DETECTION OF ILLICIT DRUGS IN VARIOUS MATRICES VIA TOTAL VAPORIZATION SOLID-PHASE MICROEXTRACTION (TV-SPME)

by

Kymeri Elizabeth Davis

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. John Goodpaster

Forensic and Investigative Sciences Program

Dr. Nicholas Manicke

Forensic and Investigative Sciences Program

Dr. Frédérique Deiss

Department of Chemistry & Chemical Biology

Approved by:

Dr. Christine Picard

Head of the Graduate Program

For Lance, Whisper, Abu, & myself.

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ABSTRACT

Author: Davis, Kymeri, E. MS
Institution: Purdue University
Degree Received: August 2019
Title: Detection of Illicit Drugs in Various Matrices via Total Vaporization Solid-Phase Microextraction (TV-SPME)
Major Professor: John Goodpaster

In Headspace Solid-Phase Microextraction (Headspace SPME), a sample is heated to encourage a portion of the analyte into the headspace of a vial. A coated fiber is introduced into the sample headspace and the analyte is adsorbed onto the fiber coating. Total Vaporization Solid-Phase Microextraction (TV-SPME) is a technique that is derived from this technique. In TV-SPME, liquid samples are completely vaporized allowing for better adsorption and fewer matrix effects. This method does not require any sample preparation, utilizes minimal supplies and can be automated, making it both an efficient and cost-effective method. Chapter 1 will discuss the theory of SPME and TV-SPME.

In Chapter 2, the detection of ɣ-hydroxybutyric acid (GHB) and ɣ-butyrolactone (GBL) in beverages is discussed. The detection of these compounds in beverages is of importance because these drugs may be used to facilitate sexual assault. This crime utilizes substances that cause sedation and memory loss. The derivatization of GHB as well as the properties that make GHB difficult to detect will be discussed.

Chapter 3 will discuss the detection of methamphetamine and amphetamine (as their trifluoroacetyl derivatives), GBL, and the trimethylsilyl derivative of GHB in human urine. Amphetamine is a metabolite of methamphetamine, therefore, both drugs should be identified within biological samples. GHB and GBL are metabolites of one another and interconvert when in aqueous solution. This interconversion will be discussed.

Chapter 4 will cover method optimization of the Total Vaporization Solid-Phase Microextraction method. Analytes of interest for these analyses were methamphetamine, amphetamine, GHB, and GBL. The optimal extraction temperature ranging from 60-160°C of each drug will be discussed as well as why higher temperatures may not be suitable for this method. A limit of detection study for methamphetamine and amphetamine will also be covered.

Chapter 5, the future work chapter, will discuss future analyses using the Total Vaporization Solid-Phase Microextraction method including the analysis of powder materials, plant material, and toxicological samples. Powder material will include the analysis of individual powdered drugs as well as realistic drug mixtures. Some analyses on individual powder samples has already been completed and will be shown. Plant material will include the analysis of naturally occurring compounds found in marijuana plants as well as synthetic cannabinoids. Toxicological samples will expand on previously mentioned urine samples to include drugs such as benzoylecgonine and THC-COOH.

CHAPTER 1. TOTAL VAPORIZATION SOLID-PHASE MICROEXTRACTION OVERVIEW

Introduction

While Gas Chromatography – Mass Spectrometry (GC-MS) is a frequently used technique in forensic science laboratories, it has limitations such as the need for compounds to be thermally stable as well as volatile[1]. Some compounds must undergo derivatization prior to being injected into the gas chromatograph (GC) to satisfy these requirements. Headspace Solid-Phase Microextraction (SPME) is a technique in which a sample is placed into a vial and the vial is heated to encourage a portion of the analyte to vaporize into the headspace. A SPME fiber, coated with a polymeric material such as polydimethylsiloxane-divinylbenzene (PDMS/DVB) is then introduced into the sample headspace and the analyte is adsorbed onto the fiber meaning that the analyte forms a thin coating on the fiber. The fiber is then placed inside the GC inlet for desorption.

