseudoephedrine	9.66	13.08	0.126
Ephedrine	44.39	47.65	0.732

p –values used to calculate the mean met acceptance criteria, but the concentrations quantitated below the LOQ set at 0.5 ng/mL.
*p-values<0.05 signifying a statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.
**p-values<0.01 signifying a high statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.
***p-values<0.001 signifying an extreme statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.

For sludge samples, the quantification results are displayed in Table 5. All values listed are in mg/mL. Methamphetamine, pseudoephedrine, and ephedrine were observed in all samples and quantitated. CMP, however, was only detected in the sludge from the diethyl ether One Pot syntheses.

Table 5. LC-MS/MS quantification results for sludge samples. All concentrations given in mg/mL.

Compound	E1	E2	E3	C1	C2	C3
Methamphetamine	4.46	8.78	2.66	0.31	1.14	0.78
CMP	0.31	0.35	0.19	-	-	-
Pseudoephedrine	0.94	0.57	0.82	0.39	0.45	0.61
Ephedrine	4.30	2.46	3.05	1.86	2.15	3.24

The percentages of methamphetamine, CMP, pseudoephedrine, and ephedrine found in the sludge were calculated to compare the amounts of product and precursor left behind from the methamphetamine syntheses. These percentages are summarized in Table 6. Percentages from the LC-MS/MS data were calculated by using the determined concentrations of each chemical.

Table 6. Percent of methamphetamine, CMP, pseudoephedrine, and ephedrine found in each sludge sample with LC-MS/MS. All values listed are percentages based on 100% for complete drug composition within the sludge sample.

Compound	E1	E2	E3	C1	C2	C3
Methamphetamine	44.6	72.3	39.6	12.0	30.5	16.9
CMP	3.07	2.89	2.8	0.00	0.00	0.00
Pseudoephedrine	9.35	4.66	12.2	15.3	12.2	13.1

Ephedrine	43.0	20.2	45.4	72.7	57.3	70.0

Statistical analysis of a two-population t-test, comparing average compound concentrations in sludge samples for ether and camp fuel One Pot syntheses, produced the corresponding results listed in Table 7. Based on the p-values, the mean concentrations of all drug compounds between ether and camp fuel syntheses are not statistically different. CMP was not statistically compared because this compound was not detected in the camp fuel samples.

Table 7. Statistical Comparison of Ether and Camp Fuel Sludge Samples. A two-population t-test was performed to compare the mean average concentration of ether and camp fuel for methamphetamine, psuedoephedrine, and ephedrine. A generated p-value less than 0.05 translates to a statistical difference between the two solvent types. A p-value less than 0.01 or less than 0.001, indicate a high statistical difference or extreme statistical difference, respectively.

Sludge (mg/mL)	Ether	Camp Fuel	p-value
Methamphetamine	5.30	0.74	0.068
Pseudoephedrine	0.77	0.48	0.084
Ephedrine	3.27	2.41	0.283

*p-values<0.05 signifying a statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.
**p-values<0.01 signifying a high statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.
***p-values<0.001 signifying an extreme statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.

For post-salt solvent samples, the quantification results are displayed in Table 8. All values listed are in ng/mL. Methamphetamine, pseudoephedrine, and ephedrine were found and quantitated. CMP, however, was only detected in the post-salt solvent from the diethyl ether One Pot syntheses.

Table 8. LC-MS/MS quantification results for post-salt solvents. All concentrations given in ng/mL.

Compound	E1	E2	E3	C1	C2	C3

Methamphetamine	60,600	50,600	29,700	630	20.0	808
CMP	5,800	4,030	3,420	-	-	-
Pseudoephedrine	7,370	8,630	2,900	185	6.00	424
Ephedrine	5,530	5,070	4,090	327	5.00	272

The percentage of methamphetamine, CMP, pseudoephedrine, and ephedrine found in the post-salt solvents were calculated to compare the amounts of product and impurities present in each post-salt solvent; these percentages are summarized in Table 9.

Table 9. Percent of methamphetamine, CMP, pseudoephedrine, and ephedrine found in each post-salt solvent with LC-MS/MS.

Compound	**E1**	**E2**	**E3**	**C1**	**C2**	**C3**
Methamphetamine	76.4	74.1	74.0	55.2	64.5	53.7
CMP	7.31	5.9	8.54	0.00	0.00	0.00
Pseudoephedrine	9.30	12.6	7.23	16.2	19.8	28.2
Ephedrine	6.98	7.41	10.2	28.7	15.7	18.1

Statistical analysis of a two-population t-test, comparing average compound concentrations in post-salt solvent samples for ether and camp fuel One Pot syntheses, produced the corresponding results listed in Table 10. Based on the p-values, the mean concentrations of all drug compounds between ether and camp fuel syntheses are statistically different. CMP was not statistically compared because this compound was not detected in the camp fuel samples.

Table 10. Statistical Comparison of Ether and Camp Fuel Post-Salt Solvent Samples. A two-population t-test was performed to compare the mean average concentration of ether and camp fuel for methamphetamine, psuedoephedrine, and ephedrine. A generated p-value less than 0.05 translates to a statistical difference between the two solvent types. A p-value less than 0.01 or less than 0.001, indicate a high statistical difference or extreme statistical difference, respectively

Post-Salt Solvent (ng/mL)	**Ether**	**Camp Fuel**	**p-value**

Methamphetamine	46957	486	0.007**
Pseudoephedrine	6297	205	0.025*
Ephedrine	4896	201	0.000***

*p-values<0.05 signifying a statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.
**p-values<0.01 signifying a high statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.
***p-values<0.001 signifying an extreme statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.

2.5 Discussion

Following One Pot reactions, LC-MS/MS quantitative results were obtained in the product salt, sludge, and post-salt solvent samples. Compounds that were detected included methamphetamine, pseudoephedrine, ephedrine, and CMP. Based on the results, the analytical instrument of choice was successful in characterizing each aspect of a One Pot synthesis. In addition to obtaining values of yield within the methamphetamine final product, concentrations within One Pot method waste were collected to understand contamination potentials for individuals in the immediate area. Also, contamination potential exists for the environment, assuming negligent disposal of clandestine laboratory waste. And in summary, values were obtained for two commonly used One Pot method solvents, thus providing comparisons, as well as a more in-depth and characteristic interpretation of clandestine methamphetamine laboratories.

For the product salt samples, no statistical difference in yield was present. The minimal detection of the byproduct CMP, and absence within clandestine methamphetamine samples, could indicate the solvent used within the One Pot reaction. According to the literature, “The protons required for the reduction of the hydroxyl group

and the partial reduction of the aromatic ring arise because of damp or impure solvents or even water absorbed into the reaction from the atmosphere."[25] Therefore, the amount of water present within the One Pot reaction can impact the levels of CMP produced. However, careful precautions within the methodology of this study were taken to ensure equal treatment of both solvent types. In discussions about the differences in the solvent types, diethyl ether versus camp fuel, a former DEA chemist explained the fact that any water added, present, or produced within the reaction is more easily soluble in diethyl ether.[36] As a result, any methamphetamine produced is more likely to continuing reducing into CMP when suspended in diethyl ether versus camp fuel. An equal amount of water was added to the camp fuel syntheses, which explains CMP being still produced. The byproduct concentrations are much lower in the camp fuel syntheses because the methamphetamine did not come into contact with water as readily due to camp fuel's immiscibility with water. Another contributing factor may be the duration of the One Pot reaction. All controlled syntheses were performed over a period of two hours. With knowledge that camp fuel does not allow CMP to be produced as easily, future tests of extending the reaction time may increase the formation of CMP.

The trend of lower CMP detections in the other sample types, sludge and post-salt solvent, continued with the camp fuel reactions. In fact, CMP was not detected in camp fuel waste samples. For sludge comparisons of solvent type, there were no statistically significant differences for the other compounds present. All p-values generated were above a 0.5 value. However, all sludge samples were much more concentrated with target compounds, units of mg/mL, when comparing them to the other sample types, units of ng/mL. This phenomenon cannot be fully explained and will require additional

research, but one hypothesis is that the de-pressurized One Pot allotted waiting time before the salting out process allowed the drug compounds to settle towards the bottom of the reaction vessel. Since the solvent was decanted into a separate container the following day, a higher concentration of compounds may be present in residual solvent present in the sludge samples.

For post-salt solvent comparisons of solvent type, every compound targeted was statistically different, with two compounds categorized as highly different and another categorized as extremely different. In order of statistical difference, pseudoephedrine, methamphetamine, and ephedrine had p-values of 0.025, 0.003, and 0.000, respectively. Despite the statistical differences between the diethyl ether and camp fuel post-salt solvents, the percentages of drug compounds present, at an observational level, seem to be relatively consistent. This similarity may imply that while the conversion of pseudoephedrine and ephedrine to methamphetamine was comparable, differences within the post-salt solvent samples can be attributed to another aspect of the One Pot method process. Two potential sources of the deviation are the salting out process and filtration of product from solvent. Further studies will need to be conducted to confirm and better understand the variability.

Overall, the One Pot methamphetamine method, in two variations based on solvent type, was characterized using LC-MS/MS. The syntheses performed at OSU-FTTL were representative of a clandestine laboratory procedure, adjusted slightly for safety precautions within a research facility. An important note is that clandestine laboratory reactions are likely to be highly variable in regards to the amount of ingredients used and reaction duration. Until more research is gathered, any

interpretations of illicit methamphetamine samples should focus more on the presence of specific compounds, instead of compound quantities. Based on this study's results, a signature byproduct of the One Pot reaction, CMP, as established in the literature, was successfully quantitated in diethyl ether syntheses, for both product and waste samples. CMP was also detected in trace amounts within camp fuel syntheses product samples. With the likelihood of high procedural and production variability within clandestine laboratories, the absence of CMP within this study's camp fuel waste samples cannot exclude CMP from being present in clandestine laboratories that utilize camp fuel. The study's findings contribute knowledge and awareness to both the law enforcement and scientific communities towards the goal of improving detection and tracking capabilities of One Pot clandestine laboratories. In addition, the target compounds detected in One Pot waste materials indicates a real possibility of contamination within the surrounding area and in the environment. However, the ability to detect product methamphetamine, precursor pseudoephedrine, and byproduct CMP (diethyl ether samples only) in manufacturing waste, provides an opportunity to track the location of clandestine One Pot laboratories within the community via waste disposal routes.

CHAPTER III

ONE POT WASTE DEPOSIT SIMULATION – SEWAGE EFFLUENT ANALYSIS

3.1 Introduction

Methamphetamine has manufactured into a continual problem over the past several decades. Despite the dangers and health risks commonly associated with the stimulant's use, individuals can easily produce methamphetamine in an area of their choosing, thus creating clandestine, or secret, laboratories. From 2004 to 2011, the Drug Enforcement Administration (DEA) reported an estimate of over 85,000 methamphetamine laboratory incidents.[37] Among these statistics, Tulsa County in Oklahoma proved to be the national leader of incidents over the 8-year time period. However, other areas of the United States of America have experienced similar methamphetamine popularity, even in more recent years. According to the World Drug Report, over 30,000 clandestine methamphetamine laboratories have been detected across the nation from 2011-2014.[38]

Examination of clandestine methamphetamine laboratories over the course of time has shown multiple techniques for producing the drug of interest. While new production methods may be a result of increased yield or simplicity, the major reason to develop new techniques has been after legislative restriction on the manufacturing ingredients.[33] Recently, the One Pot methamphetamine method has become the most

common manufacturing process. Along with a short and simple procedure, the One Pot method is popular due to the use of obtainable household ingredients. Pseudoephedrine, the starting material in the One Pot method, is the only item that may provide an acquisition challenge. As mentioned earlier, legislation has attempted to restrict access to methamphetamine production ingredients. Many states, in an attempt to reduce the number of clandestine methamphetamine laboratories, have limited the amount of pseudoephedrine that an individual can purchase.[39] Since pseudoephedrine is still available in limited quantities, in part for its medicinal decongestive properties, individuals are still utilizing the One Pot methamphetamine manufacturing method. In fact, most of the seized domestic laboratories in 2014 were determined to be One Pot or "Shake and Bake" methamphetamine labs.[40]

Continual popularity of methamphetamine laboratories does not reduce associated dangers. The One Pot method, like an older methamphetamine manufacturing procedure called the Birch Reduction method, explained in Chapter 2, uses lithium as an electron source to reduce the hydroxyl group of pseudoephedrine, thus creating methamphetamine.[27] Lithium and other ingredients involved in the One Pot method, like a flammable organic solvent, causes the methamphetamine production to become severely dangerous. Environmental exposure and the possibility of a fire are two risks introduced to the nearby community. Even with successful methamphetamine cooks, toxic waste disposal can have detrimental effects to the surrounding area and residents. According to some reports, approximately five to seven pounds of waste accompanies every pound of methamphetamine produced.[41] Common disposal routes include trash service, negligent dumping into outdoor areas, and introduction to the waste water

system. While waste disposal has case-by-case variability, several reports and case studies prove that methamphetamine laboratory waste has been deposited in sink drains, flushed down toilets, etc. One police detective wrote that it is not uncommon for methamphetamine laboratory individuals to pour toxic liquids down the drain in order to avoid detection.[42] Before this study, the possibility of using waste water to locate methamphetamine laboratories had yet to be determined.

Trace detection of drugs in waste water, as further discussed in the following section, has been primarily used to monitor pharmaceutical and illicit drug use in a community. Parent, or unaltered, drug compounds and metabolites have successfully been detected in small amounts utilizing various sample extractions and analytical instrumentations. While waste water analysis has contributed to understanding population drug trends and/or waste water treatment improvements, the possibility of detecting drug compounds to identify or assist in identifying clandestine laboratories has yet to be investigated. The focal points of this research are to first, identify the potential hazards and health impacts of exposure to One Pot methamphetamine and manufacturing byproducts. A second goal is to determine the possibility of monitoring One Pot methamphetamine laboratory products and waste materials after introduction to the waste water system. And the last goal is to confirm or deny the usefulness of CMP as a unique identifier of methamphetamine manufacturing.

Simulated One Pot methamphetamine laboratory products were developed and produced at the Oklahoma State University – Forensic Toxicology and Trace Laboratory (OSU-FTTL). Methamphetamine product salts were produced, filtered, and removed. All remaining reaction waste, both solids and liquids, were deposited into a local waste

water system. Time course waste water samples were collected downstream using an autosampler provided by the Savannah River National Laboratory (SRNL). Sample analysis included solid phase extraction (SPE) followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantitate target compounds within sewage effluent. Results proved the ability to detect methamphetamine, pseudoephedrine, and over-reduced methamphetamine, CMP. Analyses provided additional information, such as concentration levels and window of detection within sewage effluent. This research demonstrated the possibility and potential of monitoring clandestine One Pot methamphetamine laboratories in the community through the use waste water analysis. In combination with continued research, the following study provides a method for law enforcement to assist in proactively detecting and seizing clandestine drug laboratories in the community.

3.2 Review of the Literature

The One Pot method has become the primary method of choice for quick methamphetamine production. Similar to all drug production methods that came before, laboratory waste can be a huge concern. With the continual number of clandestine methamphetamine laboratories in communities, the possibility of environmental exposure to harmful and toxic chemicals has drawn public attention. This awareness has developed a need to locate and consequently, eliminate One Pot methamphetamine laboratories within states, cities, and neighborhoods. Identification of clandestine drug operations has been limited to first-hand reports, gathered intel, or laboratory incidents that reveal the presence of methamphetamine production. For example, a study conducted in 2013 determined that trace amounts of methamphetamine and

pseudoephedrine could be detected following a methamphetamine laboratory fire.[43] While these results provided a useful tool for investigators, any and all law enforcement action would take place post-discovery of a clandestine drug laboratory.

A need has been identified to assist in location of One Pot methamphetamine laboratories before potential environmental exposure. Proactively seeking clandestine laboratories, especially those utilizing the One Pot method, has produced limited success. Although the Drug Enforcement Administration reported over 9,000 methamphetamine laboratory discoveries in 2014, many clandestine operations may continue to be kept in secrecy.[40] The high availability of the One Pot method ingredients reduces the effectiveness of product monitoring. Therefore, other means of clandestine laboratory identification must be researched.

Of all the possible routes of disposing clandestine laboratory waste, the sewage system provides the most optimistic pathway for the law enforcement and scientific communities to achieve remote detection. As noted in the introductory section, individuals have been known to pour methamphetamine and manufacturing waste down household water drains to avoid detection. Figure 9, below, is an image taken by a police officer in Gladwin City, Michigan. According to the news report, the police discovered a One Pot methamphetamine laboratory in a household kitchen.[44]

Figure 9. Police image of One Pot laboratory within kitchen sink. Figured obtained from Dan Firnbach, 2015.

Of significant importance is the use of the sink to perform the methamphetamine synthesis. Whether the individual was pouring any unwanted materials down the drain, or just utilizing the sink as a receptacle for any chemical spills, One Pot methamphetamine waste is likely to reach the sewage system. Another case, which provides more concrete proof of clandestine laboratory waste being introduced to the sewage system comes from a former Chief of Police in Stillwater, Oklahoma. At one particular scene, the sink drain pipes were severely corroded due to methamphetamine waste exposure, to a point where the sink drain could not be used. Therefore, the individual in this circumstance configured a piece of tubing from the sink to the drain of the dishwasher so that methamphetamine waste could be washed away.[45] The configuration at this clandestine laboratory is shown in Figure 10, specifically the right side of the image where a single piece of white tubing can be observed.

Figure 10. Image taken at clandestine methamphetamine laboratory. On the right-hand side, a piece of white tubing was installed to run from the sink to the dishwasher drain. Image was obtained by Ronald Thrasher, 2016.

While case-by-case variability exists between clandestine methamphetamine laboratories, the examples provided confirm the sewage system to be a utilized method of waste disposal. And consequently, the research at hand, has sought to determine if the detection of One Pot methamphetamine waste within sewage samples is possible.

Historically, analysis of community waste water has been conducted to identify population drug trends, monitor the effectiveness of waste water treatment facilities, etc. The primary reason pharmaceuticals and illicit compounds can be detected within waste water is the simple concept that individuals use drug compounds, and then excrete said compounds through urine and feces. Therefore, a direct correlation can be made based on drug levels detected within waste water and human consumption. Primarily, the

methods utilized to perform this type of research include SPE and gas chromatography-mass spectrometry (GC-MS) or LC-MS/MS, although others techniques exist. SPE is a sample preparation technique that uses a solid packing material within a column to separate different chemical compounds within a sample.[46] In addition to the ability to separate and/or isolate target drugs, SPE typically concentrates the compounds. A larger volume of sample is added to a SPE cartridge than is eluted as a final prepared sample for analysis. Essentially, and ideally, all of the target drug compounds within a sample will be captured by the solid packing material within the SPE cartridge. As a result, the more sample added to the cartridge, the higher the concentration of drug compounds within the sample analyzed. For this reason, and the purpose of purifying dirty waste water, SPE has been the primary sample preparation technique within this area of research.

As explained above, sewage waste, commonly referred to as effluent, has been used in many instances to detect trace amounts of pharmaceuticals and illicit compounds. Among these studies include methamphetamine. When performing waste water studies, additional information, such as the drug's interaction with the body and excretion from the body, can be crucial to instrumental detection and result interpretation. Many sewage effluent drug studies include methamphetamine, but in addition to monitoring the parent compound, amphetamine levels are examined as well. "Between 30-54% of an oral dose is excreted in urine as unchanged methamphetamine and 10-23% as unchanged amphetamine. Following an intravenous dose, 45% is excreted as unchanged parent drug and 7% amphetamine."[47] With this, drug metabolism as well as route of administration are important factors in result interpretation. However, methamphetamine to amphetamine ratios in sewage water will not likely follow expected or theoretical values

due to other potential sources of these compounds. Amphetamine is available as a prescription drug. Increased use of this prescription drug in a given area or population would increase amphetamine concentrations in sewage effluent, thus creating a challenge for methamphetamine to amphetamine ratio determinations. A second possible contributor to increased concentrations include compounds that metabolize to amphetamine or methamphetamine. According to one report, benzphetamine, selegeline, and famprofazone are among this group.[47] While the aforementioned substances may not be commonly used, the knowledge of their potential impact is important to consider when reviewing waste water results.

