SEPARATION OF D- AND L-METHAMPHETAMINE IN POSTMORTEM SAMPLES VIA LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

By

APRIL DAWN MITCHELL

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SEPARATION OF D- AND L-METHAMPHETAMINE IN POSTMORTEM SAMPLES VIA LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

Thesis Approved:

Jarrad Wagner, Ph.D.

Thesis Adviser

Ron Thrasher, Ph.D.

Committee Member

Mark Payton, Ph.D.

Committee Member

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Name: APRIL DAWN MITCHELL

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Abstract:

Methamphetamine, or meth, is a commonly abused drug in the United States, especially Oklahoma. Meth exists in the form of two enantiomers, d-meth and l-meth. D-meth affects the central nervous system and causes the addictive sense of euphoria. On the other hand, l-meth is active in the peripheral nervous system and works as a decongestant. For these reasons, d-meth is available only as the prescription Desoxyn and l-meth is available over-the-counter as Vick's Vapor Inhaler.

In order to obtain meth without a prescription, meth is prepared in clandestine laboratories in the United States and Mexico. In death investigations involving meth, it may be useful to separate the enantiomers, which may indicate licit or illicit exposure to methamphetamine.

To investigate this, an LC-MS/MS method was developed to separate d-meth, l-meth, and its main metabolite, amphetamine. The method was applied to postmortem blood samples collected by the State of Oklahoma Office of the Chief Medical Examiner from November 2015 through March 2016. Of the 72 specimens extracted, 87% had a 95% or greater ratio of d-meth to l-meth, which indicates that the decedents were using prescription or illicit meth. All samples containing methamphetamine has some amount of d-meth, making over-the-counter sources unlikely.

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CHAPTER I

INTRODUCTION

In 2009, the unexpected death of actress Brittany Murphy shocked the nation. Days before her death, Murphy complained of flu-like symptoms. The toxicology report revealed many drugs present in Murphy's system at time of death: acetaminophen and hydrocodone, two pain relievers; chlorpheniramine, a cold and allergy treatment; and l-methamphetamine, the active ingredient in the nasal decongestant Vicks® VapoInhaler™.[1,2]

Like many pharmaceutical drugs, methamphetamine, or meth, is present in two forms, or enantiomers: d-methamphetamine and l-methamphetamine. Both forms have the same physical and chemical properties but the body reacts differently to each form. D-methamphetamine, a Schedule II drug, is more pharmacologically reactive and is considered "street meth." Conversely, l-methamphetamine is less reactive and is the active ingredient in Vicks® VapoInhaler™. Meth can be present in purely d- or l- forms or even a 50/50 mix of both forms.

Methamphetamine is excreted as the unchanged parent drug, but it is also metabolized into amphetamine. Like meth, amphetamine also has d- and l- enantiomers. Since amphetamine is often present in meth cases, amphetamine should be included in the analysis. Most drug screening methods simply yield a "positive" meth result and do not distinguish between the two forms;

therefore, a person innocently taking Vicks® could be accused of using meth. Also, many crime labs do not have a method for separating enantiomers. Samples requiring analysis must be sent to an off-site reference lab. For these reasons, crime labs need a method to separate the enantiomers on-site.

The separation of meth enantiomers is not a new problem in forensic toxicology. To achieve separation, existing studies use capillary electrophoresis (CE), gas chromatography mass spectrometry (GC-MS), or liquid chromatography tandem mass spectrometry (LC-MS/MS).[3–20] Aside from forensic analyses, enantiomers have been separated in environmental wastewater samples.[3,4]

Current separation methods use GC-MS, which requires derivatization. This is a technique that chemically modifies the sample to make the sample better suited for analysis. However, many derivatizing reagents can be dangerous. Additionally, the derivatization process may lengthen the analytical procedure.[5]

Of the existing LC-MS/MS studies for meth separation, most use a chiral column, which separates the enantiomers quickly without derivatization.[6–8] However, chiral columns are expensive. Crime labs with strict budgets may be unable to adopt a method with such a costly component.

While less common, derivatization can be adapted to LC-MS/MS. This method enables the use of a less expensive achiral column. Two studies examine the efficiency of Marfey's reagent as the derivatizing reagent.[9,10] Only one of two studies analyzes postmortem samples but uses absorbance spectrophotometry as a detector rather than the desired mass spectrometry.[9] The Newmeyer study analyzed oral fluid specimens using LC-MS/MS, a method that uses blood is still needed.[10]

Marfey's reagent has been proven to separate the methamphetamine and amphetamine enantiomers without the use of an expensive chiral column. The resulting data may help determine legal or illegal meth use in postmortem samples. Additionally, data may indicate the type of production method used in clandestine meth labs, which may assist law enforcement.

The purpose of this project was to develop a method to separate d- and l-methamphetamine and amphetamine via LC-MS/MS using Marfey's reagent. The method was applied to postmortem blood samples provided by the toxicology lab at the Office of the Chief Medical Examiner (OCME) in Oklahoma City, Oklahoma. The application was a historical analysis to determine whether d- or l-methamphetamine was more prevalent in postmortem samples obtained between November 2015 and March 2016. D-methamphetamine will likely be more prevalent than l-methamphetamine since d-methamphetamine is illicit "street meth."