Total Vaporization Solid-Phase Microextraction (TV-SPME) relies on the same technique as headspace SPME but completely vaporizes a liquid sample before being adsorbed onto the fiber. This allows for partitioning of the analyte between only the vapor and the coating of the fiber. With this approach, more of the sample is adsorbed onto the fiber and small sample sizes (e.g. 1 – 100 µL) may be used. This method also helps to eliminate matrix effects due to this being a two-phase system as opposed to a three-phase system like standard headspace SPME. TV-SPME is like immersion SPME in that immersion SPME is also a two-phase system.[1] With immersion SPME, a fiber is immersed into a liquid sample containing the analyte as opposed to extracting the analyte from its vapor. TV-SPME is preferred to immersion SPME because immersion SPME requires more sample volume than TV-SPME.

To perform TV-SPME, analytes are dissolved in a solvent and an aliquot of this mixture is placed into a 20 mL headspace GC vial. Specific volumes of liquid samples must be used in order to ensure total vaporization of the sample. These volumes are determined by using the Ideal Gas Law to calculate the number of moles of a solvent multiplied by the molar volume of the liquid (Equation 1).

$$V_o = \left(\frac{PV_v}{RT}\right)\left(\frac{M}{\rho}\right) \qquad \text{Equation 1}$$

where V_o is the volume of the sample (mL), P is the vapor pressure of the solvent (bar), V_v is the volume of the vial (L), R is the ideal gas constant ($0.083145\ \frac{(L*bar)}{k*mol}$), M is the molar mass of the solvent (g/mol), T is temperature (K), and ρ is the density of the solvent $(g/mL)^2$.

The Antoine equation (Equation 2) is used to account for the influence of temperature on vapor pressure:

$$log_{10}P = A - \left(\frac{B}{T+C}\right) \qquad \text{Equation 2}$$

where T is temperature and A, B, and C are the Antoine constants for the solvent. Equation 2 may be substituted into Equation 1, yielding:

$$V_o = \left(\frac{\left(10^{A-\frac{B}{T+C}}\right)V_v}{RT}\right)\left(\frac{M}{\rho}\right) \qquad \text{Equation 3}$$

Equation 3 gives the volume of the sample (V_o) that can be completely vaporized as a function of the temperature and solvent used.[2]

Derivatization may also take place during this procedure. When derivatization is needed for a GC method, a labile hydrogen on an analyte is replaced with a functional group that allows for better separation by making the analyte thermally stable and more volatile. There are three forms of derivatization: acylation, silylation and alkylation. For this work, acylation and silylation were performed. The derivatization agent used for acylation was trifluoroacetic anhydride (TFAA). A general reaction of TFAA with an amine is shown in Figure 1. To perform silylation, N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS) was used to derivatize carboxylic acids and amines. A general reaction for BSTFA + 1% TMCS is shown in Figure 2.

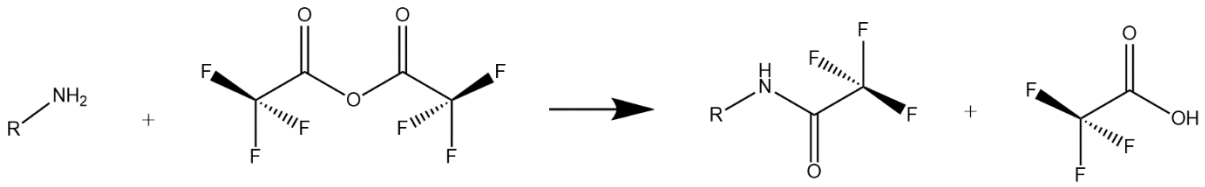


Figure 1. General reaction for TFAA with an amine.