Another important source of methamphetamine is those using the stimulant as prescribed. Desoxyn is the only brand name methamphetamine available via prescription. With LC-MS/MS analysis, Desoxyn use cannot be distinguished from illicit use. An abundance of legal Desoxyn users within a given area, may skew illicit consumption interpretations. However, a method to predict the amount of prescription methamphetamine use has been implored in the literature. A study conducted in 2011, for example, focused on the interpretation of methamphetamine concentrations in sewage effluent.[48] This group utilized DEA drug records to estimate the amount of prescription methamphetamine used within a population. Since Desoxyn is not a commonly prescribed drug and sewage effluent analysis is highly variable to begin with, conclusions from this study indicated that calculated Desoxyn intake and excretion values would, for the most part, confidently estimate the amount of prescription methamphetamine present within the total methamphetamine concentration found in waste water. The aforementioned study estimated that in a particular region of the State of Washington,

only 3-8% of the methamphetamine found in waste water could be attributed to Desoxyn.[48] Although this value is theoretical and dependent on prescribed individuals ingesting and excreting Desoxyn within the given area, it provides a relatively close estimate of the maximum prescription methamphetamine that could be introduced to sewage effluent.

The last potential contributor to methamphetamine within waste water, which is the focal point of this chapter, are clandestine production laboratories. As explained above, the One Pot methamphetamine production method is the primary technique of choice among clandestine laboratories. Additionally, each manufacture of methamphetamine has associated waste. Based on the results from Chapter 2, trace amounts of product and byproducts are contained within the waste materials. If individuals are disposing One Pot reaction waste down a sink drain, toilet, bath tub, etc., methamphetamine and related manufacturing compounds would be introduced to sewage effluent.

Despite the many factors and sources of methamphetamine into the sewage water system, the majority of studies attempting to monitor the use of methamphetamine typically report the total concentrations found. For the purposes of this review, only studies involving methamphetamine will be discussed. In 1999, Daughton, one of the pioneers of waste water research, and a co-author Ternes, published a review that essentially established the demand to detect pharmaceuticals in the environment.[49] Following this call for research, several groups, across the United States and internationally, began creating waste water projects. Recent work begins in 2008, where a group developed a special technique to combine SPE and LC-MS/MS into one

workflow system. SPE was performed in such a way that the final step of eluting the target compounds with a sample, traveled directly into the LC-MS/MS. Based on the results, Postigo et al. were able to reach a limit of detection (LOD) within waste water for amphetamine, methamphetamine, and ephedrine at 0.34, 0.28, and 0.78 ng/L, respectively. And the limit of quantitation (LOQ) within waste water for the same compounds were 0.92, 0.75, and 2.21 ng/L, respectively.[50] An important note in this project's methodology is the use of only 5 mL per sample, which is likely attributed to the combined SPE and LC-MS/MS system. The authors referenced that previous research typically used between 100 and 500 mL of sample.[50] Another impactful finding from this project was the fact that the analysis of fortified chemistry-grade water produced significantly better results than analysis of waste water samples. In conclusion, this result demonstrates that the waste water itself, is suppressing the ability to detect certain drug compounds. In 2014, research was done to further investigate the identified suppression. According to Ostman et al., a 45-78% signal repression of drugs exists within waste water analysis when compared to chemistry-grade water.[51] Further research, such as altering methodology, will need to be completed to avoid or reduce the suppression.

While the research projects above demonstrated signal suppression and potential drug LOD and LOQ values in waste water utilizing LC-MS/MS, another research group utilized GC-MS/MS to perform a similar study. Gonzalez-Marino et al. compared and determined the LOD of various drug compounds for different water sample types: river water, treated waste water, and raw waste water. The LOD values for amphetamine were determined to be 0.8, 3, and 7 ng/L, respectively, while the LOD values for

methamphetamine were 2, 7, and 7 ng/L, respectively.[52] The detection cutoffs are not as low as seen with LC-MS/MS, but this research demonstrates the ability to use different instrumentation to achieve analytical detection. An additional benefit, as noted by the authors, is that GC-MS/MS allows for a full spectra scan, which may be helpful in research designed to detect as many drug compounds as possible. For a more direct comparison of GC-MS and LC-MS/MS, Mwenesongle et al. performed a study in 2013 comparing the two instruments in regards to the ability to monitor drugs of abuse and their metabolites using SPE followed by GC-MS. The results showed that GC-MS, in this instance, produced better results than the LC-MS/MS. The LOQ for methamphetamine was 0.33 picograms using GC-MS, and 208 picograms with LC-MS/MS. Although this finding contradicts previous literature, it confirms the ability of both instruments to detect trace amounts of methamphetamine within waste water.

In addition to research performed to determine the capabilities of analytical instrumentation to quantify drugs of interest within waste water, other projects were being conducted to determine drug consumption trends. In 2009, Banta-Green et al. utilized waste water analysis to survey drug trends, particularly cocaine, methamphetamine, and ecstasy, within a chosen population. Study design and results primarily focused on regions within the population, and categorized them as urban, suburban, and rural areas. Indicators and metabolites of cocaine and ecstasy were more prominent in urban areas, but methamphetamine was present equally in all areas.[53] Not only do these findings demonstrate a successful campaign to detect target drug compounds, but they also contribute to the confirmation of methamphetamine prevalence. Shortly after this research, another group focused on illicit substances as well, but had the

goal of determining day-to-day consumption patterns. Zuccato et al. established that "party-drugs" such as cocaine and ecstasy were more prominent in waste water collected over the weekend as opposed to weekdays. Conversely, methamphetamine demonstrated similar concentrations throughout the entire week.[54] Another finding was the upward trend of methamphetamine concentrations within waste water from 2005-2009.[54] While these two findings contribute to the capability of detecting drugs within sewage effluent, the results also elaborate on methamphetamine prevalence within the community, similar to the conclusions mentioned above.

In summary, many studies within the literature provide examples and processes of analyzing both pharmaceutical and illicit compounds in waste water samples. Additionally, several studies have focused on the amount or concentrations present for specific compounds. As for methamphetamine, the studies above demonstrate high and continued prevalence within populations. However, the methamphetamine values obtained were attributed solely to human consumption. The research groups discussed above either mentioned the potential for other sources of methamphetamine, or ignored the fact completely. As stated earlier, another source of methamphetamine into sewage effluent is clandestine laboratories. Whether individuals are dumping methamphetamine product down the drain to avoid detection, or discarding manufacturing waste, indicators of such activity could be identified via waste water analysis. Again, the overall goal of this chapter is to determine the possibility of detecting One Pot method identifiers within waste water as a means to locate the presence of a clandestine laboratory.

The One-Pot methamphetamine waste has only been quantitatively characterized in the findings presented in Chapter 2. Based on LC-MS/MS results, the target

compounds to identify the presence of methamphetamine manufacturing would be methamphetamine, pseudoephedrine, ephedrine, and CMP. Since three of the four compounds present within One Pot manufacturing waste are commonly used and excreted drugs, a challenge exists in distinguishing drug consumption versus drug production. On the other hand, methamphetamine detection, in conjunction with the detection of other manufacturing byproducts, could indicate the presence of methamphetamine manufacturing. Since CMP was also identified within One Pot waste, the detection of this byproduct may prove to be a key indicator of a clandestine laboratory. The waste water analysis also needs to examine amphetamine, due to the result of metabolism. Methamphetamine, in most instances, will be excreted 50% unchanged or un-metabolized, while 10-20% of the methamphetamine will be converted to amphetamine before excretion.[47] Although the focal point of the waste water results will be on the concurrent detection of methamphetamine and CMP, the amount of amphetamine present may reveal the quantity of methamphetamine that should be attributed to human consumption. Based on review of the literature, no research has been conducted on sewage effluent to detect the presence of methamphetamine manufacturing. As explained earlier, the prevalence of One Pot methamphetamine has created a public and environmental safety demand to proactively find clandestine laboratories. With knowledge of negligent dumping of hazardous reaction waste into sewage effluent, waste water analysis may provide a means to locate and consequently remove One Pot clandestine laboratories from communities before they can cause more harm to innocent bystanders.

3.3 Methodology

Two side-by-side One Pot reactions were completed over a course of four days for a total of 8 syntheses, labeled EE1-EE4 and CE1-CE4; "EE" and "CE" respectively indicate "Ether Effluent" or "Camp Fuel Effluent" and the number indicates the sequence. Following each reaction, lithium was removed and placed into 250 mL water in a container labeled with the appropriate One Pot reaction designation and deemed "lithium-in-water solution." Sludge and post-salt solvent samples were collected as described above in the methodology section of Chapter 2. Pre-salt solvent was completely purged with hydrogen chloride gas and the filtered product salts were removed and stored solely for laboratory analysis. In total, the One Pot reaction waste materials recovered from each of the 8 methamphetamine salt syntheses included:

1) lithium-in-water solution,
2) sludge,
3) post-salt solvent.

These three liquid solutions together constituted the full methamphetamine production waste from each One Pot reaction. EE1-EE3 and CE1-CE3 materials were set aside for subsequent, controlled deposit into a municipal sewage water system. EE4 and CE4 were stored for laboratory characterization analysis as described in Chapter 2.

A municipal lift station located within a mixed zone of commercial and industrial buildings was chosen for sewage water sampling to attempt detection of the three prepared liquid components of One Pot laboratory waste generated per synthesis. The gravity-fed lift station is described as low flow (roughly 1500-3000 gallons per day) and discharges to a 4-inch forced main. The lift station is comprised of an underground

concrete catchment tank, or wet well, which collects sewage water from the surrounding commercial-industrial zone. The wet well is open to atmosphere and is fitted with a ground level hatch opening, raised in the interior image seen in Figure 11.

Figure 11. Interior view of wet well at low flow lift station.

The wet well is equipped with submerged pumps to periodically pump the sewage water to a higher elevation, ultimately directed towards the sewage water treatment plant, with at least one additional and larger lift station downstream ahead of the waste treatment plant in the municipality.

Lift station historical data logging statistics from the one-month time period prior to the field study indicate approximately 3-4 pumpdowns of the wet well occur per day. A pumpdown is the initiation of water pumps that activate when the water level in the wet well reaches a certain maximum level. The pumps will continue pushing water out of the lift station until a minimum water level is reached, thus ceasing the pump down. 3-4 pumpdowns is an average number for this site, but 6 discrete pump starts were reported to have occurred prior to one study day. Also available was an estimated discharge of 2666 gallons over the 6 reported pumpdowns for that day, so 444 gallons may be calculated as an approximate volume per pumpdown. A monthly average suggests a similar value of 438 gallons per pumpdown. An important note is the presence of a large unknown volume of sewage water remaining in the wet well after each pumpdown. Provided lift station design drawings suggest if roughly 400-450 gallons are pumped out of the wet well as reported each pump cycle, that would closely match the volume that always remains in the bottom of the well, serving, in part, as an important cooling fluid surrounding the submersible pumps. Therefore, any compound concentrations generated from laboratory results may potentially be skewed due to this unknown variable.

Prior to any experiments, several tests were performed to ensure experimental success. A 24-cartridge automated waste water sampler (Teledyne ISCO, Model 3700, Lincoln, NE)

was installed. The autosampler was placed at ground level beside the lift station wet well hatch opening, see Figure 12.

Figure 12. Automated waste water sampler placed at ground level beside wet well.

The autosampler was modified to integrate a level transmitter and small programmable electronic controller to sense falling water in the wet well and direct the sampler to collect one sample during each pumpdown of the wet well; however, this modification was unsuccessful during the study. Another autosampler program is collection of waste water samples at designated time intervals, which demonstrated repeated success when set to collect a sample every 15 minutes. Next, dye tests were performed to identify sewage water travel time to the lift station from the selected manhole One Pot waste

material deposit site. Fluorscein Green Dye (Brainerd Chemical Company, Tulsa, OK) was deposited into the sewage manhole located 646 feet upstream, and visible indication of dyed water entry to the wet well, see Figure 13, was observed 21.5 minutes later.

Figure 13. Lift station wet well sewage inflow point during dye test.

A second dye test was performed the following day to ensure a similar flow pattern and rate from the designated manhole upstream to the lift station. No visual time recordings were documented, but observation of the autosampler bottles indicated a similar travel time between 15-30 minutes. Figure 14, below, demonstrates the observation of green dye present in the fourth autosampler bottle. Bottle 1 was collected prior to the dye test, Bottle 2 was collected at the initiation of the dye test, and Bottle 3 was collected 15 minutes. Since Bottle 4 was collected 30 minutes post-initiation, the green dye reached the lift station at some moment between Bottle 3 and 4, i.e. 15-30 minutes. Following success of autosampler installation and programming, and subsequent dye tests, simulated One Pot methamphetamine waste disposal could be explored.

Figure 14. Collected Waste Water Samples Within Autosampler Rack. Green dye test initiated at the collection of Bottle 2, followed by 15-minute interval collections. Green dye present in Bottle 4 indicates a manhole to lift station travel time of 15-30 minutes.

One Pot methamphetamine reaction wastes were deposited once per day, over a Tuesday, Wednesday, and Thursday period, into the street manhole located 646 feet upstream of the low flow lift station over two consecutive weeks; diethyl ether reaction wastes, EE1-EE3, were deposited the first week, camp fuel wastes, CE1-CE3, the following week. One Pot reaction wastes were deposited each morning immediately following collection of a background sewage water sample with the 24-cartridge automated waste water sampler mentioned above.

After a background sample was collected each morning, One Pot waste liquids were then deposited. The deposits included three One Pot waste products previously explained, lithium-in-water solution, sludge, and post-salt solvent. Following initiation of each experiment, 500 mL of sewage water were collected at 15-minute intervals from the downstream lift station wet well for a period of three to four hours, resulting in 12-16 "Time Course" samples collected. Time Course samples were labeled with the corresponding synthesis designation, EE or CE and reaction number, followed by a period and autosampler bottle number. The bottle number corresponded to the chronology at which the sewage water sample was collected. Bottle 1 was collected as a background sample pre-deposit, Bottle 2 was collected at time equals 0 minutes, Bottle 3 was collected at time equals 15 minutes, and so on, in chronological order.

At mid-day, the Time Course sampling was halted and the autosampler was then set to collect a sample upon receiving a "Pulse" signal from detection of falling water in the wet well, i.e. a pumpdown event, reducing the sample collection burden over the remainder of a 24-hour total collection period per waste deposit. However, this modification was unsuccessful during the study as previously mentioned. The Pulse

program of the autosampler was inconsistent, and resulted in variable collection of samples, if any, during each deposit experiment. Any Pulse samples collected were labeled following the same chronological pattern as mentioned above, but the bottle numbers began sequentially after the last Time Course sample collected for that specific deposit. For example, if the last Time Course sample was collected in bottle 12, the first Pulse sample collected would be labeled as 13, with of course, additional designating information like solvent type and deposit number.

A sampling summary, of both Time Course and Pulse samples, can be found in Table 11. All samples listed below were transferred to new and separate containers for transport back to OSU-FTTL. Each sample was stored in a freezer until laboratory analysis.

Table 11. Sewage Water Sample Collection. Included for each One Pot methamphetamine waste deposit are the date, time of deposit, sample collection start time, number of time course samples collected, and number of pulse samples collected.

Deposit	Date	Deposit Start Time	Sample Collection Start Time	Time Course Samples	Pulse Samples
EE1	7/26/16	8:20 AM	8:05 AM	12	0
EE2	7/27/16	7:35 AM	7:24 AM	15	7
EE3	7/28/16	7:26 AM	7:18 AM	16	24
CE1	8/2/16	7:29 AM	7:19 AM	12	0
CE2	8/3/16	7:29 AM	7:21 AM	12	10
CE3	8/4/16	7:29 AM	7:23 AM	12	16

For SPE, the following materials and machines were utilized: Oasis MCX 3 cc cartridges (Waters Corporation, 60 mg, 30 μm), CEREX 48 Flow Control (SPEware Corporation, Baldwin Park, CA), and CEREX 48 Sample Concentrator (SPEware). The following solutions were utilized: Internal Standard Mix (1000 ng/mL solution of all four deuterated internal standards in LC-MS grade water), 10 mM hydrochloric acid solution prepared with 37% HCl and LC-MS grade water, LC-MS grade methanol, and Mobile Phase A. For every sample, 10 μL of Internal Standard Mix and 200 μL of 10 mM hydrochloric acid solution were added to 2 mL of sample. Following sample preparation, Table 12 ,below, outlines each section of the solid phase extraction procedure. SPE cartridges were conditioned prior to sample addition. After a rinse step, cartridges were dried under positive pressure for 20 minutes at approximately 80 psi. After being vacuum dried, elution buffer was added and the eluent was collected into labeled test tubes. Samples were dried to complete dryness under nitrogen at 40 ^{o}C. Reconstitution buffer was added to each test tube and following thorough vortexing, every sample was transferred to an LC injection vial for LC-MS/MS analysis. The LC-MS/MS instrument methodology is the same as described in Chapter 2.

Table 12. Solid Phase Extraction Procedure.

SPE Step	**Parameter**
Sample Preparation	10 μL Internal Standard Mix 200 μL 10 mM HCL
Condition	2 mL LC-MS grade methanol

	2 mL 10 mM HCL
	2 mL 10 mM HCL
Sample Addition	2 mL sample
Rinse	2 mL 10 mM HCL
Cartridge Dry Down	20 min at ~80 psi
Elution	2 mL 2% ammonium hydroxide in methanol
Elution Dry Down	Under nitrogen at 40ºC
Reconstitution	100 μL Mobile Phase A

The validation of the SPE method contained three tests: linearity, accuracy and precision, and matrix effects. Each of these is explained below. The quantitation ratios, the ratio of the larger transition area to the internal standard transition area, from the calibrators that met the identification criteria were plotted versus concentration. After the data were plotted, they were fitted with a best fit line, and weightings were adjusted to assure the best correlation, or highest R-squared or R^2 value. The R^2 for this line was required to be greater than 0.9. For the calibration points to be included in this study, they had to have an accuracy and precision within 30% when applied to the line of best fit. The limit of quantitation, or LOQ, was allowed to be within 30% for both accuracy and precision, though its instrument response had to be at least five times greater than the response of a blank. The linear range for all non-internal standard compounds in the LC-MS/MS method mentioned above contained the following calibrator points: 100, 50, 25, 5, 1, 0.5, and 0.1 ng/mL of each drug compound.

Six replicates of the calibration curve were extracted and concentrations values were calculated for each calibrator. Accuracy for each calibrator was calculated by averaging the concentration of the six replicates and dividing that average by the theoretical concentration of each calibrator. Precision for each calibrator was calculated by dividing the standard deviation average by the calculation average and subtracting that value from 1. R^2 values for each calibration curve were obtained after applying a best fit line, and all values averaged for a given compound. The concentration values, mentioned above, were obtained utilizing MultiQuant software (SCIEX, Foster City, CA), which is specifically designed for LC-MS/MS result analysis. All other values and statistical parameters were obtained by utilizing Microsoft Excel (Microsoft Corporation, Redmond, WA).

An accuracy and precision study was performed to provide information regarding the accuracy and precision, or variability, in the SPE process. To pass the accuracy and precision study, the average values for each point had to be within 30% of the true value. Two quality control (QC) points were used for the accuracy and precision study. QCA had a concentration of 50 ng/mL and QCB had a concentration of 1 ng/mL. Each QC was analyzed four times a day for six days. The first two analyses occurred at the beginning of the day and the second two occurred at the end of the day. Values for QC points were obtained using MultiQuant software. Concentrations for each QC analysis were used in Microsoft Excel to obtain accuracy, within-run precision, between run precision, intraday precision, interday precision and within-laboratory precision.

Matrix effects are a type of assay interference caused by ion suppression or ion enhancement in the matrix. To test for matrix effects, the analytical results of five

injections of compounds that did not undergo extraction, labeled as "neat," were compared to injections of five individual water samples that were from different sources, were known to be free of the compounds of interest, and were fortified with compounds after extraction. The neat and post-extraction fortified samples were designated as sample set 1 and sample set 2, respectively. Matrix effect (ME) was calculated by dividing the average peak area of the water samples fortified after extraction by the average peak area of the compound in neat solution and multiplying by 100. The ME value takes into account any ion suppression or enhancement. The 10 ng/mL calibrator was used in this experiment. To prepare sample set 1, methamphetamine, CMP, pseudoephedrine, ephedrine, and amphetamine drug standards, mentioned above, were diluted in deionized water at 200 ng/mL. Although this concentration does not match the calibrator being using in this study, the fortification of the neat sample is increased to 200 ng/mL to account for the simulation of a sample with 100% extraction efficiency, being a 2 mL sample at 10 ng/mL. For sample set 2, the known blank water samples were spiked with 200 ng/mL of the same five compounds used in sample set 1.