CHAPTER II

REVIEW OF THE LITERATURE

2.1 Overview

Methamphetamine, or meth, is a central nervous system stimulant that is classified as a phenethylamine compound. Other stimulants in this class include amphetamine, MDMA, or ecstasy, and MDEA.[11] Meth is a highly addictive drug that can be ingested, smoked, snorted, or injected. However, meth does have some therapeutic uses; it is available as the prescription Desoxyn to treat asthma, narcolepsy, obesity, depression, and attention-deficit/hyperactivity disorder (ADHD).[11,21]

A review of the literature demonstrates that methamphetamine is a complex drug with many published studies. However, a method for analyzing postmortem blood samples without the use of a chiral column is still needed. The goal of this chapter is to provide the reader with an overview of methamphetamine. History, clandestine methods, pharmacology, and analytical methods will be discussed.

2.2 History of Methamphetamine

Methamphetamine was first created in 1893 in Japan.[21] Widespread methamphetamine use began during World War II. Japanese kamikaze pilots received injections of

methamphetamine before taking off on their final missions.[22] American, German, and Japanese soldiers also used meth to increase endurance, suppress appetite, and reduce fatigue.[21,23] After the war, amphetamine use increased in the United States.

In 1970, Congress passed the Controlled Substances Act. This law introduced a scheduling system, which placed drugs into categories I-V based on medical value, harmfulness, and potential for dependence and/or misuse and abuse.[24] Schedule I drugs, such as heroin, have no medicinal value and high potential for abuse.[24] Schedule II drugs, including methamphetamine, have a high potential for abuse, but some therapeutic use.[24] Conversely, Schedule V drugs are easily obtainable with a refillable prescription.

2.3 Clandestine Laboratory Methods

As the U.S. government increases regulations on meth, illicit laboratories and methods emerge. One illicit method uses phenylacetone as the starting material for meth production.[21] Called the P2P method, the phenylacetone is combined with methylamine to create methamphetamine.[12] However, the P2P method is a lengthy process which produces a less potent form of meth.[21]

The U.S. government eventually classified phenylacetone as a Schedule II drug due to its neurotoxic effects. As a result, pseudoephedrine and ephedrine, common ingredients in cold medicines, became the new precursors for meth. The Red Phosphorous and Birch methods emerged.

First, the Red Phosphorous method uses hydroiodic acid and red phosphorous to reduce the ephedrine or pseudoephedrine molecules.[12] Red phosphorous can be obtained from matchbook strikers, and hydroiodic acid can be easily created using iodine.

Second, the Birch method reduces ephedrine or pseudoephedrine using lithium in liquid ammonia.[12] Lithium can be obtained from batteries, and liquid ammonia can be purchased from agricultural suppliers.[12]

In 2005, the U.S. federal government passed the Combat Methamphetamine Epidemic Act aimed at reducing the availability of pseudoephedrine.[23] As a result, pharmacies removed products containing pseudoephedrine from the shelves and placed them behind the counter. To this day, many states require a driver's license and a signature in order to buy products containing pseudoephedrine.

Despite the new limitations on pseudoephedrine, meth "cooks" adapted and began to make meth using the One-Pot method, which is a modified version of the Birch method. This method produces very small batches of the drug which can be made in two liter soda bottles.[22] Small reaction containers allow cooks to produce methamphetamine anywhere.

The regulations on pseudoephedrine caused domestic meth production to decrease significantly. As a result, much methamphetamine in the United States is currently imported from Mexico.

2.4 Enantiomerism

Methamphetamine has two forms, called enantiomers: d- and l-. Both forms have the same physical and chemical properties, but are different. Hands are a great analogy for enantiomerism. While the left and right hands look and function similarly, they are not the same. Hands are actually mirror images of each other. D- and l-methamphetamine are also mirror images of each other.

Enantiomers differ in the direction they rotate plane-polarized light and are described as either dextrorotatory (d-) or levorotatory (l-).[25] Levorotatory compounds rotate polarized light to

the left and are “left-handed.” Conversely, dextrorotatory compounds rotate polarized light to the right and are “right-handed.”[13] Enantiomers can exist independently of one another but can also exist equally as racemates.

Illicit methamphetamine cooks will create different forms of the drug. The P2P method yields a racemic 50/50 mixture of d- and l-methamphetamine; the Red Phosphorous, Birch, and One-Pot methods yield purely d-methamphetamine.[25] Therefore, d-methamphetamine is considered illegal “street meth.” Discernment of the cook method can help law enforcement understand current cook trends and illicit use.

2.4.1 Pharmacology of Enantiomers

Methamphetamine creates a sense of alertness, euphoria, and well-being by increasing norepinephrine and dopamine in the central nervous system (CNS).[21] The high from methamphetamine lasts longer than the high from cocaine. [21] When meth is smoked or injected, the effects of the drug are instantaneous; however, full effects of meth occur 5 minutes after snorting and 20 minutes after ingestion.[21]

In addition to affecting the CNS, meth also affects the peripheral nervous system by producing the “fight-or-flight” response.[21] Symptoms of this response include increased heart rate, body temperature, blood pressure, and breathing rate.[21] Meth can also lead to cardiovascular complications such as irregular heartbeat, high blood pressure, and heart attack.[21] Meth users also exhibit signs of mental health problems, skin infections, and poor oral hygiene.