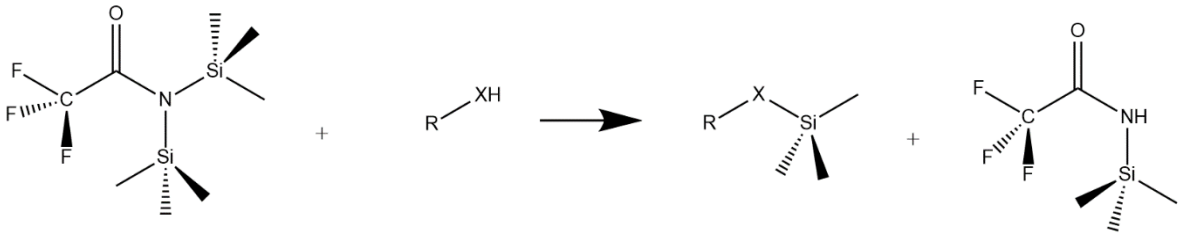


Figure 2. General reaction for BSTFA + 1% TMCS where X can be either an oxygen or a nitrogen atom.

To perform derivatization with TV-SPME, the SPME fiber is first exposed to a vial containing the derivatization agent for a predetermined amount of time depending on the analyte. The SPME fiber is then exposed to a new vial containing the analyte of interest. This vial is heated inside of a heated agitator. The analyte is then adsorbed onto the fiber with the derivatization agent. The derivatization of the analyte and/or the matrix takes place on the fiber before being inserted into the GC inlet for desorption. The derivatization vial was kept at room temperature when TFAA was used and the vial is heated when BSTFA + 1% TMCS was used. This is because TFAA evaporates much more quickly than BSFTA + 1% TMCS. A depiction of TV-SPME may be seen in Figure 3 below.

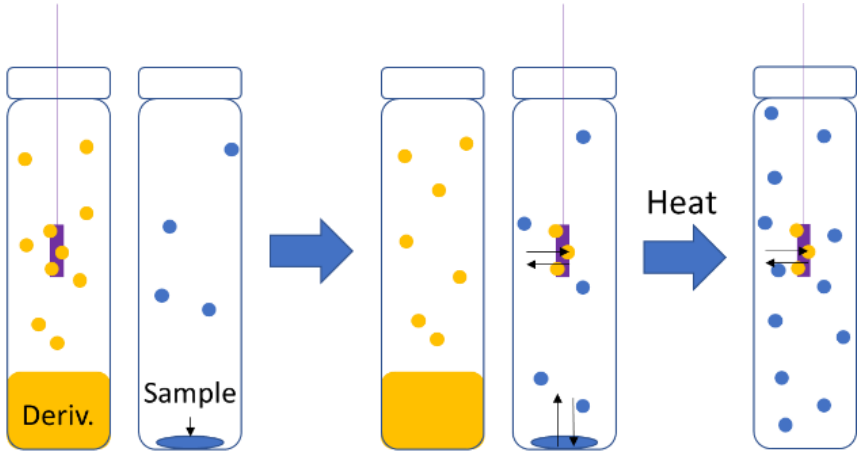


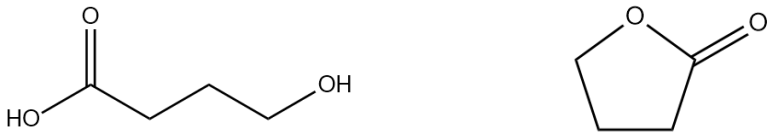
Figure 3. Depiction of the TV-SPME procedure[2].

TV-SPME is beneficial because it allows for the analyte to be derivatized during the extraction process which reduces analysis time. Other methods, such as liquid injection, require that the analyte react with the derivatizing agent in solution prior to being injected into the GC. TV-SPME also requires little to no sample preparation. A matrix containing an analyte may be placed directly into the headspace vial and analyzed. Extraction procedures are not necessary because any nonvolatile compounds will remain in the headspace vial and will not be adsorbed onto the fiber. This method also utilizes small sample sizes. Most sample volumes range from 2.4 µL – 24 µL depending on the solvent used. Currently, TV-SPME is a qualitative technique and not quantitative.