During the matrix effects study, recovery efficiency (RE) was also performed. RE demonstrates how well the sample is recovered during SPE. To test for RE, the analytical results of five individual water samples from different sources, which were known to be free of the compounds of interest and were fortified at the 10 ng/mL level before extraction, designated as sample set 3, were compared to sample set 2 in the ME study. RE was calculated by dividing the average peak area of water samples fortified before the extraction by the average peak area of water samples fortified after extraction and multiplying by 100.

Process efficiency (PE) or how well the SPE process functions in the way of detecting the compounds of interest, was also determined during the matrix effects study. To test for PE, the analytical results of five injections of compounds that were in sample set 1 were compared to injections of five individual water samples that were in sample set 3. PE was calculated by dividing the average peak area of water samples fortified before extraction by the average peak area of the compounds in neat solution and multiplying by 100.

Currently, there is no defined limit for acceptable matrix effects, recovery efficiency, or process efficiency. For this study, a range of 60-140% was considered acceptable. If values were out of the plus or minus 40% range when averaged across each matrix source, the compounds were evaluated in the other precision and accuracy studies to determine that there was sufficient sensitivity and selectivity in the method for sewage water analysis. In summary, Table 13 outlines sample specifics in each sample set.

Table 13. Matrix Effects Study Sample List. Table includes the water sample source, as well as the concentration at which each sample was fortified with drug compounds of interest.

Sample Set	Water Sample	Drug Concentration
1	LC-MS Grade	200 ng/mL, un-extracted
2	Source 1	200 ng/mL, post-extraction
	Source 2	200 ng/mL, post-extraction
	Source 3	200 ng/mL, post-extraction
	Source 4	200 ng/mL, post-extraction

	Source 5	200 ng/mL, post-extraction
3	Source 1	10 ng/mL, pre-extraction
	Source 2	10 ng/mL, pre-extraction
	Source 3	10 ng/mL, pre-extraction
	Source 4	10 ng/mL, pre-extraction
	Source 5	10 ng/mL, pre-extraction

Sewage water samples, selectively chosen from each One Pot waste deposit, were sent to SRNL for analysis. The samples included a background sample from each deposit as well as two samples that were likely to exhibit the largest concentration of target compounds. After arriving at SRNL, the waste water samples were dissolved and heated in 5% nitric acid in preparation for lithium analysis; sample weights and volumes are as listed in Table 14. In addition, the table provides a list of which samples were sent to and analyzed by SRNL staff. The dissolutions were analyzed on an Agilent 7700x Inductively Coupled Plasma Mass Spectrometer (ICP-MS) equipped with a helium collision cell. High Purity standard solutions that consisted of 52 elements at concentrations of blank, 1, 5, 10, 25, and 50 parts-per-billion (ppb) in 2% nitric acid were used to calibrate the instrument. The dissolutions were diluted by a factor of ten in 2% nitric acid for analysis on the instrument.

Table 14. Weight and volume of sewage water samples prepared for ICPMS analysis.

Sample	**Sample Weight (g)**	**Total Volume (mL)**
CE 1-1	4.83	100

CE 1-4	4.75	100
CE 1-5	4.72	100
CE 2-1	4.78	100
CE 2-4	4.77	100
CE 2-5	4.75	100
CE 3-1	3.72	100
CE 3-4	4.71	100
CE 3-5	4.81	100
EE 2-1	4.78	100
EE 2-4	4.84	100
EE 2-5	4.42	100
EE 4-1	4.75	100
EE 4-4	4.71	100
EE 4-5	4.70	100
EE 6-1	4.83	100
EE 6-4	4.84	100
EE 6-5	4.80	100

3.4 Findings

Solid phase extraction with LC-MS/MS analysis was validated. Tests included calibration and linearity, accuracy and precision, and matrix effects. The following calibrators met criteria of linearity by having accuracy and precision values within 10%: 100, 50, 25, 5, 1, and 0.5 ng/mL. Table 15-Table 19 below demonstrate the accuracy and precision for all calibrator levels of methamphetamine, pseudoephedrine, ephedrine, amphetamine, and CMP. The "Average" column refers to the average concentration, in ng/mL, of the 6 replicate runs. Accuracy and precision are reported as percentages, with 100% considered to be absolute. Any value below or above true accuracy or precision is considered a suppression or enhancement of calibrator concentration, respectively. All average concentrations of the blank were under 5 times that of the LOQ, which was determined to be 0.5 ng/mL for all compounds. In the following sections, any value outside the calibration curve range are estimates based on the slope of each best fit line, but must meet identification criteria in order to be reported. The best fit line was determined to be a power fit with no weighting.

Table 15. Methamphetamine Linearity

Methamphetamine Calibrator (ng/mL)	**Average**	**Standard Deviation**	**Overall Accuracy**	**Overall Precision**
100	92.8	8.22	93%	92%
50	53.1	7.09	106%	88%
25	25.4	1.86	102%	93%

5	4.55	0.45	91%	91%
1	0.94	0.10	94%	90%
0.5	0.54	0.04	107%	94%
Blank*	0.01			
R^2	0.98			

Table 16. Pseudoephedrine Linearity

Pseudoephedrine Calibrator (ng/mL)	**Average**	**Standard Deviation**	**Overall Accuracy**	**Overall Precision**
100	96.6	9.37	97%	91%
50	52.2	6.37	104%	89%
25	26.0	2.34	104%	92%
5	4.97	0.55	99%	90%
1	0.91	0.10	91%	90%
0.5	0.49	0.03	98%	94%
Blank*	0.00			
R^2	0.99			

Table 17. Ephedrine Linearity

Ephedrine Calibrator (ng/mL)	**Average**	**Standard**	**Overall**	**Overall**

		Deviation	Accuracy	Precision
100	102	9.83	102%	91%
50	52.3	3.49	105%	94%
25	25.0	1.95	100%	93%
5	4.65	0.30	93%	94%
1	0.95	0.08	95%	93%
0.5	0.52	0.04	104%	92%
Blank*	0.08			
R^2	1.00			

Table 18. Amphetamine Linearity

Amphetamine Calibrator (ng/mL)	Average	Standard Deviation	Overall Accuracy	Overall Precision
100	96.6	8.98	97%	92%
50	51.6	3.74	103%	93%
25	25.6	2.51	102%	91%
5	4.85	0.27	97%	95%
1	0.98	0.07	98%	93%
0.5	0.53	0.06	107%	89%

Blank*	0.00
R^2	0.99

Table 19. CMP Linearity

CMP Calibrator (ng/mL)	Average	Standard Deviation	Overall Accuracy	Overall Precision
100	106	17.73	106%	85%
50	56.5	6.06	113%	90%
25	25.4	3.69	102%	87%
5	5.05	0.82	101%	85%
1	1.03	0.11	103%	90%
0.5	0.52	0.05	104%	92%
Blank*	0.00			
R^2	0.98			

All QC averages except within-run precision and within-laboratory precision for CMP fell within 20% of the true value. CMP's within-run precision was 23.2% and its within-laboratory precision was 20.4%. Additionally, all accuracy values for the QC points were within 15% of the true value. See Table 20-Table 22, below, for all QC precision and accuracy values. For all values listed, the precision results are that of imprecision, meaning a value of 0 is considered absolute precision. Therefore, the closer

a value to zero, the more precise that QC point is for that specific compound. For accuracy, a value of 100% is considered absolute.

Table 20. Within-Run and Between Run Precision for QC points.

	Within-Run Precision		**Between Run Precision**	
Compound	**QCA**	**QCB**	**QCA**	**QCB**
Methamphetamine	6.26	15.0	12.2	10.1
Pseudoephedrine	5.72	4.79	9.16	5.83
Ephedrine	2.20	5.28	0.10	0.03
CMP	16.5	23.2	0.13	0.09
Amphetamine	3.33	8.19	7.57	3.96

Table 21. Interday and Intraday Precision for QC points.

	Interday Precision		**Intraday Precision**	
Compound	**QCA**	**QCB**	**QCA**	**QCB**
Methamphetamine	3.96	6.44	11.4	3.36
Pseudoephedrine	8.81	8.94	8.22	4.74
Ephedrine	3.02	12.6	10.0	1.66
CMP	3.94	8.56	6.22	14.0
Amphetamine	10.5	16.1	7.19	0.00

Table 22. Within-Laboratory Precision and Accuracy for QC points.

	Within-Laboratory Precision		Accuracy	
Compound	**QCA**	**QCB**	**QCA**	**QCB**
Methamphetamine	13.6	16.0	105%	89%
Pseudoephedrine	13.3	11.2	104%	97%
Ephedrine	10.7	13.6	105%	99%
CMP	18.1	20.4	113%	112%
Amphetamine	13.2	17.6	106%	100%

All five compounds of interest passed the matrix effect (ME) study, but the same is not true for recovery efficiency (RE) and process efficiently (PE). Methamphetamine and CMP both failed RE and PE tests, while amphetamine only failed PE. All values listed in Table 23 were calculated using the peak area for all compounds. All matrix effect samples had ME, RE, and PE corrected by dividing the peak area observed for each compound by the peak area of the corresponding internal standard. The resulting ratio, known as the quantitation ratio, was then used in place of the respective sets used in ME, RE, and PE calculations. The quantitation ratios obtained from the internal standard correction passed all ME, RE, and PE studies. All ME, RE, and PE percentages for the peak area comparisons and the quantitation ratio comparisons are given in Table **24**.

Table 23. Matrix Effects, Recovery Efficiency, and Process Efficiency. All calculations used peak areas for methamphetamine, pseudoephedrine, ephedrine, CMP, and amphetamine.

Area

Compound	Matrix Effects	Recovery Efficiency	Process Efficiency
Methamphetamine	84%	55%	46%
Pseudoephedrine	101%	78%	78%
Ephedrine	93%	75%	70%
CMP	99%	32%	32%
Amphetamine	93%	62%	58%

Table 24. Matrix Effects, Recovery Efficiency, and Process Efficiency. All calculations used quant ratios for methamphetamine, pseudoephedrine, ephedrine, CMP, and amphetamine.

Quant Ratio

Compound	Matrix Effects	Recovery Efficiency	Process Efficiency
Methamphetamine	103%	105%	109%
Pseudoephedrine	99.0%	96.5%	95.5%
Ephedrine	98.4%	97.3%	95.8%
CMP	117%	65.4%	76.4%
Amphetamine	103%	98.9%	102%

Time course samples were collected for every One Pot methamphetamine waste deposit. These included three reactions using diethyl ether and three using camp fuel, labeled EE1-3 and CE1-3, respectively. Table 25-Table 30 below lists all time course sample concentrations following SPE and LC-MS/MS analysis. For all tables, samples are labeled with deposit type and number, followed by the autosampler bottle number

after the period. As explained previously, Bottle 1 was collected before each deposit and the consecutive bottle numbers were collected at succeeding 15 minute-intervals. All values listed are in ng/mL. Any result listed below a value of 0.5 ng/mL, or the LOQ, is outside of the calibration range; however, an estimated value was listed if the chromatographic peak met identification criteria. These values are denoted with a single asterisk in the tables below. All negative samples are listed as 0.00.

Table 25. EE1 Time Course Samples. All values reported in ng/ml.

Sample	Time from Deposit (min)	Methamphetamine	Pseudoephedrine	Ephedrine	CMP	Amphetamine
EE1.1	-15	0.10*	0.09*	0.05*	0.00	0.25*
EE1.2	0	0.09*	0.04*	0.02*	0.00	0.14*
EE1.3	15	0.11*	0.05*	0.05*	0.00	0.20*
EE1.4	30	5.37	1.78	7.67	0.70	0.18*
EE1.5	45	2.16	1.02	3.65	0.35*	0.13*
EE1.6	60	1.71	0.63	2.36	0.28*	0.17*
EE1.7	75	1.13	0.43*	1.75	0.26*	0.12*
EE1.8	90	1.24	0.43*	1.74	0.23*	0.15*
EE1.9	105	1.17	0.36*	1.63	0.21*	0.16*

EE1.10	120	0.96	0.32*	1.26	0.17*	0.18*
EE1.11	135	0.91	0.66	1.14	0.14*	0.14*
EE1.12	150	0.80	0.38*	1.11	0.17*	0.38*

Table 26. EE2 Time Course Samples. All values reported in ng/ml.

Sample	**Time from Deposit (min)**	**Methamphetamine**	**Pseudoephedrine**	**Ephedrine**	**CMP**	**Amphetamine**
EE2.1	-15	0.34*	1.19	0.53	0.00	0.33*
EE2.2	0	0.28*	1.54	1.08	0.00	0.29*
EE2.3	15	0.27*	1.03	0.48*	0.00	0.28*
EE2.4	30	0.31*	0.99	0.29*	0.00	0.30*
EE2.5	45	3.09	1.73	5.74	0.28*	0.33*
EE2.6	60	2.47	1.50	4.45	0.25*	0.30*
EE2.7	75	5.10	1.97	10.0	0.44*	0.33*
EE2.8	90	5.86	2.42	12.5	0.56	0.25*
EE2.9	105	6.05	2.19	11.4	0.57	0.25*
EE2.10	120	5.38	2.00	10.2	0.51	0.24*
EE2.11	135	4.46	1.72	8.70	0.46*	0.24*

EE2.12	150	4.50	1.81	8.35	0.43*	0.24*
EE2.13	165	3.24	1.38	5.99	0.33*	0.22*
EE2.14	180	1.40	0.59	2.61	0.18*	0.18*
EE2.15	195	1.33	0.62	2.43	0.14*	0.53

Table 27. EE3 Time Course Samples.

Sample	**Time from Deposit (min)**	**Methamphetamine**	**Pseudoephedrine**	**Ephedrine**	**CMP**	**Amphetamine**
EE3.1	-15	0.62	0.40*	1.01	0.00	0.26*
EE3.2	0	0.60	0.34*	1.00	0.00	0.26*
EE3.3	15	0.45*	0.29*	0.69	0.00	0.21*
EE3.4	30	0.74	0.51	1.45	0.05*	0.28*
EE3.5	45	6.90	2.41	13.8	0.35*	0.24*
EE3.6	60	6.56	2.50	12.5	0.38*	0.19*
EE3.7	75	4.23	1.35	7.35	0.24*	0.26*
EE3.8	90	2.98	0.97	5.66	0.16*	0.19*
EE3.9	105	2.91	1.06	5.42	0.17*	0.19*
EE3.10	120	2.71	0.96	4.93	0.18*	0.21*

EE3.11	135	2.43	0.80	4.53	0.15*	0.24*
EE3.12	150	2.19	0.73	3.87	0.13*	0.20*
EE3.13	165	1.89	0.72	3.63	0.12*	0.17*
EE3.14	180	1.62	0.54	2.97	0.13*	0.16*
EE3.15	195	1.54	0.57	3.16	0.09*	0.61
EE3.16	210	1.32	0.54	2.47	0.10*	0.39*

Table 28. CE1 Time Course Samples

Sample	**Time from Deposit (min)**	**Methamphetamine**	**Pseudoephedrine**	**Ephedrine**	**CMP**	**Amphetamine**
CE1.1	-15	0.96	0.84	1.28	0.00	0.18*
CE1.2	0	0.92	0.87	0.98	0.00	0.16*
CE1.3	15	0.87	0.79	0.84	0.00	0.13*
CE1.4	30	1.12	0.85	1.02	0.00	0.16*
CE1.5	45	41.4	18.1	68.9	0.25*	0.21*
CE1.6	60	18.0	7.11	26.3	0.17*	0.23*
CE1.7	75	16.3	6.59	24.4	0.17*	0.21*
CE1.8	90	15.4	5.78	22.2	0.13*	0.20*

CE1.9	105	14.8	8.37	22.0	0.15*	0.21*
CE1.10	120	9.60	7.41	14.3	0.10*	0.16*
CE1.11	135	7.83	6.83	11.2	0.08*	0.17*
CE1.12	150	7.18	4.81	10.7	0.08*	0.14*

Table 29. CE2 Time Course Samples.

Sample	**Time from Deposit (min)**	**Methamphetamine**	**Pseudoephedrine**	**Ephedrine**	**CMP**	**Amphetamine**
CE2.1	-15	0.74	1.53	0.94	0.00	0.15*
CE2.2	0	0.74	1.41	0.90	0.00	0.14*
CE2.3	15	0.55	1.08	0.64	0.00	0.10*
CE2.4	30	1.43	2.38	3.55	0.00	0.14*
CE2.5	45	17.1	18.9	88.4	0.19*	0.16*
CE2.6	60	7.78	10.6	36.4	0.11*	0.14*
CE2.7	75	6.50	7.48	25.1	0.10*	0.26*
CE2.8	90	5.81	6.96	23.7	0.08*	0.24*
CE2.9	105	5.06	7.05	21.7	0.05*	0.24*
CE2.10	120	5.01	6.45	21.7	0.07*	0.22*

CE2.11	135	5.04	6.61	21.0	0.09*	0.22*
CE2.12	150	3.78	5.37	15.4	0.00	0.19*

Table 30. CE3 Time Course Samples.

Sample	Time from Deposit (min)	Methamphetamine	Pseudoephedrine	Ephedrine	CMP	Amphetamine
CE3.1	-15	0.32*	2.22	1.95	0.00	0.13*
CE3.2	0	0.43*	1.70	1.95	0.00	0.09*
CE3.3	15	0.41*	1.57	1.70	0.00	0.11*
CE3.4	30	15.0	24.6	102	0.08*	0.12*
CE3.5	45	11.9	18.7	75.2	0.10*	0.13*
CE3.6	60	9.13	16.1	57.8	0.08*	0.13*
CE3.7	75	7.62	12.4	53.2	0.07*	0.15*
CE3.8	90	7.76	11.2	51.9	0.07*	0.15*
CE3.9	105	6.99	11.0	47.6	0.06*	0.14*
CE3.10	120	5.37	9.01	40.6	0.04*	0.12*
CE3.11	135	4.56	6.76	30.5	0.00	0.11*

CE3.12	150	4.14	6.87	37.0	0.00	0.10*

Additionally, Pulse samples were collected for deposits EE2, EE3, CE2, and CE3. EE1 and CE1 experienced sampling errors during the pulse collection period and no Pulse samples were collected by the autosampler. Table 31-Table 34 below lists all Pulse sample concentrations following SPE and LC-MS/MS analysis. For all tables, samples are labeled with deposit type and number, followed by the autosampler bottle number after the period. The bottle number begins after each deposit's Time Course sample count. As noted previously, Pulse samples are chronological, but not associated with a known time within the final 20-21 hour collection window. All values listed are in ng/mL. Any result listed below a value of 0.5 is outside of the calibration range; however, an estimated value was listed if the chromatographic peak met identification criteria. These values are denoted with a single asterisk in the tables below. All negative samples are listed as 0.00.

Table 31. EE2 Pulse Samples.

Sample	Methamphetamine	Pseudoephedrine	Ephedrine	CMP	Amphetamine
EE2.16	0.32*	0.19*	0.76	0.00	0.25*
EE2.17	0.29*	0.10*	0.34*	0.00	0.19*
EE2.18	0.48*	0.26*	0.56	0.00	0.24*
EE2.19	0.19*	0.08*	0.14*	0.00	0.12*
EE2.20	0.22*	0.09*	0.18*	0.00	0.19*

EE2.21	0.28*	0.11*	0.33*	0.00	0.30*
EE2.22	0.18*	0.09*	0.17*	0.00	0.19*

Table 32. EE3 Pulse Samples.