The enantiomers affect the body differently depending on the form. D-methamphetamine, or street meth, is more potent to the CNS whereas l-methamphetamine, the active ingredient in Vicks® VapoInhaler™, is more potent to the peripheral nervous system.[25] Therefore, d-meth is significantly more addictive.

The forms of meth will also impact the high that the user experiences. Meth produced by the P2P method is less potent since the drug is a 50/50 mixture of d- and l-methamphetamine.[12] Pure d-methamphetamine produced from pure ephedrine or pseudoephedrine will lead to a more potent drug and a better high.[12] The information can be seen in Table 1.

Table 1. Illicit methamphetamine cook methods, precursor drugs, and corresponding enantiomer products.

Illicit Meth Cook	Precursor Drugs	Product
P2P	Phenyl-2-propanone	d- and l- methamphetamine
Red Phosphorous	l-ephedrine or d-pseudoephedrine	d-methamphetamine
Birch	l-ephedrine or d-pseudoephedrine	d-methamphetamine
One-Pot	l-ephedrine or d-pseudoephedrine	d-methamphetamine

2.5 Analytical Methods

The separation of meth enantiomers is not a new topic in forensic science. As technology advances, more efficient analytical techniques emerge. Since most drug screening methods do not distinguish between d- or l-methamphetamine, a reliable confirmatory test is needed. A variety of confirmatory instruments will be discussed in this section, including gas and liquid chromatography. While both instruments can be used with a variety of detectors, this paper will focus on mass spectrometry (MS) as the detector. Although capillary electrophoresis (CE) has been used to separate enantiomers, more research and development is needed before this method can become more viable.

2.5.1 Gas Chromatography

Gas chromatography, or GC, has been the instrument of choice for methamphetamine analysis.[12] However, this instrument has a disadvantage. During GC analysis, drug samples are

exposed to extreme heat. Many drugs are unstable at high temperatures and require derivatization, which is the addition of a special chemical that makes the sample more stable during analysis. Many different types of special chemicals, or derivatizing reagents, can be used.

For enantiomer analysis, derivatization is a very common method. Chemicals containing fluorine are popular derivatives for methamphetamine separation. Trifluoroacetyl-L-prolyl chloride (L-TPC) and heptafluorobutyryl obutyrl-L-prolyl chloride have been used to separate MDMA enantiomers in rat and human specimens.[13,26]

2.5.2 Liquid Chromatography

Liquid chromatography, or LC, has become more popular in forensic labs due to its ability to detect low levels of drugs.[5] In general, LC methods require less sample preparation and derivatization than GC; less sample preparation may decrease overall analysis time.[5]

For enantiomer separation using LC, there are two options: enantiomer-specific (chiral) columns and derivatization. A chiral column completely eliminates the need for derivatization. The drug sample simply undergoes sample preparation and is ready for analysis. Many published LC methods use chiral columns to separate the meth enantiomers.[6–8]

The Astec Chirobiotic™ column from Sigma-Aldrich has effectively separated the meth enantiomers. Common biological specimens of interest include urine and oral fluid.[6–8] The high cost of this chiral column is a major disadvantage. Toxicology labs with strict budgets may be unable to adopt a method with such an expensive component.

The expense of chiral columns creates a need for a more cost-effective separation method. Derivatization, which may be adapted to LC, enables the use of less expensive achiral columns. Studies show that (−)-1-(9-fluorenyl)ethyl chloroformate (FLEC) and (1-fluoro-2,4-

dinitrophenyl-5-l-aniline amide (Marfey's reagent) are effective derivatizing reagents for meth separation.[9,10]

2.6 Other Applications

Enantiomeric separation of methamphetamine also has clinical and environmental applications. Patients at pain management clinics must take frequent urine tests in order to prove compliance with the drug program. A patient testing positive for meth may claim the result was due to the l-methamphetamine in Vicks® VapoInhaler™.

Many drugs find their way into wastewater and sewage through human excretion.[5] Several studies have successfully separated enantiomers found in wastewater residues.[3,4] This information can lead to more studies on the human effects of trace level consumption of drug enantiomers.

2.7 Conclusion

Methamphetamine is an old drug with a rich history. Regulation of meth precursor drugs has led to a variety of illicit meth cooks, such as P2P, Red Phosphorous, Birch, and One-Pot methods. The P2P method yields a 50/50 racemic mixture of d- and l-meth that is less potent. The Red Phosphorous, Birch, and One-Pot methods yield more potent d-methamphetamine.

Separating the meth enantiomers can aid drug-related investigations by law enforcement. The enantiomers can provide information about clandestine lab production methods. However, the separation does not provide definitive conclusions about drug origin since meth does have some therapeutic uses.

Several methods using CE, GC, and LC have successfully separated the meth enantiomers. Methods for LC are becoming popular. However, most LC studies use an expensive chiral column. The purpose of this research is to develop a more cost-effective method using

Marfey's reagent to separate d- and l-meth in postmortem blood samples via LC-MS/MS. The method will then be applied to case samples provided by the OCME toxicology lab.

CHAPTER III

METHODOLOGY

3.1 Overview

The purpose of this project was to develop a method to separate the two forms of methamphetamine via LC-MS/MS using Marfey's reagent for pre-column derivatization. The method was then applied to casework from the toxicology lab at the Office of the Chief Medical Examiner (OCME) in Oklahoma City. The application determined whether d- or l-methamphetamine was more prevalent in postmortem blood samples by calculating a relative percent ratio, which may help determine legal or illegal meth use in postmortem samples. Data may also indicate the type of production method used in clandestine meth labs, which can assist law enforcement. The project analyzed postmortem samples, which are not subject to IRB oversight. Additionally, any identifying information has been anonymized.