CHAPTER 2. DETERMINATION OF ɣ-HYDROXYBUTYRIC ACID & ɣ-BUTYROLACTONE IN ALCOHOLIC BEVERAGES

Introduction

ɣ-Hydroxybutyric acid (GHB) and ɣ-butyrolactone (GBL), seen in Figure 4 are compounds that may be found in cases of drug facilitated sexual assault (DFSA). DFSA occurs when one person uses a drug to incapacitate an individual and then proceeds to commit a sexual assault. This may be done by intentionally giving the victim the drug without their knowledge or if the victim has self-administered the drug and therefore was incapable of giving consent. GHB and GBL are ideal drugs for DFSA because they cause sedation, short-term memory loss, and are quickly metabolized in the body. GHB and GBL are colorless, odorless, and are often tasteless. GHB is a Schedule I drug and is often produced in domestic and foreign clandestine laboratories. GBL is a List 1 chemical and is sold as an industrial product such as fish tank cleaner.[3] A List 1 chemical is a regulated chemical that can be used to manufacture an illegal substance.[4] DFSA is underreported because victims may not be aware that they were assaulted due to memory loss caused by the drug. While it is not known how many DFSA occurrences happen each year, the United States Department of Justice states that these occurrences are increasing.[5]



(a) (b)

Figure 4. Molecular structure for a) GHB (104.1 g/mol) and b) GBL (86 g/mol).

GHB has a half-life of 30-50 minutes in the human body.[6] Exogenous GHB is cleared from the blood stream within 5 hours and from urine within 3-10 hours.[6, 7] GBL also has a short half-life of 30-52 minutes within the body.[8] The detection of exogenous GHB is difficult because it is a naturally occurring compound in mammals.[6] When biological samples suspected of containing GHB are tested, a clean sample of the alleged victim's urine must also be collected to account for their naturally occurring GHB levels. This means that an additional sample must be collected from the alleged victim roughly 12 hours after an attack has occurred. Therefore, the detection of GHB and GBL in suspected beverages is highly important as it gives a more reliable concentration of the exogenous drug used.

Gas Chromatography - Mass Spectrometry (GC-MS) is commonly used for the detection of GHB and GBL. GHB may be found in hair, blood, and urine.[9, 10] Other methods used to detect GHB include High Performance Liquid Chromatography, Liquid Chromatography-MS, Headspace Solid-phase Microextraction, and Nuclear Magnetic Resonance Spectroscopy. Solid samples of GHB and GBL may be analyzed using Infrared Spectroscopy. GBL may be detected using GC-MS and Raman Spectroscopy may be used to detect GHB and GBL simultaneously.[11-13] Unlike TV-SPME, Raman Spectroscopy analysis cannot be automated because it requires manual adjustment to view the sample. GHB is often detected with GBL so these methods require an extraction step which can be time consuming and labor intensive.[14] TV-SPME does not require any sample preparation and GHB and GBL may be analyzed simultaneously.

GHB cannot be detected by GC-MS methods "as is" due to its hydrophilicity via the two hydroxyl groups on either end of the molecule and its thermal instability. A compound must be stable at temperatures at or above 200°C.[1] Therefore, derivatization of the compound must be performed. GHB is a carboxylic acid so N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) +

1% trimethylchlorosilane (TMCS) may be used to derivatize GHB into ɣ-hydroxybutyric acid-trimethylsilyl$_2$ (GHB-TMS$_2$), producing a more stable compound that may be detected by GC-MS methods. The reaction of BSFTA + 1% TMCS with GHB is shown in Figure 5**.** GBL does not require derivatization to be detected by the GC, however, when spiked into beverages with a high concentration of water, such as alcoholic beverages, derivatization of the aqueous matrix is needed to achieve the desired results. This is because water in not an ideal solvent for GC analysis due to its high vapor pressure. Therefore, the GBL samples were also derivatized with BSTFA + 1% TMCS to derivatize the water in the matrix. The derivatization agent does not affect the GBL compound.