Sample	Methamphetamine	Pseudoephedrine	Ephedrine	CMP	Amphetamine
EE3.17	0.62	0.15*	0.42*	0.00	0.17*
EE3.18	0.43*	0.12*	0.39*	0.00	0.14*
EE3.19	0.50	0.15*	0.46*	0.00	0.16*
EE3.20	1.59	0.81	0.28*	0.00	0.16*
EE3.21	1.02	0.55	0.29*	0.00	0.17*
EE3.22	1.38	0.71	0.33*	0.00	0.20*
EE3.23	0.35*	0.18*	0.25*	0.00	0.11*
EE3.24	0.47*	0.19*	0.28*	0.00	0.13*
EE3.25	0.45*	0.22*	0.27*	0.00	0.13*
EE3.26	0.73	0.41*	0.47*	0.00	0.13*
EE3.27	1.37	0.22*	0.45*	0.00	0.36*
EE3.28	0.65	0.12*	0.32*	0.00	0.18*
EE3.29	1.26	0.36*	0.57	0.00	0.32*
EE3.30	0.62	0.49*	0.35*	0.00	0.19*

EE3.31	0.25*	0.25*	0.24*	0.00	0.15*
EE3.32	0.30*	0.26*	0.24*	0.00	0.11*
EE3.33	0.25*	0.28*	0.24*	0.00	0.10*
EE3.34	0.19*	0.28*	0.24*	0.00	0.15*
EE3.35	0.17*	0.27*	0.27*	0.00	0.17*
EE3.36	0.16*	0.31*	0.29*	0.00	0.14*
EE3.37	0.18*	0.40*	0.30*	0.00	0.12*
EE3.38	0.26*	0.56	0.26*	0.00	0.18*
EE3.39	0.13*	0.43*	0.27*	0.00	0.14*
EE3.40	0.20*	0.80	0.36*	0.00	0.17*

Table 33. CE2 Pulse Samples.

Sample	Methamphetamine	Pseudoephedrine	Ephedrine	CMP	Amphetamine
CE2.13	1.26	2.85	5.11	0.00	0.34*
CE2.14	0.57	1.16	1.84	0.00	0.18*
CE2.15	0.45*	1.05	1.62	0.00	0.15*
CE2.16	0.44*	0.87	2.13	0.00	0.15*
CE2.17	0.54	1.60	1.92	0.00	0.22*
CE2.18	1.47	3.62	5.01	0.00	0.30*

CE2.19	1.28	4.14	4.80	0.00	0.32*
CE2.20	0.30*	0.67	0.51	0.00	0.15*
CE2.21	0.32*	0.63	0.69	0.00	0.18*
CE2.22	0.31*	0.78	0.94	0.00	0.11*

Table 34. CE3 Pulse Samples.

Sample	Methamphetamine	Pseudoephedrine	Ephedrine	CMP	Amphetamine
CE3.13	4.63	7.59	21.9	0.00	0.27*
CE3.14	3.21	5.17	17.7	0.00	0.22*
CE3.15	2.50	4.78	12.7	0.00	0.23*
CE3.16	2.32	4.18	9.70	0.00	0.25*
CE3.17	1.68	2.73	5.52	0.00	0.22*
CE3.18	1.03	1.77	2.96	0.00	0.19*
CE3.19	2.92	5.06	5.71	0.00	0.37*
CE3.20	3.34	5.04	5.24	0.00	0.19*
CE3.21	3.48	5.07	5.32	0.00	0.18*
CE3.22	1.57	2.54	2.59	0.00	0.30*
CE3.23	1.18	1.81	2.27	0.00	0.19*
CE3.24	3.32	1.39	1.89	0.00	0.31*

CE3.25	2.01	0.64	0.84	0.00	0.32*
CE3.26	2.30	0.55	0.79	0.00	0.34*
CE3.27	2.24	0.61	0.79	0.00	0.36*
CE3.28	0.71	0.88	0.45*	0.00	0.23*

In comparing the two solvent types, the highest or maximum concentrations for each One Pot methamphetamine waste deposit type, diethyl ether or camp fuel, are listed in Table 35 below. Any result listed below a value of 0.5 ng/mL is outside of the calibration range; however, an estimated value was listed if the chromatographic peak met identification criteria. These values are denoted with a single asterisk in the table below. All values are listed in ng/mL.

Table 35. Average maximum concentrations (ng/mL) observed for methamphetamine, CMP, pseudoephedrine, ephedrine, and amphetamine in sewage water samples collected after diethyl ether and camp fuel One Pot waste deposits.

Compound	EE Max Conc.	CE Max Conc.
Methamphetamine	6.11	24.50
CMP	0.55	0.18*
Pseudoephedrine	2.33	20.53
Ephedrine	11.32	86.43
Amphetamine	0.51	0.21*

Statistical analysis of a two-population t-test, comparing the average maximum compound concentrations of EE1-EE3 and CE1-CE3, produced the corresponding results

listed below in Table 36. Calculated p-values demonstrated no significant difference in the maximum concentration detected for methamphetamine, but the remaining compounds proved to be statistically different. CMP and amphetamine were not statistically compared because the average value of Camp Fuel samples was below the LOQ.

Table 36. Statistical Comparison of Ether and Camp Fuel Effluent Samples. All values listed in the "EE" and "CE" are in ng/mL and represent the average maximal concentration of the corresponding drug detecting in waste water. A two-population t-test was performed to compare the mean average concentration of ether and camp fuel for methamphetamine, pseudoephedrine, and ephedrine. A generated p-value less than 0.05 translates to a statistical difference between the two solvent types. A p-value less than 0.01 or less than 0.001, indicate a high statistical difference or extreme statistical difference, respectively

Sewage Effluent (ng/mL)	**EE**	**CE**	**p-value (two-tailed)**
Methamphetamine	6.11	24.50	0.10
Pseudoephedrine	2.33	20.53	0.0009***
Ephedrine	11.32	86.43	0.002**

*p-values<0.05 signifying a statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.

**p-values<0.01 signifying a high statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.

***p-values<0.001 signifying an extreme statistical difference between the concentrations observed in the ether cooks and the camp fuel cooks.

For lithium analysis, Table 37 below lists lithium concentration in microgram per gram for autosampler bottles 1, 4, and 5 of each deposit. These bottles were collected at time equals -15, 45, and 60 minutes, respectively.

Table 37. Lithium Concentration from ICP-MS analysis

Sample	***Lithium Concentration (µg/g)***
CE 1-1	ND
CE 1-4	ND
CE 1-5	0.25
CE 2-1	ND
CE 2-4	ND
CE 2-5	0.30
CE 3-1	ND
CE 3-4	0.23
CE 3-5	0.17
EE 1-1	ND
EE 1-4	0.12
EE 1-5	0.08

EE 2-1	0.01
EE 2-4	ND
EE 2-5	0.06
EE 3-1	0.02
EE 3-4	0.02
EE 3-5	0.18

Figure 15-Figure 19 shows graphical representation of the concentration (ng/mL) of methamphetamine, pseudoephedrine, ephedrine, CMP, and amphetamine observed in the sewage water time course samples collected after the ether One Pot waste deposits. EE1 and EE3 followed the same rise-fall pattern, where the highest concentration of each drug, except amphetamine, was observed between 45 and 60 minutes. After this peak, the concentration dropped significantly for 30 minutes and then the rate of the decline began to lessen over time. For example, EE2 showed a large increase in all drug concentrations, except amphetamine, over the first two hours and then a large decrease in all drug concentrations, except amphetamine, over the second two-hour period. For all three ether One Pot waste deposits, amphetamine remained at the same concentration, visually, throughout the 3 to 4-hour timeframe.

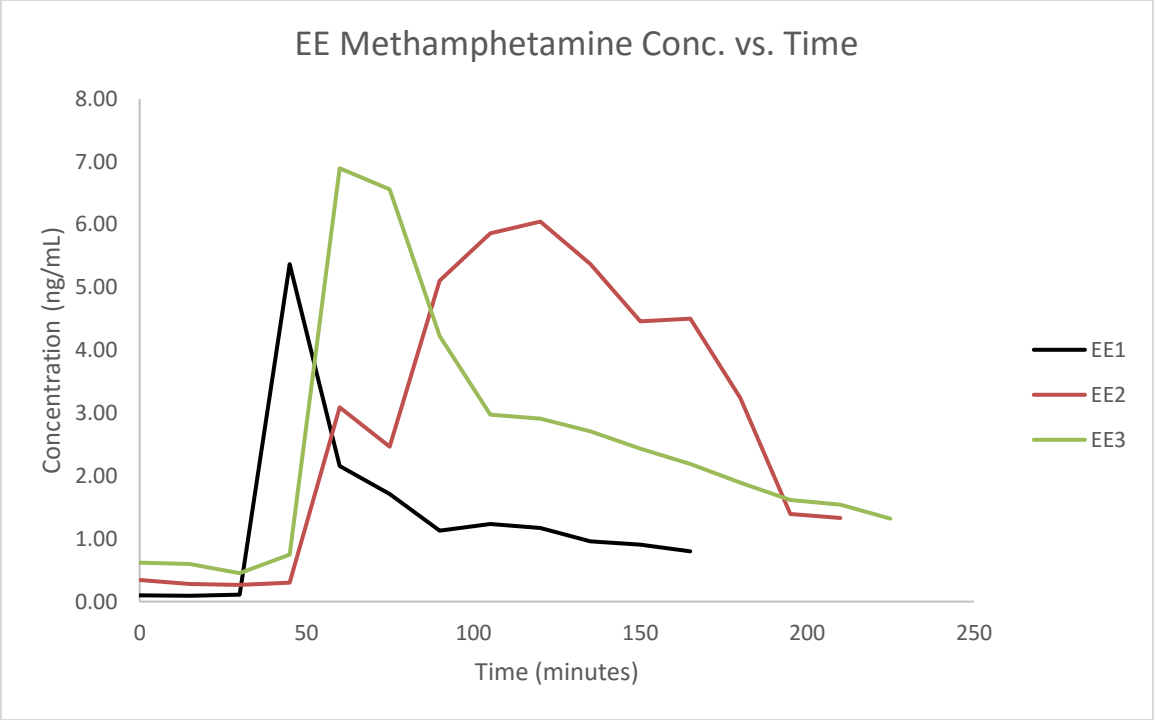


Figure 15. Change in methamphetamine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after ether cook waste deposits.

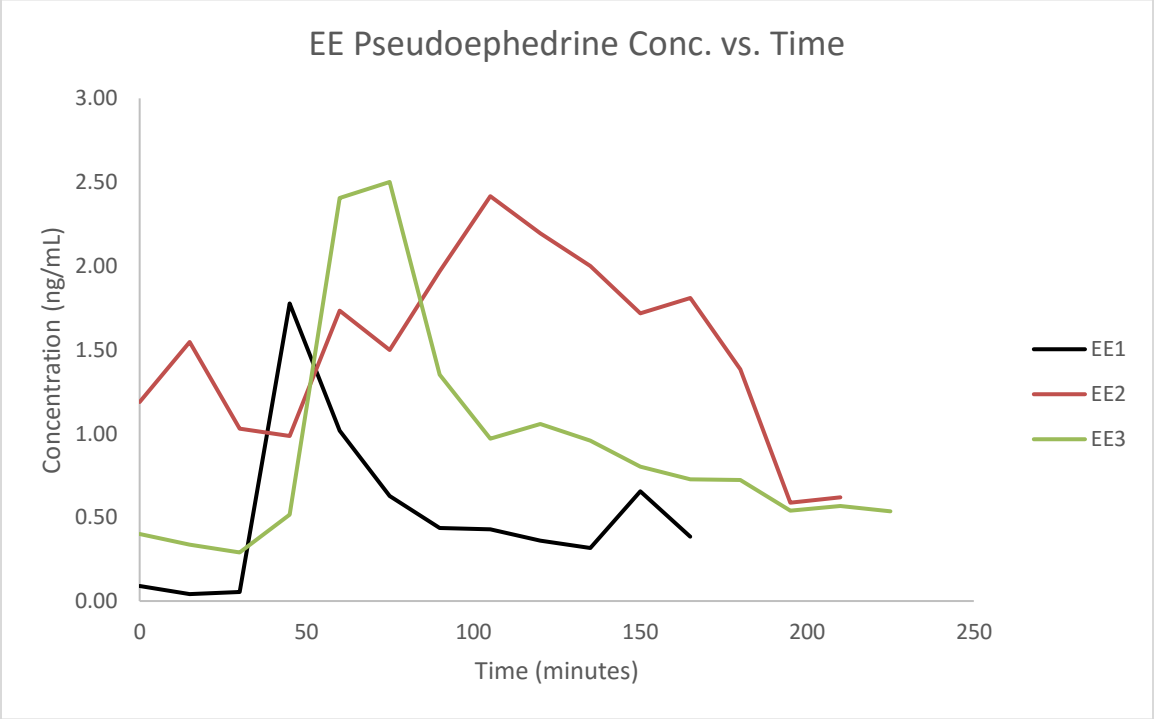


Figure 16. Change in pseudoephedrine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after ether cook waste deposits.

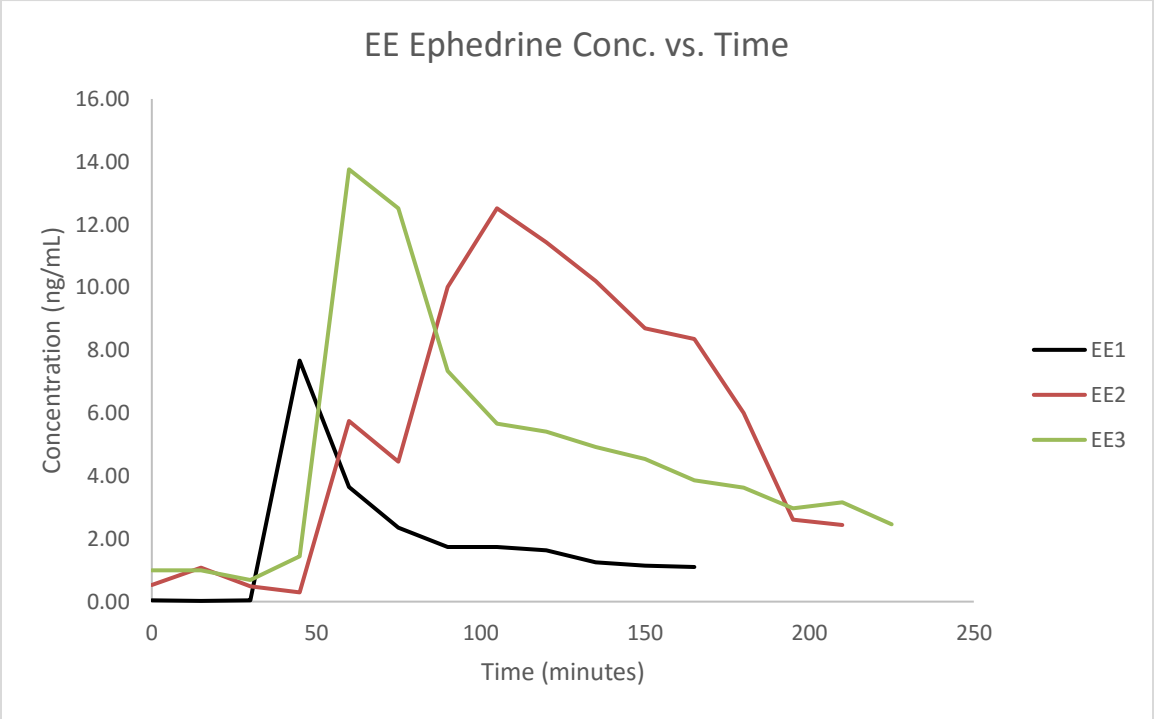


Figure 17. Change in ephedrine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after ether cook waste deposits.

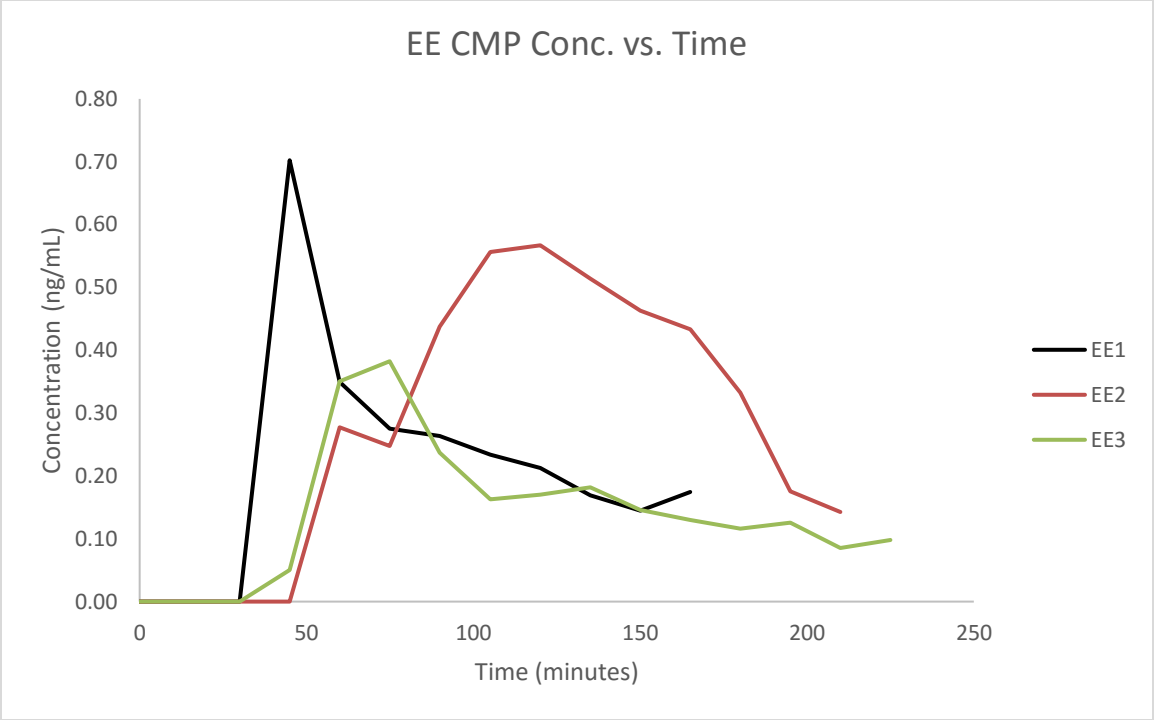


Figure 18. Change in CMP concentration (ng/mL) observed in sewage water samples taken every 15 minutes after ether cook waste deposits.

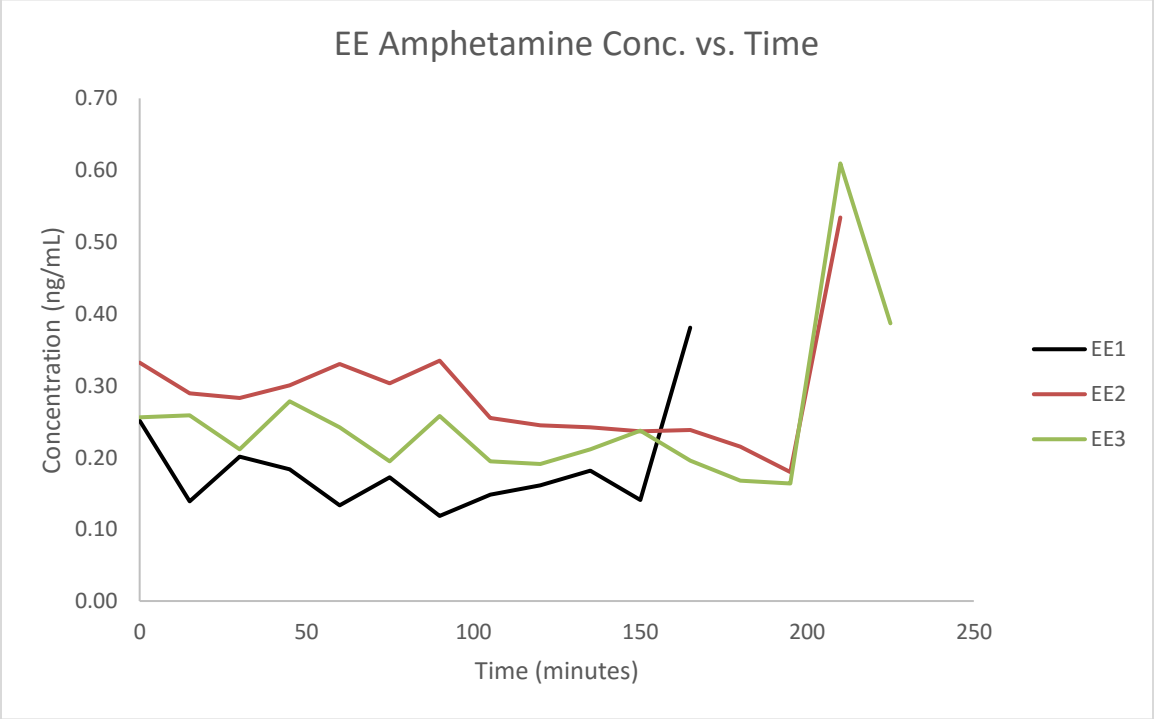


Figure 19. Change in amphetamine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after ether cook waste deposits.