This chapter outlines the method for the separation of d- and l-methamphetamine via LC-MS/MS using Marfey's reagent. Topics discussed in this chapter include: chemicals and equipment, extraction and derivatization methods, data analysis, and validation.

3.2 Chemicals and Equipment

3.2.1 Materials

D-methamphetamine, l-methamphetamine, ± methamphetamine, d-amphetamine, l-amphetamine, ± amphetamine (1 mg/mL), and internal standards (ISTD) ± d_{11}-methamphetamine and ± d_{11}-amphetamine (1 mg/mL) were purchased from Cerilliant (Round Rock, TX, USA). The following chemicals were purchased from Fisher Scientific (Waltham, MA, USA): sodium bicarbonate, methanol, ammonium hydroxide, n-butyl chloride, sulfuric acid, and hydrochloric acid. Marfey's reagent was purchased from Sigma-Aldrich (St. Louis, MO, USA), and deionized water was produced on-site.

3.2.2 Reagents and Solutions

A variety of solutions was needed throughout the method. These solutions include: concentrated ammonium hydroxide (pH >10), n-butyl chloride, 1 N sulfuric acid, 0.10% methanolic hydrochloric acid, 1.0M sodium bicarbonate, 1.0M methanolic hydrochloric acid, 60:40 methanol/water solution, and 0.1% (w/v) Marfey's reagent in acetone. The Marfey's reagent solution was stored at 4 °C in an amber vial for up to one month. Other solutions were prepared as needed and stored at room temperature.

3.2.3 Instrumentation

The system consisted of a 1290 Infinity LC with a 6420 Triple Quad mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Data were acquired using MassHunter version B.07.00 software. Nitrogen evaporation was completed using an N-EVAP 116 (Organomation Associates, Inc., Berlin, MA, USA).

3.2.4 Postmortem Samples

Postmortem blood samples were provided by the toxicology lab at the Office of the Chief Medical Examiner (OCME) in Oklahoma City, Oklahoma. The OCME database was used to compile a list of samples that been confirmed positive for methamphetamine via GC-MS. Samples were obtained between November 2015 and March 2016. Age, sex, cause and manner of death, and other significant autopsy results were documented.

3.3 Sample Preparation

3.3.1 Sample Extraction

The blood samples were extracted using the OCME alkaline drug liquid-liquid extraction method which is used to screen for weak bases and their metabolites. With all sample batches, four control samples in blood were included at the beginning of the run: a negative control with 100 ng/mL internal standard (ISTD), a 100 ng/mL calibrator, a 100 ng/mL positive control, and a double blood blank.

In 15 mL screw cap tubes, 100 µL of 1 µg/mL ISTD solution and 1 mL of sample blood were added. If a full milliliter was not available, 500 or 800 µL of sample blood was placed in the test tube and filled to 1 mL with control blood. Next, 500 µL concentrated ammonium hydroxide was added and vortexed, followed by 7.5 mL n-butyl chloride. The samples were roto-extracted for 10 minutes and centrifuged at 2800 rpm for 5 minutes.

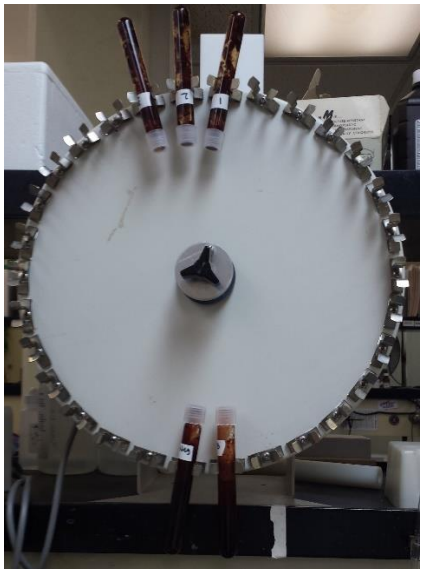

Figure 1. The roto extract apparatus spins and inverts the samples over 10 minutes, ensuring complete mixture.

Next, the upper organic layer was transferred to a clean glass tube. The bottom blood layer was discarded. After transferring, 2.5 mL 1 N sulfuric acid was added. The samples were roto-extracted for 10 minutes and centrifuged at 2800 rpm for 5 minutes.

After centrifugation, the organic upper layer was aspirated to waste. Five-hundred microliters of concentrated ammonium hydroxide were added and vortexed thoroughly before adding 2.5 mL of n-butyl chloride. The samples were roto-extracted for 10 minutes and centrifuged at 2800 rpm for 5 minutes.

Finally, the upper organic layer was carefully transferred to a 7 mL conical glass tube. The solution was taken to dryness under a stream of nitrogen to approximately half volume, then 20 µL of 0.1% methanolic hydrochloric acid was added and evaporated to complete dryness at 40 °C. The vials were removed from the nitrogen stream immediately after drying.

3.3.2 Sample Derivatization

The derivatization method was adapted from Newmeyer.[10] The samples were reconstituted in 100 µL of water and 20 µL of 1M sodium bicarbonate, then vortexed. Next, 100 µL of 0.1% Marfey's reagent was added and vortexed for 10 seconds.