Figure 20-Figure 24 show a graphical demonstration of the concentration (ng/mL) of methamphetamine, pseudoephedrine, ephedrine, CMP, and amphetamine observed in the time course sewage water samples collected after the camp fuel One Pot waste deposits. All three camp fuel deposits followed the same rise-fall pattern, where the highest concentration of each drug, except amphetamine, was observed between 30 and 60 minutes. After this, the concentration dropped significantly for 30 minutes and then the rate of decline began to lessen over time. For all three camp fuel One Pot waste deposits, amphetamine remained at the same concentration, visually, throughout the 3 to 4-hour timeframe.

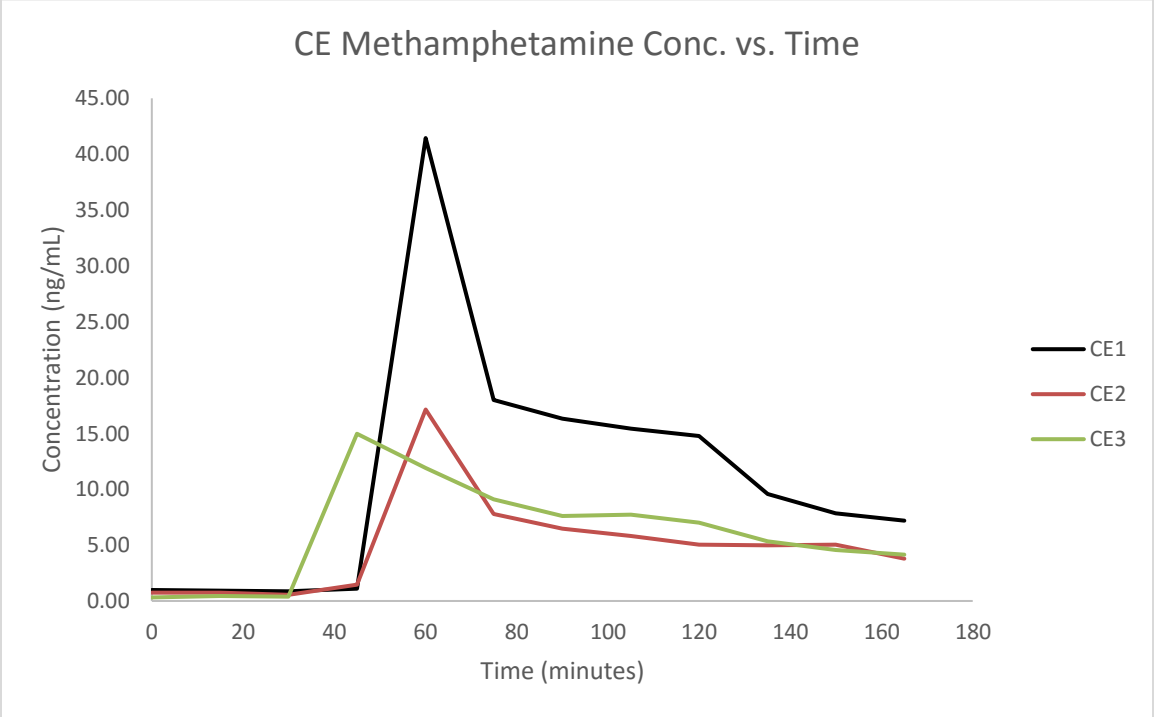


Figure 20. Change in methamphetamine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after camp fuel cook waste deposits.

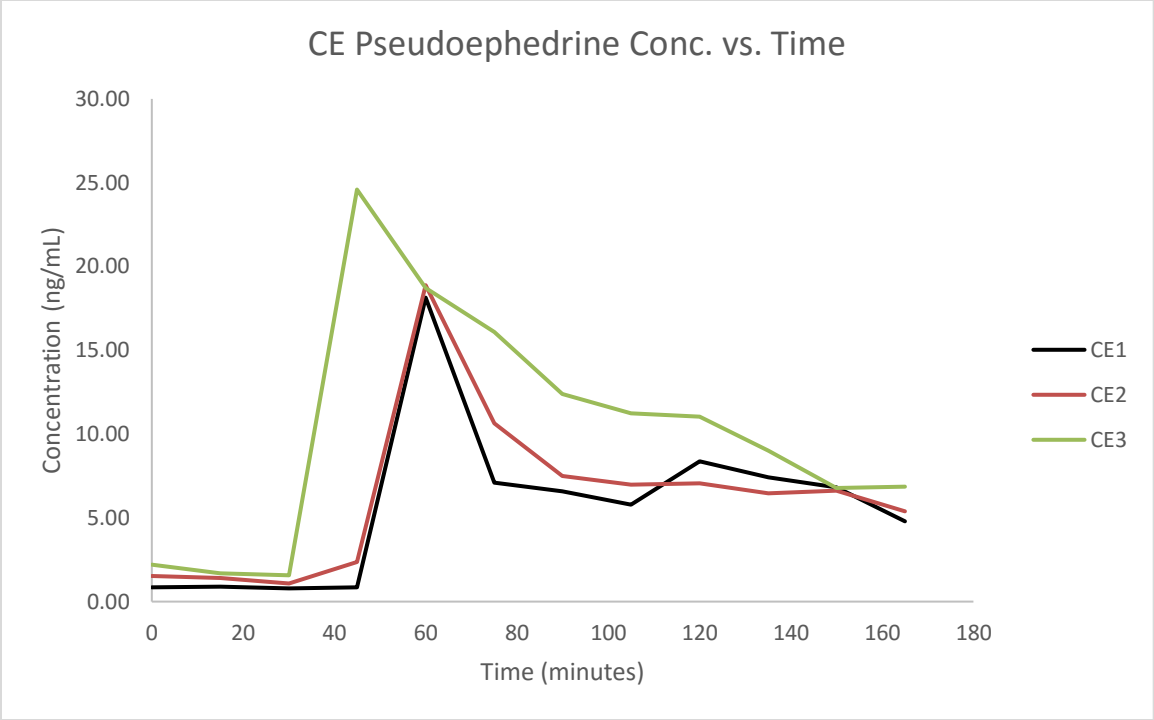


Figure 21. Change in pseudoephedrine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after camp fuel cook waste deposits.

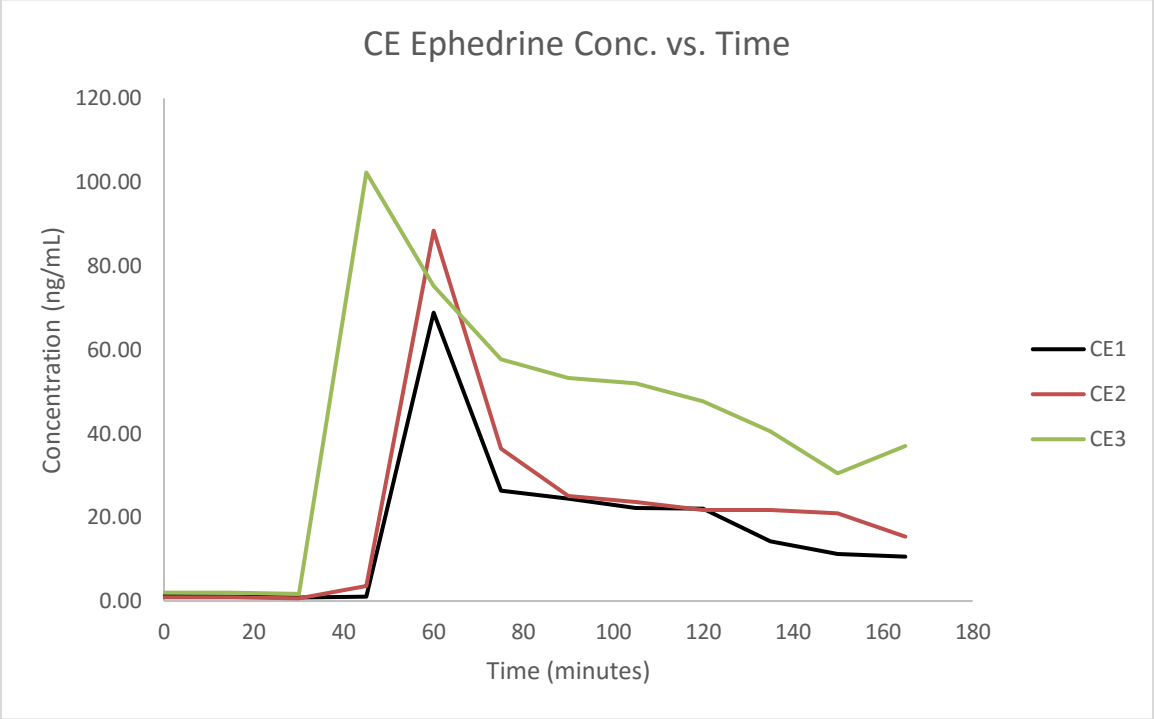


Figure 22. Change in ephedrine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after camp fuel cook waste deposits.

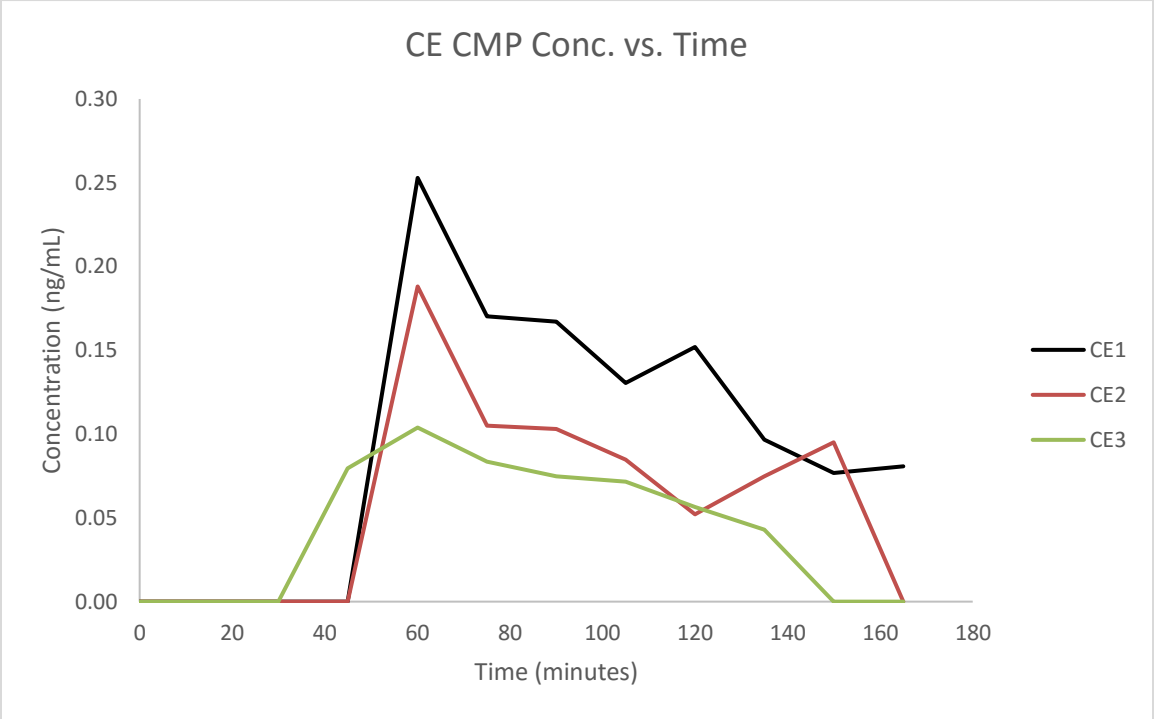


Figure 23. Change in CMP concentration (ng/mL) observed in sewage water samples taken every 15 minutes after camp fuel cook waste deposits.

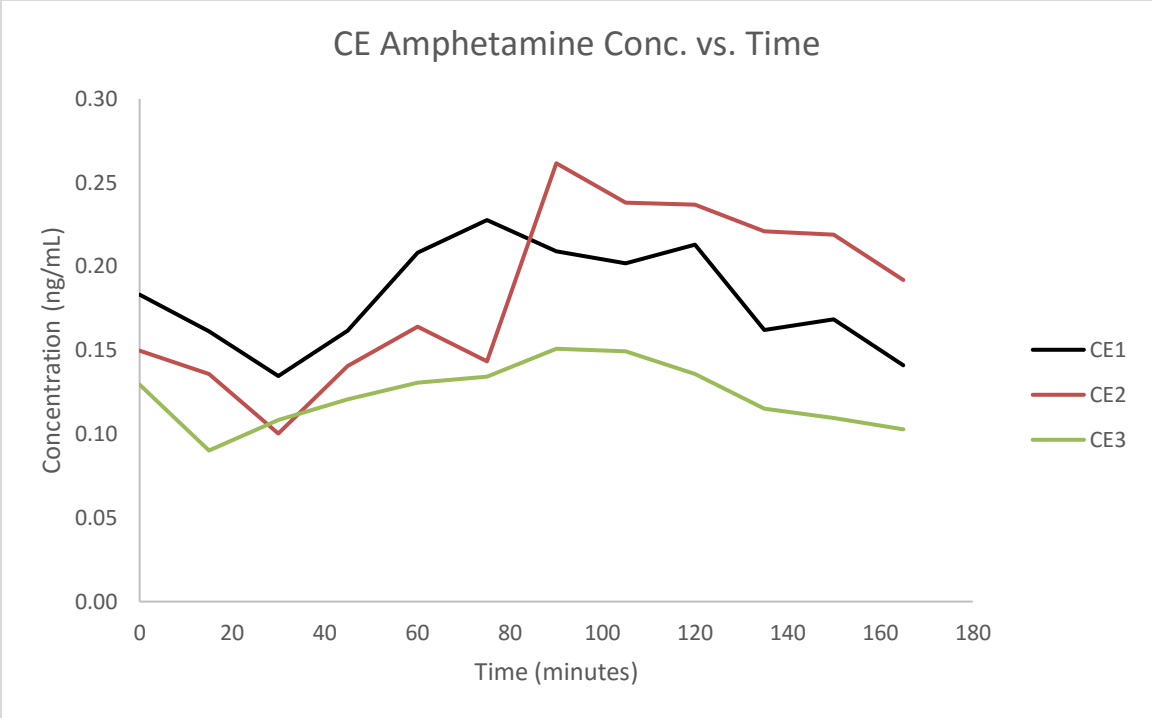


Figure 24. Change in amphetamine concentration (ng/mL) observed in sewage water samples taken every 15 minutes after camp fuel cook waste deposits.

Figure 25-Figure 34 are alternating figures. The odd numbered figures show the concentration (ng/mL) of methamphetamine, pseudoephedrine, ephedrine, CMP, and amphetamine observed in the time course sewage water samples collected after all six One Pot waste deposits. The even numbered figures compare the average concentrations (ng/mL) of these compounds observed in sewage water samples after the ether One Pot waste deposits to the average concentrations observed in sewage water samples after the camp fuel One Pot waste deposits. Higher methamphetamine, pseudoephedrine, and ephedrine concentrations were observed, visually, in sewage water samples collected after camp fuel One Pot waste deposits.

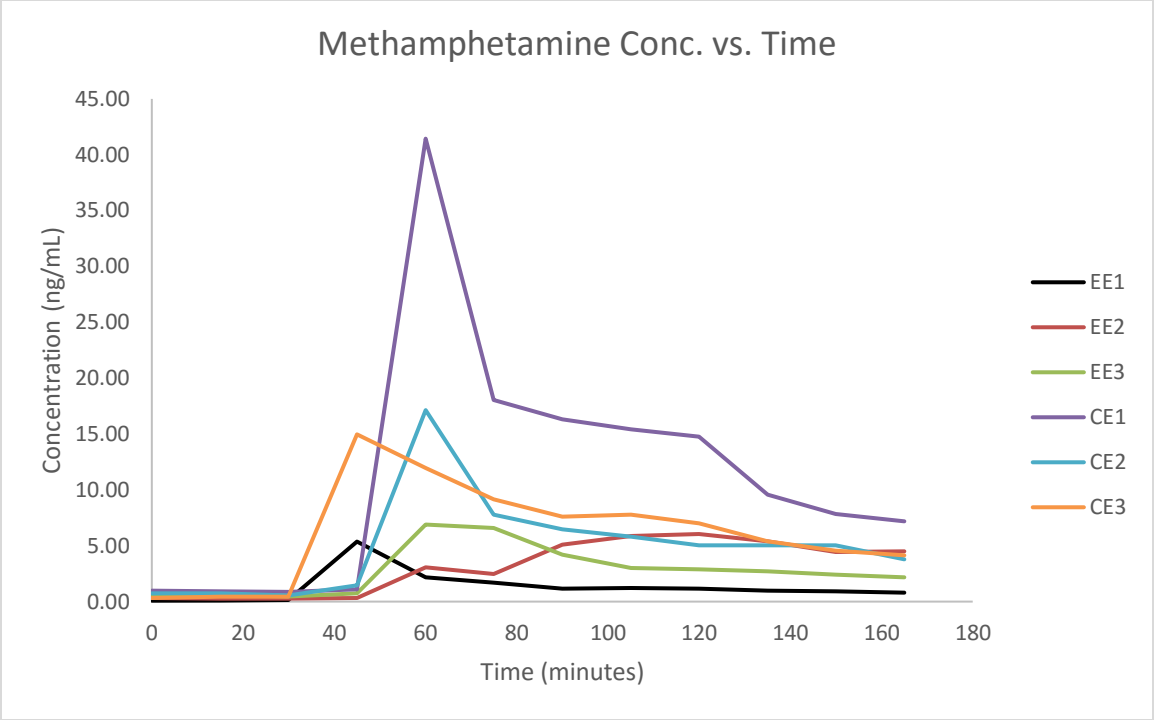


Figure 25. Comparison of the concentration of methamphetamine (ng/mL) observed in sewage water samples every 15 minutes for all One Pot waste deposits.

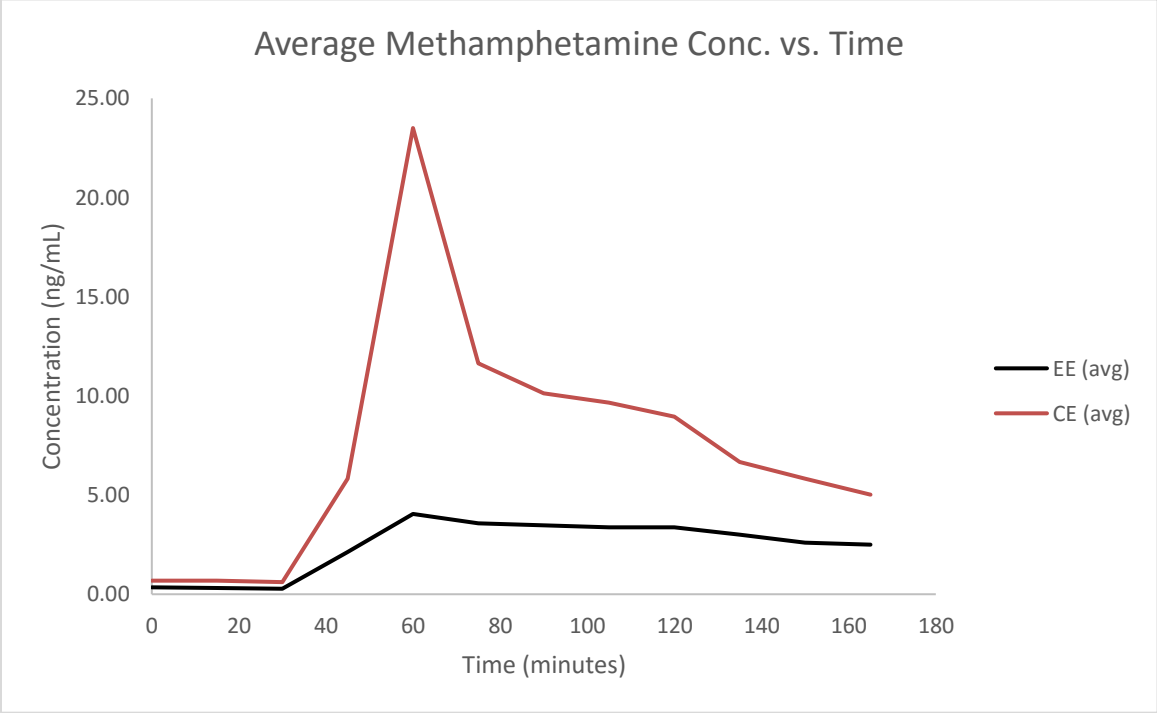


Figure 26. Average concentration of methamphetamine (ng/mL) observed every 15 minutes after One Pot waste deposits.