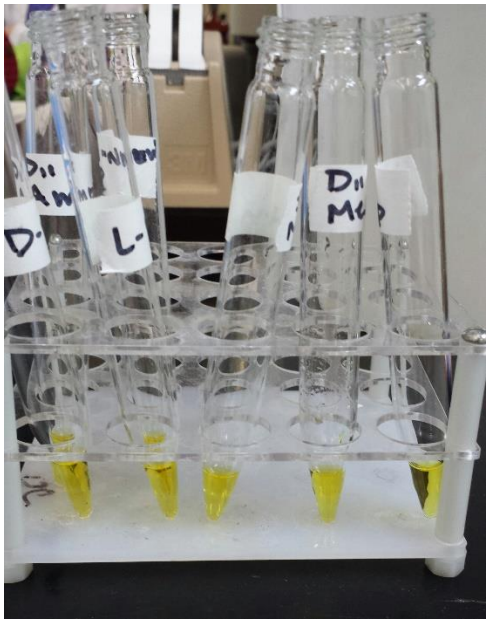

Figure 2. Marfey's reagent solution is bright yellow.

The samples were heated at 45 °C for 1 hour. After 60 minutes, the samples cooled to room temperature before the addition of 40 µL of 1M HCl. Afterward, the samples were taken to dryness. Once fully evaporated, the samples were reconstituted in 200 µL of methanol/water (60:40) and vortexed. The samples were centrifuged for 5 minutes at 2800 rpm. A filter was used to transfer the samples to injection vials for instrumental analysis.

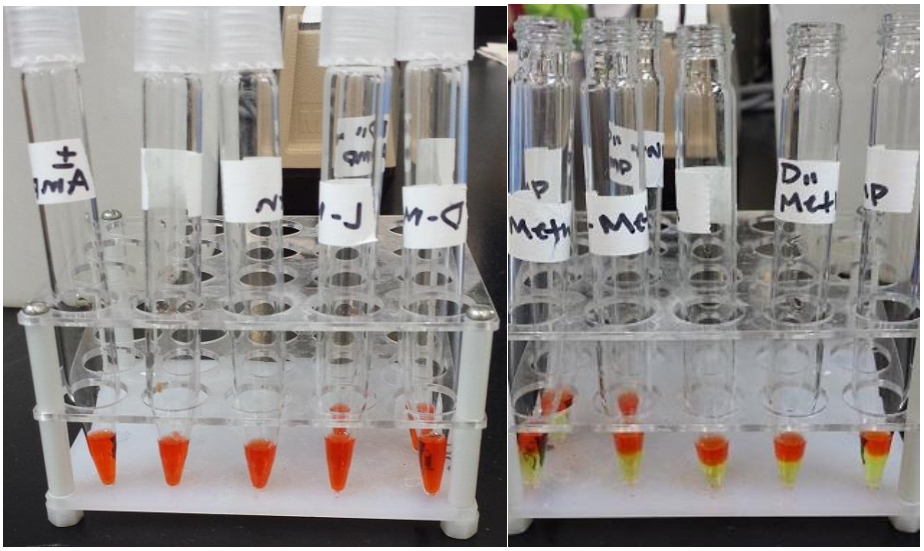

Figure 3. (Left) The heating process causes the samples to change from yellow to red.
Figure 4. (Right) The addition of HCl creates the bottom yellow layer.

3.3.3 LC-MS/MS

Chromatographic separations were completed using an Agilent Poroshell 120 EC-C18 column (2.7 µm, 2.1 x 150 mm) under isocratic conditions with 35% water (mobile phase A) and 65% methanol (mobile phase B) for 10 minutes. HPLC flow was diverted to waste the first 3 minutes of analysis. The oven temperature was 30 °C with a flow rate of 0.3 mL/minute and an injection volume of 5 µL.

Mass spectrometry data were acquired using ESI negative mode. The gas temperature was set to 350 °C with a gas flow of 11.0 L/min. The nebulizer was set to 15 psi and the capillary to 4000 V. MRM transitions and MS/MS details can be seen in Table 3.

3.4 Validation

Since the case samples were previously confirmed positive for methamphetamine using GC-MS, a multi-point calibration curve was not used to establish linearity at this time. Instead, a single point calibrator with a concentration of 100 ng/mL was extracted alongside the samples.

The limit of detection was determined by derivatizing a mix of drug standards in water at a concentration of 1 ng/mL. The derivatized standard mix was injected onto the LC-MS/MS at injection volumes of 2, 5, and 10 µL. The 1 ng/mL standard peaks could be differentiated from noise with an injection volume of 5 µL.

Validation studies according to SWGTOX guidelines will be conducted by the OCME at a later date.

3.5 Data Analysis

Data were analyzed using MassHunter version B.06.00 to determine the relative percent ratio of d- to l-methamphetamine in each individual sample. Using Microsoft Excel, the percentage of d-methamphetamine was calculated by dividing the concentration of l-methamphetamine by the concentration of d-methamphetamine. GraphPad Prism software (San Diego, CA) was used to calculate the 95% confidence interval.

3.6 Conclusion

This chapter outlines the method for the separation of d- and l-methamphetamine by LC-MS/MS using Marfey's reagent. Chemicals, equipment, and optimization were discussed. Postmortem blood samples were extracted using a liquid-liquid method and were derivatized using Marfey's reagent. Using the internal standard area, a percentage of l- to d-methamphetamine and l- to d-amphetamine were determined. The method will be fully validated in accordance with SWGTOX guidelines by the OCME toxicology lab at a later date .[27]

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Presentation of Results

A total of 72 decedent blood samples were extracted, derivatized, and analyzed. The samples fell into two categories: >95% l- to d-methamphetamine and other. Sixty-three samples fell into the >95% d-meth category while 9 fell into the “other” category. Concentrations below the LOD were considered negative. Table 2 shows the results of the samples that fell into the “other” category.

Table 2. Data in the "Other" category

Case	Result
18	Negative for methamphetamine and amphetamine
20	49% d-methamphetamine
22	89% d-methamphetamine
29	75% d-methamphetamine
36	81% d-methamphetamine
56	63% d-methamphetamine
57	63% d-methamphetamine
63	Negative for methamphetamine
64	85% d-methamphetamine

The MRM transitions and MS/MS parameters for derivatized methamphetamine and derivatized amphetamine can be seen in Table 3.

Table 3. Retention time (RT), precursor and product ions (m/z), collision energy (CE), fragmentor (Frag), and cell acceleration voltage (CAV) for derivatized methamphetamine and amphetamine enantiomers.

Analyte	RT (min)	Precursor ion	Product ions	CE (V)	Frag (V)	CAV (V)
Derivatized d-amphetamine	7.41	386.1	325	15	120	4
			308	20	120	4
Derivatized d-amphetamine D_{11}	7.12	397.1	336	15	135	4
			319	20	135	4
Derivatized d-methamphetamine	5.54	400.1	338.8	15	135	4
			323.8	15	135	4
Derivatized d-methamphetamine D_{11}	5.46	411.1	350	15	135	4
			335	15	135	4
Derivatized l-amphetamine	6.81	386	325	15	120	4
			308	20	120	4
Derivatized l-amphetamine D_{11}	6.55	397	336	15	135	4
			319	20	135	4
Derivatized l-methamphetamine	5.15	400	338.8	15	135	4
			323.8	15	135	4
Derivatized l-methamphetamine D_{11}	5.05	411	350	15	135	4
			335	15	135	4

4.1.2 Case Studies

In addition to decedent demographic information, the author also had access to the narratives provided by OCME death scene investigators. Two narratives from the >95% d-methamphetamine category and two narratives from the "other" category will be presented here. All identifying information has been anonymized.

Case 1

The decedent was a 32-year-old white female with a history of asthma. Decedent was found supine on the floor, gasping for air. When EMS arrived, the decedent was in respiratory distress. Upon arrival at the hospital, the decedent was unresponsive with no improvement. The decedent had a history of drug abuse and was known to smoke meth. Specimens were taken at the

hospital, which tested positive for methamphetamine, THC, and benzodiazepines. The initial physician believed the decedent had been manufacturing meth in the residence, but this claim was unsupported. Cause of death was anoxic encephalopathy due to asthma and complicated by meth use. The sample contained >95% d-methamphetamine.

Case 2

The decedent was a 36-year-old white male whose medical history was unknown. Law enforcement received conflicting stories. The decedent was either an occupant in a vehicle that became stranded while driving through a flooded low water crossing or was attempting to swim out to rescue the vehicle's occupants and was caught in the current. The decedent was found 236 yards from the vehicle in approximately 3 feet of water. There was a positive history of methamphetamine use. The cause of death was drowning and the sample contained >95% d-methamphetamine.

Case 3

The decedent was a 51-year-old white female with a history of hypertension. The decedent was complaining of a burning chest and trouble breathing and drove herself to the ER where she later died. The husband said the only medical history was hypertension and the decedent took medication to treat it. However, the decedent was positive for amphetamines and opiates. The husband denied any history of drug or alcohol abuse. No trauma was noted to the decedent and no foul play was suspected. The cause of death was cardiac tamponade due to hemopericardium due to ruptured ascending aortic dissection due to hypertension and atherosclerotic cardiovascular disease with methamphetamine toxicity as a contributing factor. D-methamphetamine was determined to be 75%.

Case 4

Decedent was a 39-year-old white female driver of a vehicle with a male passenger in the front seat. The vehicle was travelling westbound on a four lane divided highway with a center median cable barrier. The driver attempted to make an improper U-turn from the shoulder of the highway when the vehicle was impacted on the driver's side door by a westbound vehicle. The decedent was pinned in the driver's seat and pronounced dead at the scene by EMS. No foul play was suspected, and the deceased driver was ruled to be at fault for the accident due to improper action. The cause of death was multiple blunt force injuries and the sample contained 63% d-methamphetamine.

4.2 Discussion

4.2.1 Analysis

The purpose of this research was to develop a qualitative method to separate d- and l-meth via LC-MS/MS and then apply the method to postmortem blood samples provided by the OCME toxicology lab for an historical analysis. The research hypothesized that d-methamphetamine would be more prevalent since most methamphetamine cooks produce d-meth and d-meth is commonly abused in Oklahoma.