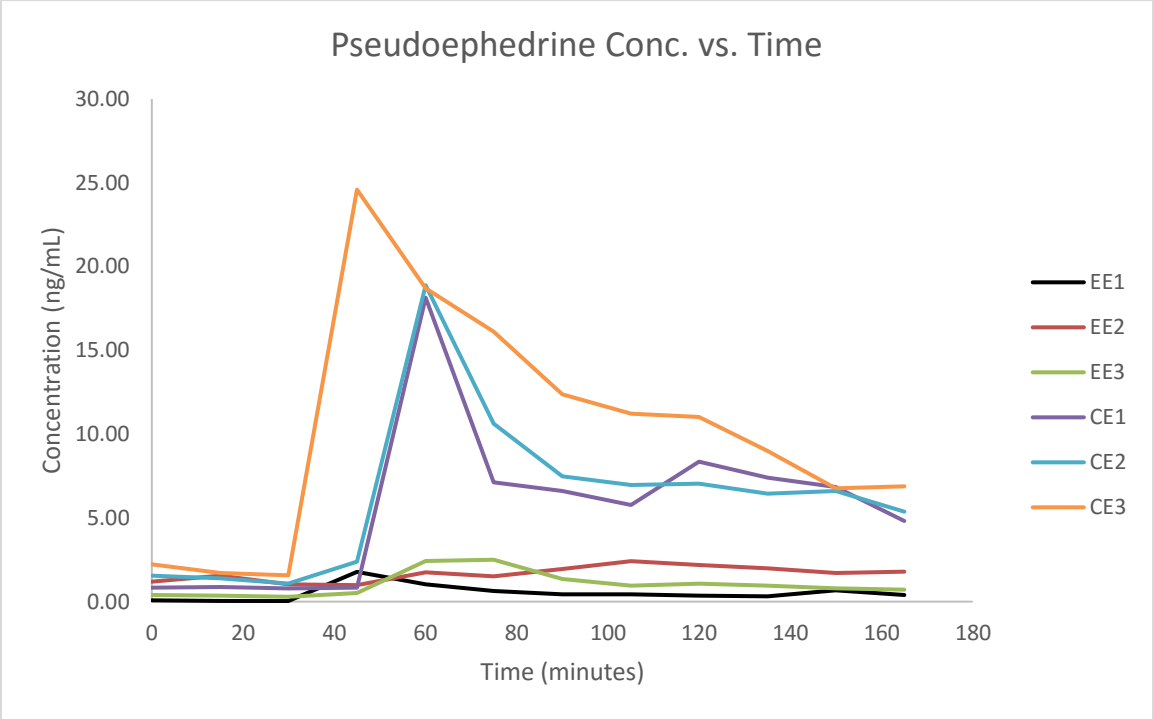


Figure 27. Comparison of the concentration of pseudoephedrine (ng/mL) observed in sewage water samples collected every 15 minutes for all One Pot waste deposits.

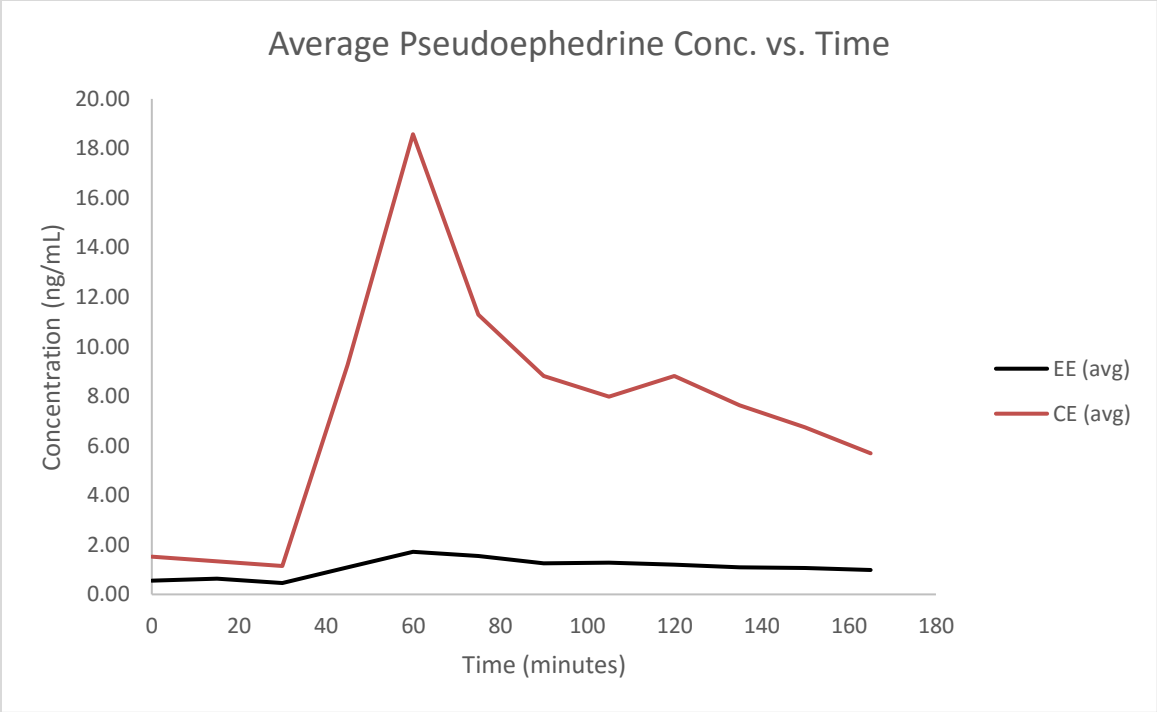


Figure 28. Average concentration of pseudoephedrine (ng/mL) observed every 15 minutes after One Pot waste deposits.

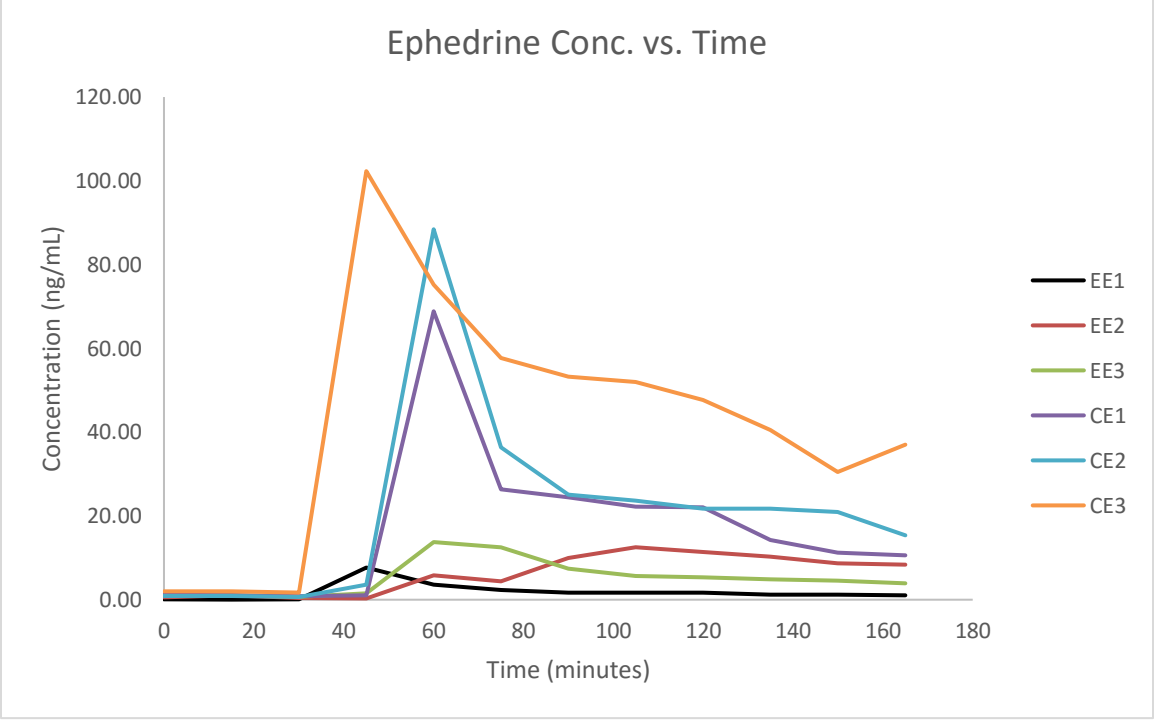


Figure 29. Comparison of the concentration of ephedrine (ng/mL) observed in sewage water samples collected every 15 minutes for all One Pot waste deposits.

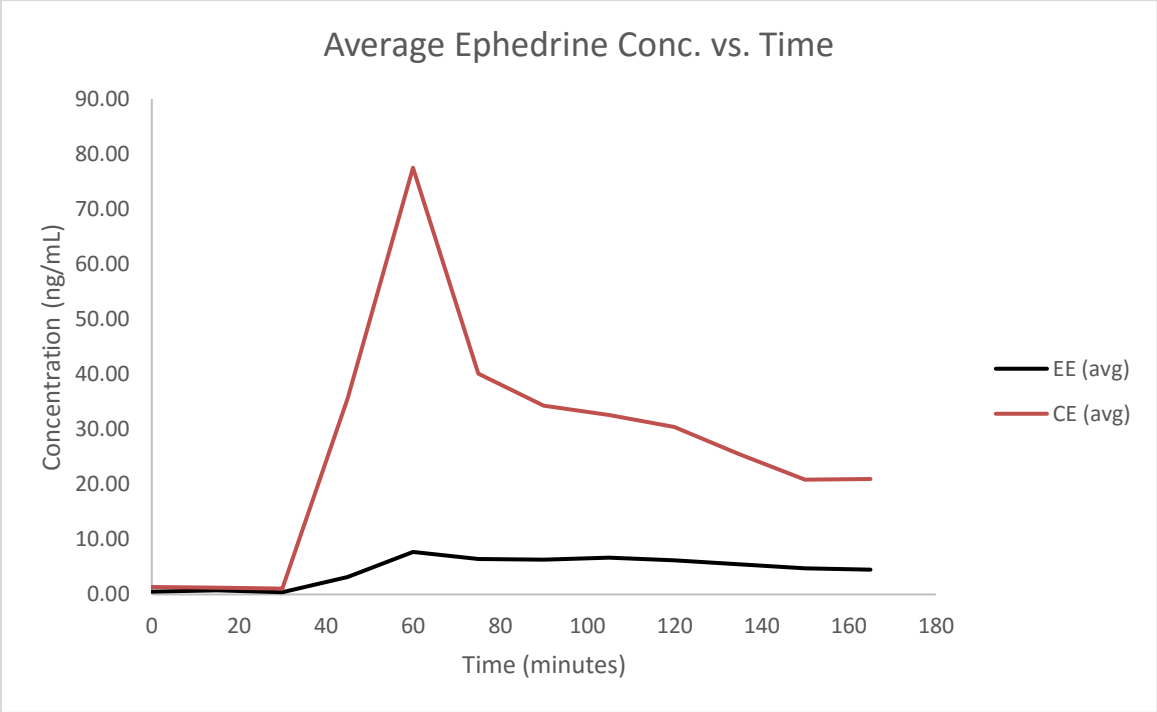


Figure 30. Average concentration of ephedrine (ng/mL) observed every 15 minutes after One Pot waste deposits.

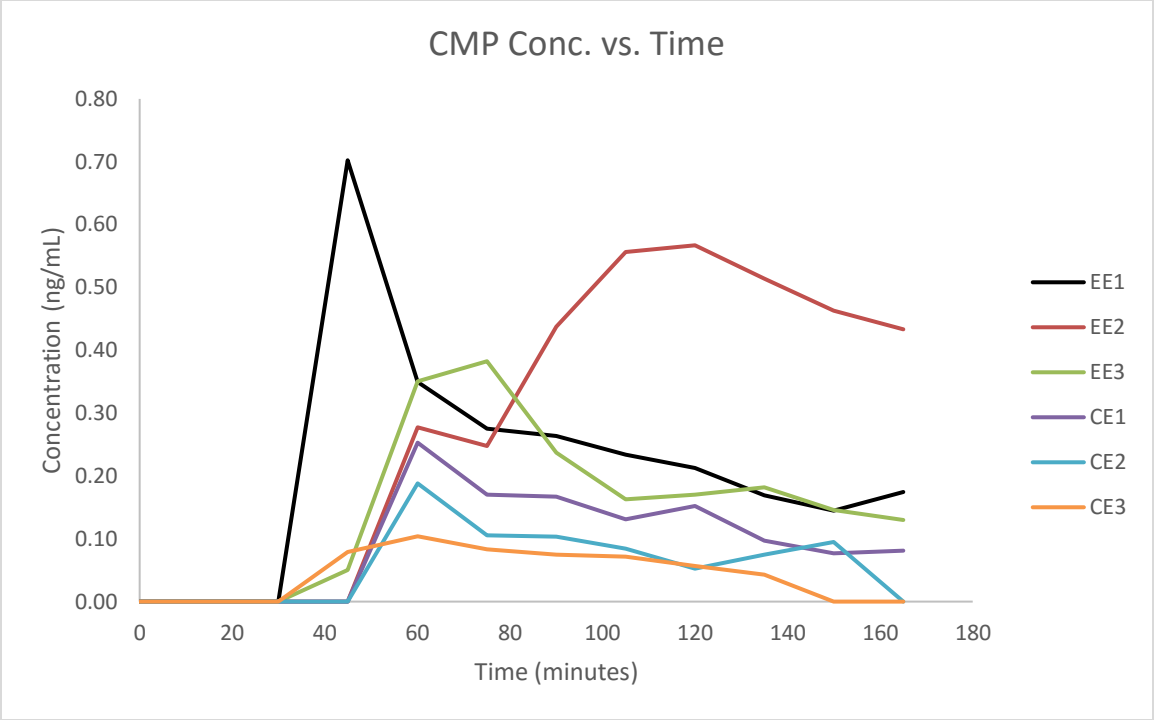


Figure 31. Comparison of the concentration of CMP (ng/mL) observed in sewage water samples collected every 15 minutes for all One Pot waste deposits.

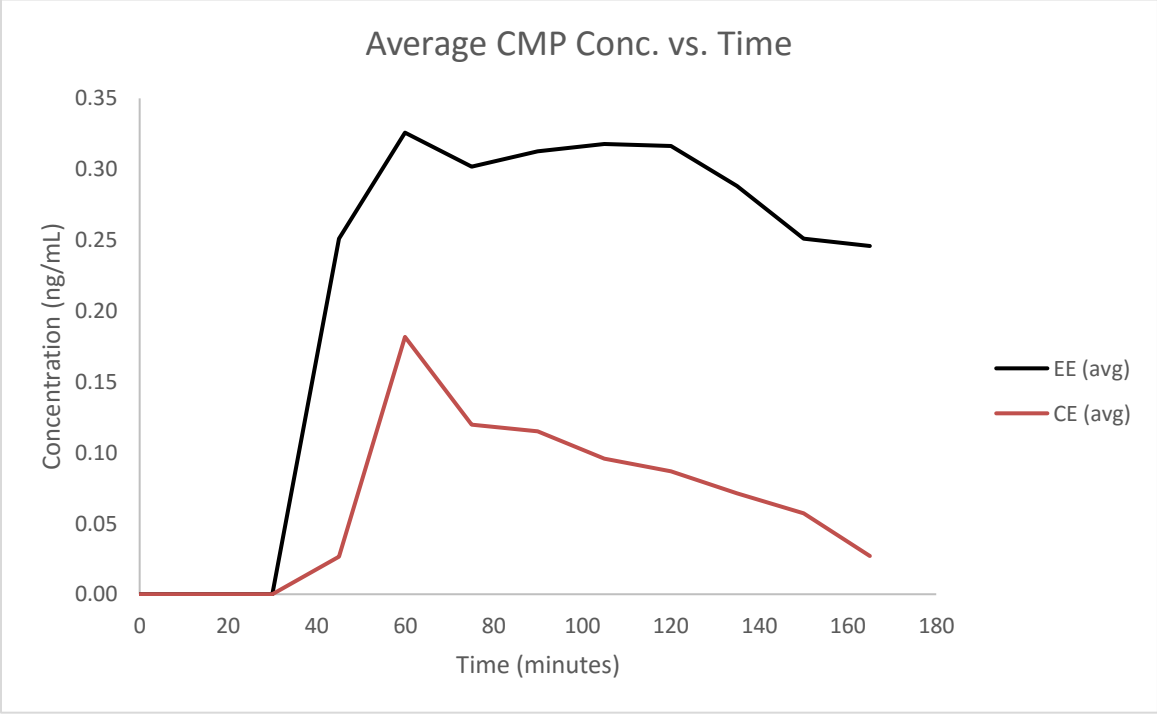


Figure 32. Average concentration of CMP (ng/mL) observed every 15 minutes after One Pot waste deposits.

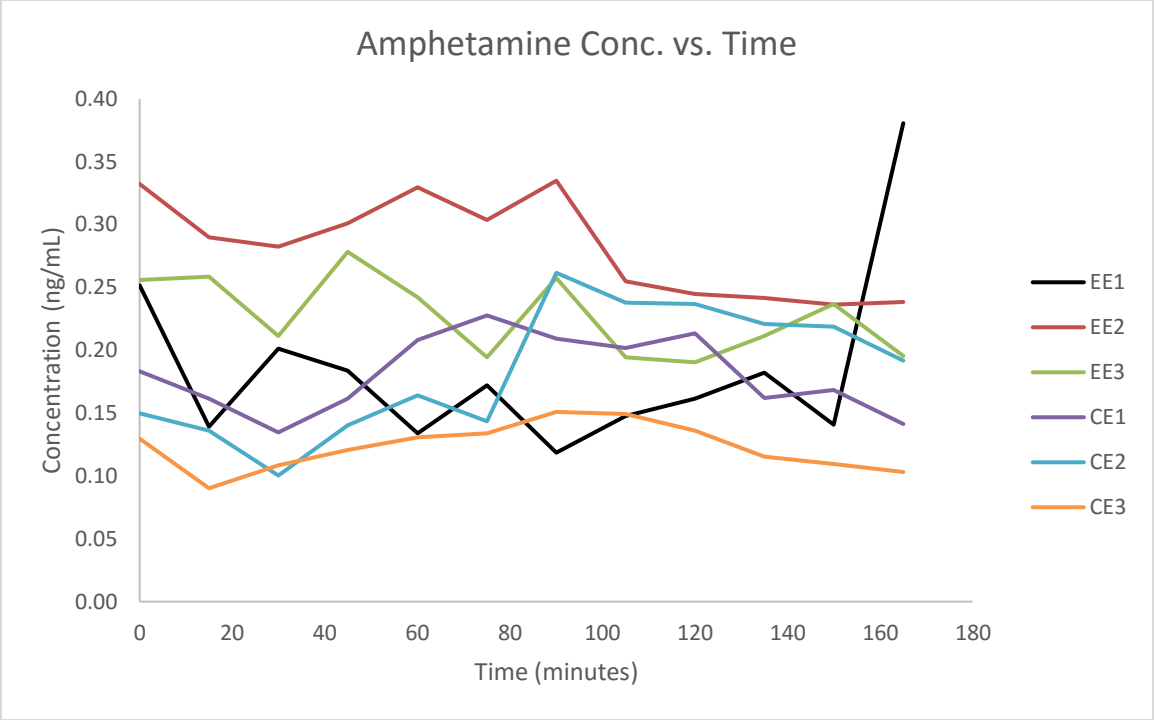


Figure 33. Comparison of the concentration of amphetamine (ng/mL) observed in sewage water samples collected every 15 minutes for all One Pot waste deposits.

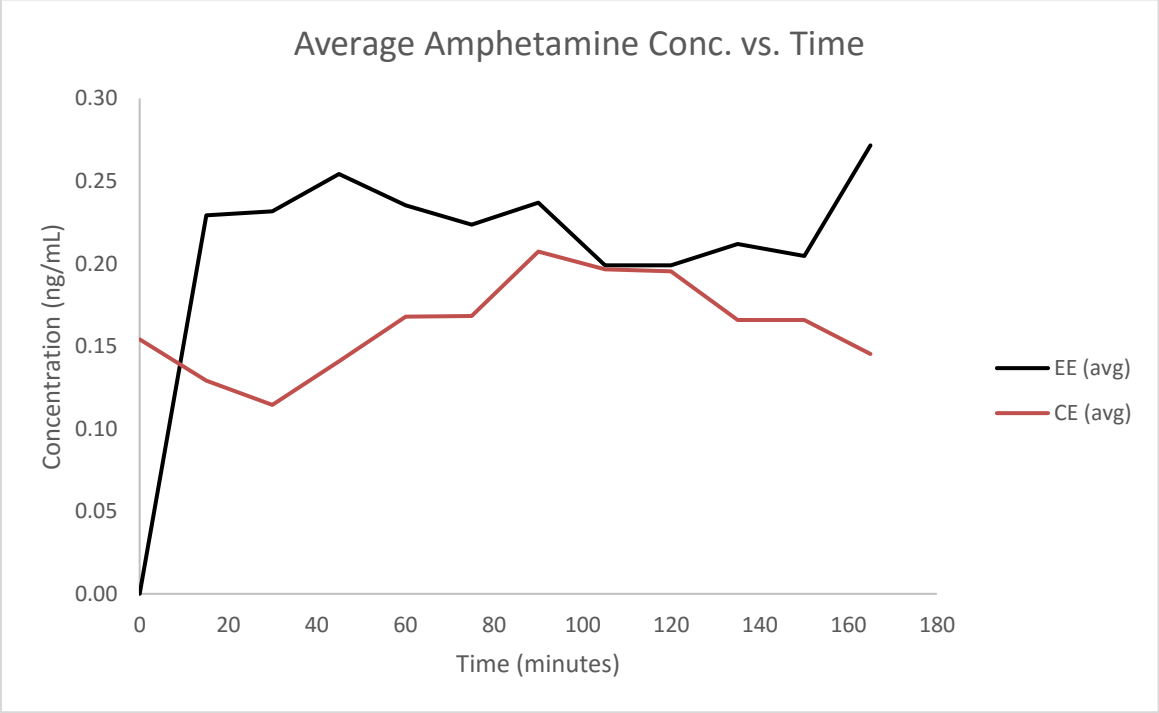


Figure 34. Average concentration of amphetamine (ng/mL) observed every 15 minutes after One Pot waste deposits.

The final laboratory results pertain the samples obtained from EE4 and CE4 syntheses. As mentioned in the methodology section, post-salt solvent, sludge, and product salts samples were stored for laboratory analysis, and not to be used within waste water deposit experiments. The characterization results from Chapter 2 provided drug compound concentrations within One Pot reaction waste. However, an additional synthesis of each solvent type was conducted to verify the results established in the previous chapter. Upon LC-MS/MS analysis, the following results were observed, as listed in Table 38.

Table 38. Characterization results from EE4 and CE4 reaction waste samples. All values listed in ng/mL.

	Post-Salt Solvent		**Sludge**		**Product Salt**	
Compound	**EE4**	**CE4**	**EE4**	**CE4**	**EE4**	**CE4**
Methamphetamine	102.4	2698	14.52	10.33	32.12	42.56
CMP	10.17	5.05	0.98	0.34	2.45	0.52
Pseudoephedrine	7.17	410.9	0.44	1.23	1.38	4.56
Ephedrine	28.7	1570	3.75	4.16	12.47	26.84
Amphetamine	0.00	0.00	0.00	0.00	0.00	0.00

The results indicate a much greater presence of drug compounds, with the exception of CMP, within the CE4 post-salt solvent than the EE4 post-salt solvent. As for the sludge and product salt samples, the two solvent types produced visually similar results.

3.5 Discussion

Based on the results of the SPE validation, a successful method was developed and implemented within OSU-FTTL to detect and quantitate One Pot methamphetamine related compounds. A LOQ was successfully established at 0.5 ng/mL for all target compounds. Accuracy and precision of both the calibrator and quality control levels fell within plus or minus 30% of the actual concentration. The matrix effects study demonstrated success with plus or minus 20% calculated values for all compounds. Additionally, recovery and process efficiencies were calculated for increased intel regarding the extraction process.

Following method validation, One Pot methamphetamine syntheses, were successfully performed. Overall, eight reactions were conducted, four utilizing diethyl ether and four utilizing camp fuel as the reaction solvent. As described above, the simulated laboratory waste solutions that were combined to create the total One Pot waste deposit were a lithium-in-water solution, sludge, and a post-salt solvent. After waste water collection, lithium was detectable with ICP-MS analysis. Based on the samples tested for the presence of lithium, a baseline sample, or sample collected prior to deposit, was compared with a peak sample, or sample likely to contain the highest abundance of the target compound. The peak samples were determined based on the results of the green dye tests, which provided approximate travel times of the One Pot manufacturing waste from the deposit site to the sampling location. According to the results obtained by SRNL ICP-MS analysis, lithium was not detected in two thirds of the base line samples. The only positive detections were EE2.1 and EE3.1, with concentrations of 0.01 and 0.02 ug/g, respectively. On the other hand, every deposit detected lithium in one or both of

the peak samples tested. The highest detected concentration of lithium was 0.30 ug/g, while the average of all detections, excluding baseline samples, was 0.16 ug/g. More research will need to be conducted, but based on the ICP-MS results, a spike in lithium concentrations within waste water could contribute to the identification of a One Pot methamphetamine laboratory.

Based on the waste water analysis of SPE with LC-MS/MS, methamphetamine, pseudoephedrine, ephedrine, CMP, and amphetamine were detected following One Pot waste deposit. As shown in the graph figures above, the time course samples demonstrated a low baseline of target compound, a sharp spike or increase, and then gradual decrease in concentration. Continued research is needed to determine if a similar spike in One Pot signature compounds and byproducts can be observed in uncontrolled or community situations.

A statistical test was performed on Time Course samples, with this analysis comparing EE and CE results of the average maximum concentrations obtained from each deposit. According to the results, every compound, with the exception of methamphetamine, was significantly different when comparing the two solvents commonly used in the One Pot method. For pseudoephedrine and ephedrine, a great statistical divide was experienced with p-values of 0.009 and 0.002, respectively. A similar argument can be made for methamphetamine, but based solely on visual observation of the concentrations obtained. For these three compounds, concentrations within the camp fuel effluent were much higher than those within the diethyl ether effluents. CMP and amphetamine, were not included in the statistical comparison, but demonstrated higher concentrations in favor of the diethyl ether deposits. The LC-

MS/MS characterization of EE4 and CE4 provides some explanation for the statistical differences observed within the two solvent type deposits. In contrast to the characterization results in Chapter 2, with the diethyl ether samples demonstrating a higher concentration of target compounds, the results from EE4 and CE4 exhibited the opposite. For the post-salt solvent sample, in particular, methamphetamine, pseudoephedrine, and ephedrine were roughly ten times the concentration within the CE4 sample when compared to the EE4 sample. Since these two One Pot reactions were performed in conjunction with EE1-EE3 and CE1-CE3, a safe assumption is that the reactions used for each waste water deposit, contained similar drug concentrations. The identification of the increased concentrations within CE post-salt solvents is a probable cause for the statistical difference of the average maximum concentration of methamphetamine, pseudoephedrine, and ephedrine observed following waste water analysis.

Additional waste water samples tested using LC-MS/MS analysis included those referred to as Pulse samples. As mentioned above, the Pulse feature on the waste water autosampler did not function properly. A varying number of samples were collected following each deposit, if any at all. For the samples collected at each pumpdown of the lift station, methamphetamine, pseudoephedrine, ephedrine, and amphetamine were detected at varying concentrations post-deposit. No noticeable trends were observed in the results, as concentrations would increase and decrease sporadically over the course of sample collection. An additional finding is that CMP was not detectable after an extended period of time. Since CMP was not detected in any of the Pulse samples, the Time Course samples were examined for the longest detection window of CMP in this

study. For diethyl ether and camp fuel deposits, the largest detection window is 195 and 150 minutes, respectively. The true detection window would require an additional test with an increased volume of time course samples. Knowledge that a window of detection exists for CMP could potentially be problematic for the implementation of the current reported methodology into the law enforcement community.

An important note based on all the LC-MS/MS waste water results is that the volume of sample utilized was only 2 mL, as compared to common sample volumes in the literature ranging up to hundreds of milliliters. With the controlled experimental setting and relatively small waste water system, 2 mL of sample was sufficient to detect compounds of the One Pot methamphetamine method. However, continued research, specifically increased sample volume extraction, will enhance the detection capabilities. For the signature manufacturing byproduct, CMP, improvements in methodology would only increase the window of detection for this compound. Future studies will not only need to conduct waste water testing for all target compounds within community settings, but confirm, or help to assign, drug concentrations within waste water being attributed to the disposal of One Pot methamphetamine waste material. As of now, the primary demand for continuing research lies with the determination of any other possible sources of CMP introduction into sewage effluent.

In summary, a simulated One Pot waste disposal experiment was successful in detecting the target compounds of methamphetamine manufacturing, which include, methamphetamine, pseudoephedrine, ephedrine, and CMP. The main metabolite of methamphetamine, amphetamine, was included in the detection method as a potential indicator of methamphetamine concentrations attributed to human consumption. Based

on the results, collected waste water over time demonstrated a dramatic increase or spike in all target compounds of the One Pot method. Additionally, no similar increase in amphetamine was observed. This successful experiment demonstrates the potential to implement waste water analysis as means to track and monitor clandestine laboratories within communities. Further research into this matter will increase detection capabilities, assist in assigning the source of the target compounds, and potentially, help locate and remove dangerous One Pot methamphetamine laboratories throughout the country. Consequently, this removal would also eliminate the likely harm that the hazardous and toxic chemicals associated with the One Pot method would cause to innocent bystanders and the environment.

CHAPTER IV

CMP DETECTION IN URINE SAMPLES

4.1 Introduction

1-(1',4'-cyclohexadienyl)-2-methylaminopropane, or CMP, has been identified as a signature byproduct of both the Birch Reduction and One Pot methamphetamine manufacturing methods, as explained in Chapter 2. The results from the second chapter confirmed the presence of CMP within One Pot reaction products, as well as waste material. In Chapter 3, the focus was centered around the ability to detect One Pot target compounds within waste water as a means to identify the location of clandestine laboratories within a community. Although CMP was detectable in sewage effluent following simulated waste disposal, a significant uncertainty exists in regards to whether the detection of the byproduct definitively identifies the presence of One Pot reaction waste. If CMP is detectable in trace amounts within the final methamphetamine salt that individuals are illicitly using, and potentially abusing, it is possible that CMP would excreted from the human body, thus contributing to any concentrations found within waste water.

One Pot methamphetamine product will potentially contain three compounds: methamphetamine, pseudoephedrine, and CMP. Depending on a variety of manufacturing factors, the amount of pseudoephedrine and CMP may be great or small.

Also, a product salt may have the possibility of not containing any starting material or byproduct. The results from Chapters 2 and 3, demonstrate a fairly large amount of pseudoephedrine following a 2-hour reaction. However, the OSU-FTTL One Pot method, as mentioned, is a scaled-down version for safety purposes. In a clandestine setting, an individual may use excess materials to ensure a more quality product. Additionally, due to the wide-range of One Pot procedures available online, a multitude of yields is likely present within the clandestine laboratory community. CMP, on the other hand, demonstrated higher amounts in One Pot reactions that utilized diethyl ether. Camp fuel syntheses, as discussed above, will not produce CMP as readily as diethyl ether due to increased immiscibility with water. Regardless of the amounts of pseudoephedrine and CMP within a One Pot product salt, the compounds, in addition to methamphetamine, may be consumed with illicit use. And as a result, knowledge of the consumed compounds' metabolism and excretion characteristics is paramount to any waste water analysis designed to detect or locate the presence of One Pot laboratory waste.

As previously discussed, methamphetamine is primarily excreted unchanged, with only approximately 10-20% metabolizing into amphetamine. Pseudoephedrine is excreted approximately 70% as the parent compound, and minor amounts are metabolized into nor-pseudoephedrine.[55] While the pharmacokinetics of starting material and the illicit product are well understood, CMP has not been studied within living subjects. The majority of research within the literature pertaining to CMP are focused on detection within the environment or clandestine laboratories. "Chemicals associated with clandestine drug laboratories are often disposed of covertly into soil, sewerage systems,

or public waste management facilities."[8] Typically, the studies are conducted as a means to identify potential pollution within the environment. While several studies have been focused on detecting manufacturing byproducts within soil and monitoring degradation, little to no research has been conducted that relate to the last two disposal routes mentioned in the quote above. Human consumption and excretion, as proven with the many studies discussed in Chapter 3, have demonstrated drug and metabolite contamination of the waste water system. As a result, any consumed manufacturing byproducts would be excreted, providing a detection potential in sewage effluent. However, a void in the literature creates uncertainty as to how the primary One Pot manufacturing byproduct interacts, i.e. metabolism and excretion patterns, within the human body.

Knowledge of the signature byproduct's interactions within the body, may not only identify other potential compounds to monitor within waste water, but may substantiate the significance of a CMP detection. If CMP is metabolized completely or primarily, detection in sewage effluent provides evidence of One Pot methamphetamine manufacturing. CMP metabolism has yet to be fully investigated. The following study was conducted to test urine samples that had previously tested positive for methamphetamine. Anonymized samples were collected from three different sources, two being pain management laboratories and another from a medical examiner's office. In total, 47 urine samples were received. OSU-FTTL, as part of another service, provides urine drug testing, therefore each sample received underwent a modified extraction process of the validated procedure. LC-MS/MS analysis provided drug detection results for amphetamine, methamphetamine, pseudoephedrine, and CMP only. Results

demonstrate the potential for CMP to be consumed and excreted, as a result of illicit methamphetamine use, which has yet to be confirmed in the literature. However, the very low prevalence of CMP within the urine samples indicates that CMP detection in waste water may still verify the presence of a One Pot clandestine laboratory.

4.2 Review of the Literature

Since the first discoveries of 1-(1',4'-cyclohexadienyl)-2-methylaminopropane, CMP has been the focal point of several research projects. As mentioned above, this byproduct is signature of methamphetamine manufacturing that utilizes a lithium and ammonia reduction.[27] The associated clandestine procedures that have been proven to result in CMP are the Birch Reduction and One Pot methods. While the Birch Reduction has the potential to produce greater amounts of CMP, the One Pot method, as demonstrated in Chapter 2, can have detectable amounts of the byproduct within the final methamphetamine salt. Individuals using an illicit substance produced from either of the two manufacturing methods, could potentially be ingesting CMP.

Analysis of urine for the presence of drug compounds is not a new endeavor. Urine drug testing can be applied to many scenarios, including, but not limited to, clinical, research, and forensic settings. Additionally, urine is typically analyzed to ensure individuals within a workplace setting are not abusing drugs that are illegal and/or may impair their ability to perform daily tasks. In fact, in 2003, more than 90% of U.S. companies decided to use urine as the specimen to be analyzed for drugs.[56] Other bodily fluids can be analyzed as well, such as blood or oral fluid, but urine testing is well established. The two primary confirmatory instruments to quantitate drugs within urine is GC-MS and LC-MS/MS. While GC-MS may be the most common, a rise in LC-

MS/MS prevalence and preference has been documented.[57] For the purposes of this review, only LC-MS/MS will be discussed as it is the instrument of choice for this study.

One of the first uses of LC-MS to analyze illicit drugs in human urine was validated in 1996. Tatsuno et al. developed an LC-MS method due to the challenges observed with GC-MS analysis. The challenges referenced included the demands of extensive sample treatment and/or derivatization to detect nonvolatile compounds.[58] The sample preparation implored for the LC-MS analysis was SPE. Results indicated successful analysis of illicit compounds within urine and demonstrated advantages over GC-MS.[58] Following initial research determining the possibility and potential of LC-MS, many research projects have been conducted to not only improve detection capabilities, but also expand the number of compounds that can be detected in a single run. According to one report, many groups continued to use traditional GC-MS methodologies despite learning of successful implementation of LC-MS. The reasoning behind this "considerable resistance" was not discussed by the authors.[60] However, possible sources could include immediate and already implemented availability of GC-MS, cost and time to acquire and validate LC-MS, and issues with sample interference of closely related drug compounds. Regardless, the discovery of LC-MS/MS, an increase in analytical confirmation, and its potential in drug testing became the focal point of many research projects, and helped evade the resistance seen with LC-MS. In 1999, a research group utilized LC-MS/MS to analyze several illicit drug compounds in urine samples. The group reported, in describing the instrumentation setup, "a rapid, simple, sensitive LC-ESI-MS-MS analysis…using specific transitions for each compound of interest."[59]

Enhanced sensitivity, with the addition of specific transitions, proved to play an important role in the growth of LC-MS/MS analysis prevalence in urine drug testing.

With the increasing implementation of LC-MS/MS in the drug testing community, many research groups sought to improve detection capabilities, expand the number of drugs that could be tested in a single run, and decrease overall analysis time. The majority of these goals are not surprising from a business perspective. LC-MS/MS methodologies were developed within laboratories to have the ability to detect many numerous drug compounds, both of illicit and pharmaceutical nature. However, other methodologies were developed to focus on the detection of a select group of related compounds and/or metabolites. For instance, Ming-Ren Fuh et al., in 2006, developed a method to detect amphetamine and methamphetamine using SPE with LC-MS-MS, and successfully reached a detection limit of 1 ng/mL.[57] Another group later expanded the amphetamine and methamphetamine group to include 3,4-methylenedioxymethamphetamine or MDMA, and 3,4-methylenedioxyamphetamine or MDA. For these four compounds, Andersson et al. reported LOD values of 43, 8, 8, and 2 ng/mL, respectively.[61] Variable detection limits are pursued depending on the nature of the research or business goals. For instance, a drug testing method designed to analyze prescription methamphetamine, Desoxyn, may not necessarily need a low limit of detection or quantification, if the goal is to simply confirm or deny an individual's use of the prescription. On the other hand, if a drug testing method is developed for sewage effluent analysis, such as those discussed in Chapter 3, the limit of detection for methamphetamine would need to be much lower to detect trace amounts of the illicit substance within a vast waste water system.

Despite the many varying limits of detection reported for amphetamine and methamphetamine, LC-MS/MS analysis of these two compounds within the literature is extensive. For example, method development and LC-MS/MS product design has moved beyond the mere detection and quantification of drug compounds. Several groups have conducted studies in regards to enantiomeric separation. Foster et al., in 1998, developed a method to separate, quantitate and compare the presence of d- and l-amphetamine and methamphetamine within urine samples. Results indicated successful separation with an ingredient called Marfey's reagent, and produced limits of detection and quantitation of 0.16 and 0.40 mg/L, respectively for each enantiomer.[62] The successes of this study have helped pave the way for research in regards to comparing the amount of illicit versus over-the-counter use of methamphetamine. For instance, a study conducted in 2014 attempted to separate and quantitate enantiomers to substantiate any claims that a positive methamphetamine result was due to use of a nasal decongestant containing the l-methamphetamine form of the compound. Newmeyer et al., although not testing urine samples, was successful in distinguishing the enantiomer of methamphetamine consumed.[63] These findings indicate the potential to apply a similar procedure to a multitude of samples types such as urine or even waste water.

Besides methamphetamine and amphetamine, the One Pot manufacturing product and metabolite, the precursor pseudoephedrine has also been successfully detected within urine. A recent LC-MS/MS study, conducted in 2015, was able to reach 0.1 ng/mL limit of detection for pseudoephedrine, all while analyzing for over 70 other compounds.[64] Other research groups have had similar success. In fact, the methodology in the following section was adopted from an OSU-FTTL method that was developed and is

currently being validated to detected a multitude of illicit and pharmaceutical drugs, including pseudoephedrine.

The literature discussed above relates to compounds that will, methamphetamine and amphetamine, and may be, pseudoephedrine, detected following the use of One Pot methamphetamine product. However, and as previously mentioned, no research has been conducted in regards to detecting CMP, the Birch Reduction and One Pot signature byproduct, in urine specimens. A demand exists for this research to not only potentially gather intelligence about the source of the methamphetamine product an individual consumed, but also confirm or deny the importance of detecting CMP in waste water as a means to identify the presence of a clandestine laboratory. Until this study, no research has been published with regards to CMP's interaction within the human body. Further and more extensive research is needed to fully understand CMP metabolism and potentially identify metabolites that can differentiate methamphetamine consumption versus manufacturing waste.