The results are consistent with the hypothesis. Sixty-three out of 72 case samples (87.5%) had >95% d-methamphetamine to l-methamphetamine. Cases 18 and 63 had no methamphetamine, and were therefore considered 0% d-meth. Only 7 cases out of 72 had less than 95% d-meth and can be seen in Table 2. The mean of the data was 95.12%. The 95% confidence interval was 91.71%-98.53%, which supports the hypothesis that d-methamphetamine would more prevalent in postmortem blood samples obtained in Oklahoma between November 2015 and March 2016.

The prevalence of d-methamphetamine in the results indicates that decedents were either using prescription meth or illegal meth. The lack of l-methamphetamine indicates that over-the-counter sources, such as Vick's, were unlikely.

Based on the literature review, the expected ratios of d-meth to l-meth would be 0%, 50%, and >95%. However, 6 samples ratios did not fit into the expected categories. One reason for the unexpected ratios was possible instrument saturation, which occurs when the concentration of d-meth is too great and overwhelms the instrument. Saturation might lead to a lower ratio of d-meth. A saturated peak can be detected by unusually broad peak width.

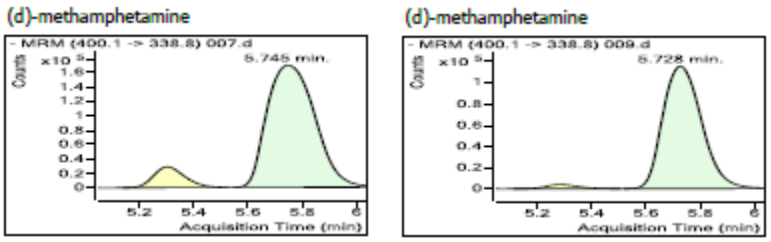

Figure 5. A saturated d-methamphetamine peak (left) compared to an unsaturated d-methamphetamine peak (right). The saturated peak is more broad than the unsaturated peak.

Although three of the six "other" samples had the appearance of saturation, the other three samples had normal peak shape. The unexpected ratios could be due to human error during analysis or contamination. Another possibility is that the methamphetamine had unexpected ratios of d- to l-methamphetamine at the time of consumption. Without testing the meth itself, the possibility cannot be ruled out.

4.2.2 Validation

Full validation according to SWGTOX guidelines was intended for this method.[27] However, a malfunction rendered the instrument unusable for 6 weeks during the intended validation period. Because of the malfunction, a full validation was no longer possible in the

available timeframe. Steps were taken to ensure that the runs were accurate, precise, and without carryover.

The blood samples were previously quantitated for methamphetamine, so multi-point calibration curve was not used. Instead, a single-point calibrator with a concentration of 100 ng/mL was used. Accuracy was measured by running a calibrator and a positive control at 100 ng/mL. The concentration of the positive control was compared to the concentration of the calibrator. For each of the sample runs, the positive control sample was within 15% of the first calibrator.

To assess precision, the calibrator and positive control were run at the beginning of each sample batch. A total of seven batches were run over seven days. For all batches, the positive control concentrations were within 15% of the calibrator concentrations.

To assess potential carryover, the double blood blank was run after the calibrator and positive control samples. No drugs were present in the blank, which would indicate that no carryover occurred at a concentration of 100 ng/mL.

Before using the method outside of research purposes, the OCME toxicology lab will complete a full validation according to SWGTOX guidelines. Method validation is required to make sure the results are accurate and will stand up in court. A full validation will include these studies: accuracy, precision, carryover, selectivity, matrix effects, recovery, processed sample stability, sensitivity, and a calibration model.

4.3 Benefits of the Study

One of the major benefits on the study is its cost-effectiveness. Rather than using an expensive chiral column, this research separated methamphetamine and amphetamine with a regular C_{18} column. The method offers an affordable in-house analysis option for forensic

laboratories facing budget cuts, which could reduce the need for sending samples to a reference lab.

For this study, the derivatization process was adapted from Newmeyer and combined with the liquid-liquid extraction used by the OCME toxicology lab.[10] The process was simple and did not require any specialized chemicals or reagents. Additionally, the derivatization can easily be paired with a different extraction method, such as solid-phase, which will allow the forensic lab to adapt the analysis based on the needs of the lab.

Although this study focused on postmortem application, the method could easily be used in antemortem and clinical settings.

Methamphetamine use is on the rise in Oklahoma. This research provides an historical analysis on the type of methamphetamine used by decedents in the state of Oklahoma from November 2015 through March 2016. Analysis of the type of meth being used can potentially provide information about the type of cooks used to produce the methamphetamine.

4.4 Limitations

While the results may make implications as to the cook type used to produce the methamphetamine, there is no way to know where the decedents' acquired their meth. Also, the toxicology lab does not have direct access to the decedents' prescription histories, which means that a decedent could have a legitimate prescription for Desoxyn.

Another limitation was that the study did not provide an extensive historical analysis of meth use. The samples were obtained from decedents between November 2015 and March 2016. With this limited timeframe, changes in meth type trends over an extended period of time in Oklahoma cannot be studied.

In the current OCME building, the toxicology lab has limited storage space for samples. Specimens that do not have holds on them are destroyed on a monthly basis to make room for incoming specimens. These storage issues significantly limited the historical analysis of this study.

The liquid-liquid extraction and derivatization steps were very time-consuming when combined. With ten samples and four controls, the total bench time was approximately 5 hours. A forensic lab may wish to shorten the analysis time to create a more practical method. Pairing the derivatization with a different extraction method, such as solid-phase extraction, may reduce total bench time.