4.3 Methodology

Urine samples that had previously tested positive for methamphetamine with LC-MS/MS were delivered to OSU-FTTL to analyze for the presence of CMP. The sample collection process did not involve interaction with a human subject nor did it provide access to identifiable personal and private information. All urine samples collected were completely anonymized. According to 45 CFR 46.102 (d) and (f) of the Code of Federal Regulations for Human Research, this project did not qualify as human subject research and was not subject to oversight by the Oklahoma State University Institutional Review Board. Approximately 1 mL of 47 urine samples was delivered to OSU-FTTL, and

arrived in tubes labeled as "Urine" with a number designation of 1 through 47. All samples were stored in a freezer until sample preparation and extraction.

For laboratory analysis, a different extraction and instrument setup was used when compared to the characterization and waste water samples, in Chapters 2 and 3, respectively. A simpler extraction and a different LC-MS/MS platform was utilized because of prior and continued success with urine drug testing within OSU-FTTL.

LC-MS/MS calibrators and quality controls were prepared using methamphetamine, pseudoephedrine, amphetamine, and CMP drug standards mentioned previously. The calibrators included levels of 250, 150, 100, 75, 25, 10, 5, 1, and 0.5 ng/mL of each compound. Four QC levels were prepared and included 150, 25, 1, and 0.1 ng/mL of each compound, and were labeled A, B, C, and D, respectively. All dilutions for the drug levels mentioned above were performed in certified drug-free urine (UTAK Laboratories Inc., Valencia, CA). An un-fortified, drug-free urine sample was set aside to be utilized as a negative control. For an internal standard, a solution was prepared using methamphetamine-d5, pseudoephedrine-d3, and amphetamine-d5, all purchased from Cerilliant (Cerilliant Corp, Round Rock, TX).

For all calibrators, quality controls, and urine sample extractions, 50 μL of sample was aliquoted into a new tube, alongside 20 μL of the prepared Internal Standard solution. Additionally, 180 μL of sample diluent, 5% methanol in water, was added to each urine sample. The tube was then capped, vortexed for 10 seconds, and centrifuged for 10 minutes at 13,000 revolutions per minute (rpm). Following centrifugation, 200 μL of each sample was then transferred to a separate injection vial and stored in a refrigerator until LC-MS/MS analysis.

Shimadzu UFLC pumps paired with Shimadzu 80-40 MS/MS was used for the LC-MS/MS analysis, shown in Figure 35.

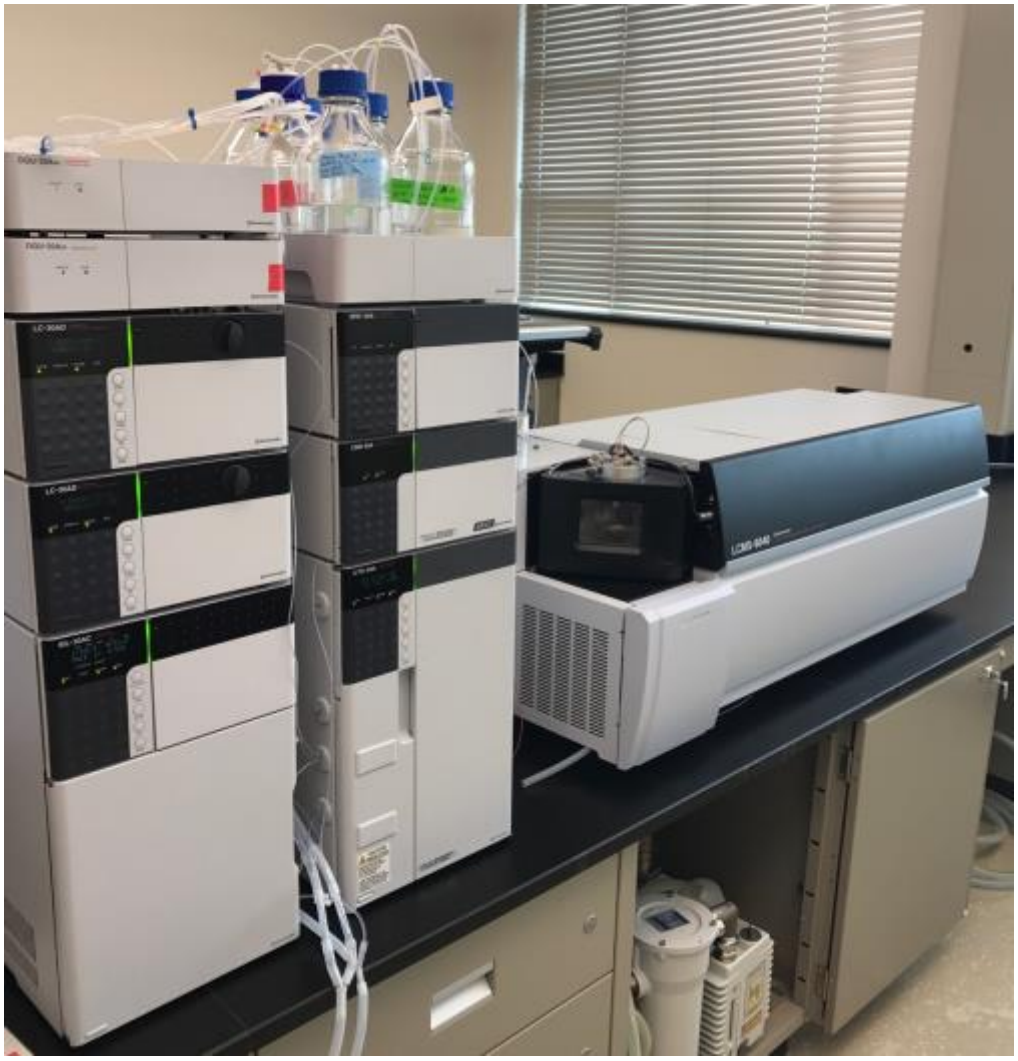


Figure 35. LC-MS/MS Instrument Setup at OSU-FTTL. Shimadzu UFLC pumps on the left and Shimadzu 80-40 MS/MS on the right.

For liquid chromatography, separation was achieved with a Restek Raptor Biphenyl 2.7 µm column (50 x 2.1 mm) with a Restek Raptor Biphenyl 2.7µm guard cartridge (5 x 3.0 mm) (Restek Corporation, Bellefonte, PA). Mobile Phase A consisted of 2mM ammonium formate and 0.1% formic acid in LC-MS grade water, while Mobile Phase B

consisted of 2mM ammonium formate and 0.1% formic acid in LC-MS grade methanol. Ammonium formate was purchased from Alfa Aesar (Alfa Aesar, Ward Hill, MA). Formic Acid was purchased from EDM (EDM Millipore Corp, Billerica, MA). Methanol was purchased from JT Baker (Avantor Performance Materials Inc., Center Valley, PA). Nanopure water was obtained using a Barnstead Nanopure Diamond laboratory water system (Thermo Scientific, Waltham, MA). The LC method had a total flow rate of 0.500 ml/min. Mobile Phase B concentration began at 5.0% and increased at a consistent rate until reaching 95% after 8.5 minutes. The concentration was then immediately increased to 100%, sustained for 45 seconds, immediately decreased back to 5.0%, and then held until the conclusion of the sample run at 11 minutes. Injections were set at 10 µL and the oven temperature was set to 30 ^{o}C.

For mass spectrometry, two product ions, fragments of the precursor ion, for each compound were selected given the mass-to-charge (m/z) ratios that produced optimal instrument response, see Table 39.

Table 39. Mass Spectrometry Parameters. Target analytes Methamphetamine, Pseudoephedrine, Amphetamine, and CMP were identified using two mass ion fragments each. Internal standards include Methamphetamine-d5, Pseudoephedrine-d3, and Amphetamine-d6.

Compound	**Precursor (m/z)**	**Product (m/z)**	**Q1 Pre Bias (volts)**	**CE (volts)**	**Q3 Pre Bias (volts)**
CMP	151.65	58.15	-30.0	-11.0	-21.0
	151.65	79.15	-30.0	-18.0	-30.0
Methamphetamine	149.75	119.05	-16.0	-25.0	-44.0

	119.00	91.20	-14.0	-13.0	-33.0
Methamphetamine-d5	154.95	92.10	-16.0	-20.0	-34.0
Pseudoephedrine	165.95	148.00	-20.0	-25.0	-14.0
	165.45	91.00	-46.0	-30.0	-34.0
Pseudoephedrine-d3	168.9	151.00	-34.0	-14.0	-28.0
Amphetamine	136.1	91.00	-14.0	-35.0	-32.0
	135.9	118.95	-16.0	-15.0	-46.0
Amphetamine-d5	140.9	93.00	-16.0	-17.0	-34.0

Similar to the other LC-MS/MS analyses, trueness of the compound identity was confirmed through comparing the areas of the two MRM transitions, resulting in an identification or ID ratio, also known as an MRM ratio. Every precursor and product ion pairing generated a chromatographic peak. MRM ratios for each compound, with the exception of internal standards, were calculated by dividing the peak area of the second pairing of each compound by the peak area of the first pairing. To build an acceptable ID ratio range, the ratios observed for every calibrator were averaged. For results to be accepted, the ID ratio must be within 30% of the ID ratio average using two significant figures for the percentage value.

4.4 Findings

Following the simple extraction of the prepared calibrator and quality control samples, LC-MS/MS results indicated accuracy and precision. Multiple injections of each calibrator and quality controls quantitated within plus or minus 30% of the actual

concentration. Additionally, all negative control samples were negative for the target compounds. Based on peak qualifying criteria, listed in the methodology section, the following limit of quantitation (LOQ) for amphetamine, methamphetamine, pseudoephedrine, and CMP were 5, 75, 5, and 0.5 ng/mL, respectively. The upper limit of quantitation (ULOQ) was 250 ng/mL for all compounds. The results obtained from LC-MS/MS analysis of all 47 urine specimens are listed in Table 40.

Table 40. LC-MS/MS results of 47 urine samples that previously tested positive for methamphetamine. The drug compounds monitored were amphetamine, methamphetamine, pseudoephedrine, and CMP. All values listed in the table are in ng/mL. Any positive value above the ULOQ, 250 ng/mL, was reported with a result of >250. All negative values or concentrations that quantitated below the LOQ for each compound, listed in row 2, are designated with five hyphen marks.

	Amphetamine Concentration	**Methamphetamine Concentration**	**Pseudoephedrine Concentration**	**CMP Concentration**
ULOQ	250	250	250	250
LOQ	5	75	5	0.5
Urine 1	>250	>250	-----	-----
Urine 2	15.536	135.208	-----	-----
Urine 3	152.729	-----	-----	-----
Urine 4	210.353	-----	-----	-----
Urine 5	>250	>250	-----	-----
Urine 6	>250	>250	-----	-----

Urine 7	223.527	>250	-----	-----
Urine 8	27.288	>250	-----	-----
Urine 9	>250	-----	-----	-----
Urine 10	74.819	>250	7.415	1.483
Urine 11	>250	>250	-----	-----
Urine 12	>250	-----	-----	-----
Urine 13	>250	-----	-----	-----
Urine 14	>250	-----	-----	-----
Urine 15	>250	>250	-----	-----
Urine 16	49.634	98.975	-----	-----
Urine 17	>250	>250	-----	-----
Urine 18	>250	>250	-----	-----
Urine 19	>250	>250	-----	-----
Urine 20	58.801	168.936	-----	-----
Urine 21	106.179	>250	-----	-----
Urine 22	-----	121.392	-----	-----
Urine 23	>250	>250	-----	-----
Urine 24	234.415	>250	-----	-----

Urine 25	179.209	>250	-----	-----
Urine 26	>250	>250	-----	-----
Urine 27	-----	204.14	-----	-----
Urine 28	>250	>250	-----	-----
Urine 29	-----	>250	-----	-----
Urine 30	>250	>250	-----	-----
Urine 31	>250	>250	-----	-----
Urine 32	>250	-----	-----	-----
Urine 33	130.768	>250	-----	-----
Urine 34	-----	>250	-----	-----
Urine 35	>250	>250	-----	-----
Urine 36	>250	>250	-----	-----
Urine 37	>250	>250	-----	-----
Urine 38	>250	>250	-----	-----
Urine 39	>250	>250	-----	-----
Urine 40	>250	>250	-----	-----
Urine 41	>250	>250	-----	-----
Urine 42	>250	>250	-----	-----

Urine 43	222.539	>250	-----	-----
Urine 44	83.463	>250	-----	-----
Urine 45	-----	-----	-----	-----
Urine 46	245.182	>250	-----	-----
Urine 47	>250	>250	-----	-----

Only one urine sample, Urine 10, demonstrated positive results for pseudoephedrine and CMP. The majority of the remaining samples tested positive for both methamphetamine and amphetamine. Urine 3-4, 9, 12-14, 32, and 45 tested negative for methamphetamine while testing positive for amphetamine. Urine 22, 27, 29, 34, and 45 tested negative for amphetamine while testing positive for methamphetamine. The only sample to test negative for every target compound was Urine 45.

4.5 Discussion

Based on the results above, a relatively low LOQ was achieved for all compounds, 5 ng/mL or less, with the exception of methamphetamine. Two important factors negate concern on this matter. The first is that the LC-MS/MS method was slightly modified from a current urine drug testing method of over 70 compounds, that had already been validated within OSU-FTTL. This methodology, in an attempt to avoid instrument saturation or over-detection, adjusted several compounds such as methamphetamine to not exhibit the optimal response in terms of sensitivity. As a result, methamphetamine is still accurately identified, but large concentrations within a patient sample will not overload the instrument detector. The second reason is that the urine

samples received had already been verified to contain methamphetamine. CMP, the focal compound of this study, had the lowest LOQ at 0.5 ng/mL. As discussed in the chapters above, CMP was determined to be present in very small amounts within One Pot methamphetamine products. Therefore, CMP was optimized on the LC-MS/MS to generate the best detection results.

The analysis of urine samples demonstrated a positive result in every sample, except for Urine 45. This specific sample was the only specimen to observe no peaks for any of the target compounds. A possible explanation for this is that the instruments used for the initial and subsequent analyses vary. While Urine 45 may have tested positive for methamphetamine initially, the results did not meet identification criteria on the instrumentation at OSU-FTTL. The majority of samples contained high concentrations of both methamphetamine amphetamine, as expected. Samples delivered to OSU-FTTL were previously reported as methamphetamine positive, and as explained above, amphetamine is a major metabolite. All remaining samples tested positive for either amphetamine or methamphetamine alone. However, one sample, Urine 10, demonstrated positive results for all four target compounds, amphetamine, methamphetamine, pseudoephedrine, and CMP. The findings within this single sample confirm the possibility that CMP can be ingested, as a byproduct of One Pot methamphetamine, and then excreted in urine. This confirmation is the only definitive interpretation of the results. Many unknowns remain unanswered. Every urine sample, with the exception of Urine 10, was negative for both pseudoephedrine and CMP. Unfortunately, this does not eliminate the consumption of One Pot methamphetamine. With the high variability reports in clandestine communities, pseudoephedrine may be completely converted into

methamphetamine. As for CMP, the levels within the consumed methamphetamine salt, of unknown origin, may be low enough to avoid detection with the current LOQ of 0.5 ng/mL. Additionally, with no prior research or evidence of byproduct's interaction within the body, the results above do not account for any metabolized or degraded CMP before excretion. Perhaps, the methamphetamine consumed in User 10 had significant levels of CMP, and thus, could be detected despite compound metabolism.

Although several unknowns still exist following this study, a few notions can be interpreted from the findings. CMP has been successful identified in a human urine specimen. While this specific finding, after continued research, may eliminate CMP as definitive identifier of a One Pot clandestine laboratory in waste water, the detection does provide the law enforcement community with an additional compound to monitor. The presence of CMP within a user's urine may help indicate or confirm the source of methamphetamine manufacturing. On the other hand, with only a single detection of CMP in 47 different urine samples, there lies a possibility that the majority of consumed methamphetamine was not produced with the One Pot method. Further research can confirm this notion, but if true, detection of CMP within sewage effluent may still uniquely identify the presence of a One Pot methamphetamine laboratory within the community.

CHAPTER V

CONCLUSION

In summary, the research projects above were designed to characterize and develop methodology to proactively detect One Pot clandestine laboratories within the population. The One Pot method has been shown to be the primary manufacturing technique over the past decade. A signature byproduct of the One Pot method was previously identified as 1-(1',4'-Cyclohexadienyl)-2-methyl-aminopropane, commonly referred to as CMP, and its presence within One Pot methamphetamine products and waste materials was confirmed. To revisit the goals of this study, they included:

1) characterize the One Pot methamphetamine method,
2) develop waste water analysis methodologies to detect One Pot waste in sewage effluent, and
3) determine the significance of detecting the primary One Pot byproduct, CMP, within environmental samples.

Based on the findings from the first study in Chapter 2, potential yields found within One Pot methamphetamine laboratories were established, and the detectable presence of target compounds within reaction waste was discovered and quantified. CMP, although demonstrating greater concentration with diethyl ether reactions, was detectable in both final product salts, as well as waste material samples like sludge and post-salt solvent.

The intelligence gathered from these findings will assist future yield and detection studies of the One Pot methamphetamine manufacturing method.

Based on the findings from the second study in Chapter 3, sewage effluent samples were collected downstream over time, following a simulated One Pot waste disposal into a local waste water system. As hypothesized, results indicated a significant increase or spike in One Pot target compounds. CMP was determined to have the smallest window of detection, but with increased detection capabilities, such as increasing sample volume, the byproduct could be detected within sewage effluent for a longer period of time following a clandestine laboratory waste disposal. Additionally, no spike in amphetamine, the major metabolite of methamphetamine, was observed. This finding indicates the possibility of monitoring other drug compounds un-related to the One Pot method as a means to distinguish methamphetamine consumption versus methamphetamine production. The results from the second study, in general, demonstrate the potential to further improve and implement waste water analysis as a means to track and locate One Pot clandestine laboratories.

Based on the results from the third study, CMP can be detected in urine following illicit One Pot methamphetamine consumption. Although a single detection within a relatively small sample size cannot provide definitive interpretations, the investigation has never been completed and/or documented within the literature. The findings from the analysis of urine samples created a demand and incentive to further study CMP within human subjects. Research projects pertaining to the byproduct's metabolism and excretion patterns, as well as improving analytical detection limits, will help substantiate CMP as a unique identifier of clandestine methamphetamine production within

environmental samples, and possibly unveil other potential metabolites to monitor during waste water analysis.

Overall, the results from the three individual, yet related, studies provide both previously unknown knowledge and a demand for continued investigation into the One Pot methamphetamine method. One Pot methamphetamine product and reaction waste yields have been reported. Although clandestine laboratory yields can be highly variable, the results established the primary compounds of interest that would be important to monitor in any detection campaign, whether pertaining to health risks or the environment. Additionally, a waste water analysis method was developed and validated to detect the target compounds of the One Pot method. Methodologies from the simulated experiment will need to be applied within communities, and detection capabilities increased. However, the successful detection of a noticeable and significant increase or spike in target compounds post-deposit create the potential to implement waste water analysis in a law enforcement setting. And lastly, urine samples, which had previously tested positive for methamphetamine, were analyzed for the presence of the One Pot precursor and primary byproduct. Results demonstrated one sample out of 47 was positive for both compounds. In addition to providing potential information in regards to the source of consumed methamphetamine, the findings could potentially indicate that the majority of methamphetamine users tested did not consume One Pot methamphetamine. Depending on the trends in the studied community or population, a CMP detection, and most certainly a spike, within sewage effluent could indicate the presence of One Pot methamphetamine waste.

Expanded research of the manufacturing process, produced target compounds and byproducts, potential sources of related compounds into the environment, and detection capabilities will continue to increase the awareness of the law enforcement and scientific communities in regards to the One Pot methamphetamine method. Due to this method's popularity and reported dangers, clandestine laboratories, from a public safety standpoint, need to be identified before the reactions and toxic chemicals cause adverse health effects for family members, friends, and neighbors. And the overall goal of continued research is to assist in the investigation and removal of clandestine laboratories from the community and environment before more harm is done. The accomplishments of this research provide the initial pathway to the development of a method to proactively detect dangerous One Pot methamphetamine clandestine laboratories.

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VITA

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