When building the method in MassHunter, the d- and l-methamphetamine and amphetamine peaks would show up in the same window. Since both d- and l- enantiomers have the same precursor ion, the software had difficulty distinguishing the correct peaks. Poor peak shape resulted and the concentrations became inaccurate. To correct this error, a value of 0.1 was added to all d- enantiomer precursor ions (See Table 3).

Due to time constraints, a full validation was not conducted. The validation must be conducted according to SWGTOX guidelines before the samples can be analyzed outside of a research setting.[27] The validation process would also include sending the samples to a reference laboratory to compare the ratios of d- to l-methamphetamine and amphetamine obtained in the forensic lab with the ratios obtained in the reference lab.

4.5 Future Research

This study presents several opportunities for additional research. First, the author was given access to the OCME database, which provided information such as cause and manner of death, age, sex, race, and narratives of the death scenes. The demographics of the decedents was not considered in this study, but could be analyzed in future research.

Second, an extensive historical analysis should be explored. Due to the limited space at the OCME toxicology lab, samples prior to November 2015 could not be analyzed. However, other forensic labs may keep specimens for longer periods of time. Such labs could conduct similar studies to track the ratios of d- and l-methamphetamine and amphetamine over longer periods of time.

Finally, different populations of methamphetamine users could be investigated. This study focused on postmortem samples in Oklahoma, but antemortem and clinical samples could also be analyzed. Examining methamphetamine cases in other states may also be beneficial. Different regions of the United States may yield different ratios, which could indicate that a different meth cook had been used.

CHAPTER V

CONCLUSION

Methamphetamine has a negative connotation even though it has legitimate uses, such as the prescription Desoxyn or Vick's Vapor Inhaler. Unfortunately, standard analytical methods do not differentiate between the d- and l-enantiomers of meth and amphetamine and will simply yield a "positive" result. A person using Vick's Vapor Inhaler could be accused of using methamphetamine, like Brittany Murphy.

Enantiomer separation analysis helped to clear Brittany Murphy's name, but the sample had to be sent to a reference lab for testing. Most toxicology labs do not currently have methods available in-house to separate enantiomers. Developing separation methods is not a new problem in forensic toxicology. Studies that use capillary electrophoresis, GC-MS, or LC-MS/MS have already been conducted on the topic.

GC-MS analysis often uses derivatization, which is labor intensive and can lengthen the analytical process. Common LC-MS/MS methods typically use a chiral column that is shortens the process, but is very expensive. Forensic labs facing budget cuts may be unable to afford such a costly component.

Although les common, derivatization can be adapted to LC-MS/MS, which allows the use of a less expensive achiral column. Two studies utilize Marfey's reagent to separate d- and l-

methamphetamine; these studies analyze urine with absorbance spectrophotometry and oral fluid with mass spectrometry.[9,10] However, a Marfey's reagent method that uses blood is still needed. The purpose of this research was to develop a method to separate d- and l-methamphetamine and amphetamine on LC-MS/MS using Marfey's reagent. The method was then applied to postmortem blood samples from the Office of the Chief Medical Examiner toxicology lab in Oklahoma City, Oklahoma. A historical analysis was also conducted on the samples, which were obtained between November 2015 and March 2016. The research hypothesized that d-methamphetamine would be a more prevalent result since the common meth cooks produce d-meth rather than l-meth.

The analytical method was developed by combining the OCME liquid-liquid extraction with the derivatization method adapted from Newmeyer.[10] The samples were run on an Agilent 1290 Infinity LC with a 6420 triple quad and an achiral column.

A total of 72 samples were analyzed and placed into 2 categories: >95% and "other." Sixty-three samples (87.5%) fell into the >95% category while the remaining 9 were placed in the "other" category. Two of the samples in the "other" category had no meth, and the other 7 samples had ratios between 49% and 89%. The results supported the hypothesis that d-meth would be more prevalent.

The prevalence of d-methamphetamine indicates that decedents were either using prescription or illicit meth. The lack of l-methamphetamine indicates that over-the-counter meth sources were unlikely for this population.

The research showed that the derivatization method can be paired with other extraction methods to separate d- and l-meth and amphetamine using Marfey's reagent and an achiral column. This method is ideal for labs with strict budgets and are unable to buy an expensive chiral column. The research also indicates the type of meth used by decedents in Oklahoma from November 2015 through March 2016.

The study also created a few limitations. The method developed in this study was labor intensive and took several hours to perform. Lengthy methods may not be practical to most toxicology labs. Next, the storage issues at the OCME prevented a more extensive historical analysis from being conducted. Conducting the analysis on samples over a longer period of time may have provided trends about the type of meth used in Oklahoma.

In conclusion, there is an option for toxicology labs wishing to separate methamphetamine and amphetamine enantiomers in-house. Labs should consider the cost of equipment and the length of analysis when deciding which analytical method to adopt.

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VITA

April Dawn Mitchell

Candidate for the Degree of

Master of Science

Thesis: SEPARATION OF D- AND L-METHAMPHETAMINE IN POSTMORTEM SAMPLES VIA LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

Major Field: Forensic Science

Biographical:

Education:

Completed the requirements for the Master of Science in Forensic Science at Oklahoma State University, Stillwater, Oklahoma in July, 2016.

Completed the requirements for the Bachelor of Science in Forensic Chemistry at Western Illinois University, Macomb, Illinois in 2013.