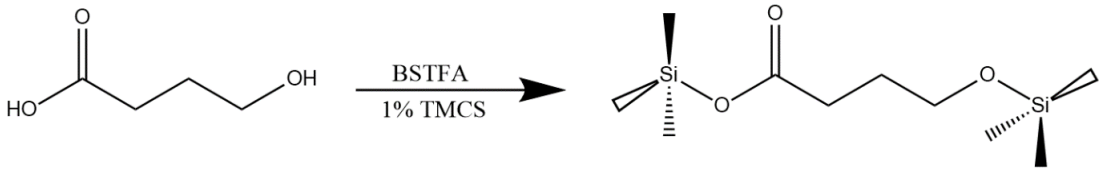


Figure 5. Reaction of GHB with BSTFA + 1% TMCS to form GHB-TMS$_2$.

GHB and GBL may be spiked into any beverage but are often spiked into alcoholic beverages at parties, raves, or night clubs without the victim's knowledge. Beer, wine, and liquor are the most commonly consumed beverages within these settings. For this work, water, beer, wine, and rum and Coke© were spiked with GHB and beer, wine, and rum were spiked with GBL and analyzed using the TV-SPME GC-MS method. GHB is very soluble in water and alcohol.[15]

Both GHB and GBL will cause sedation when 2 - 3 g is ingested.[16,17] The standard alcoholic beverage is 150 mL.[17] Therefore, a beverage spiked with 2.5 g of GHB or GBL would

have a concentration of about 16 mg/mL. For these analyses, lower concentrations of 8 mg/mL of GHB and 10 mg/mL of GBL were used.

Experimental

Materials

GHB was purchased from Cayman Chemical (Ann Arbor, Michigan) and GBL was purchased from Sigma-Aldrich (Saint Louis, Missouri). BSTFA + 1% TMCS was purchased from Thermo Fisher Scientific (Waltham, Massachusetts). Polydimethyl siloxane divinylbenzene SPME fibers, 65 µm film thickness, were purchased from Supelco (Bellefonte, Pennsylvania). The matrices, beer, wine, rum, and Coke© were purchased from local grocery stores.

Sample Preparation

GHB was spiked into water, beer, wine, and rum and Coke© with 8 mg/mL concentrations by placing 0.5 mg into a vial along with 62.5 µL of the matrix. From these samples, 2.4 µL was placed into a 20 mL headspace vial. GBL was spiked into a sample of beer, wine, and rum by placing 8.8 µL of GBL into 1 mL of each solvent for a final concentration of 10 mg/mL. From these solutions, 2.4 µL of each were placed into a 20 mL headspace vial. The PDMS/DVB fiber was first exposed to the vial containing BSFTA + 1% TMCS for 50 minutes for GHB and 10 minutes for GBL before being inserted into the vial containing the sample for 10 minutes. The fiber was then inserted into the GC inlet for desorption at 250°C.

GC-MS Parameters

An Agilent 6890N GC coupled with an Agilent 5975 inert Mass Selective Detector with an attached Gerstel PAL RTC Multi-Purpose Sampler (MPS) was used for all experiments. The GC

column was an Agilent Technologies DB-5MS column, 30 m long, 0.25 mm inner diameter, 0.25 µm film thickness.

The GC inlet was set to 250ºC and was in splitless mode. The initial oven temperature was 60ºC and was held for 1 minute. The temperature was then ramped to 250ºC at 15ºC/min and held for 1 minute at the final temperature. The mass transfer line was set to 250ºC and the flow rate was kept at 2.5 mL/min. The source was held at 230ºC and the quadrupoles were held at 150ºC. The mass range scanned was m/z 40 - m/z 550. Total ion chromatograms (TIC) were generated and extracted ion profiles were used to identify the analyte of interest in each sample. All compounds were identified using the SWGDRUG and/or NIST libraries.

Results and Discussion

GHB in Beverages

The GHB-TMS_2 chromatogram for GHB in water is shown in Figure 6. The corresponding fragmentation and mass spectrum are shown in Figure 7 below. The m/z 147 ion peak is a fragment of the derivatization agent, BSTFA. It is formed when a compound contains two functional groups such as the carboxylic acid and alcohol in GHB. GHB was detected in water, beer, wine, and rum and Coke© with concentrations of 8 mg/mL. The chromatograms for each spiked beverage are shown in Figures 8-10.

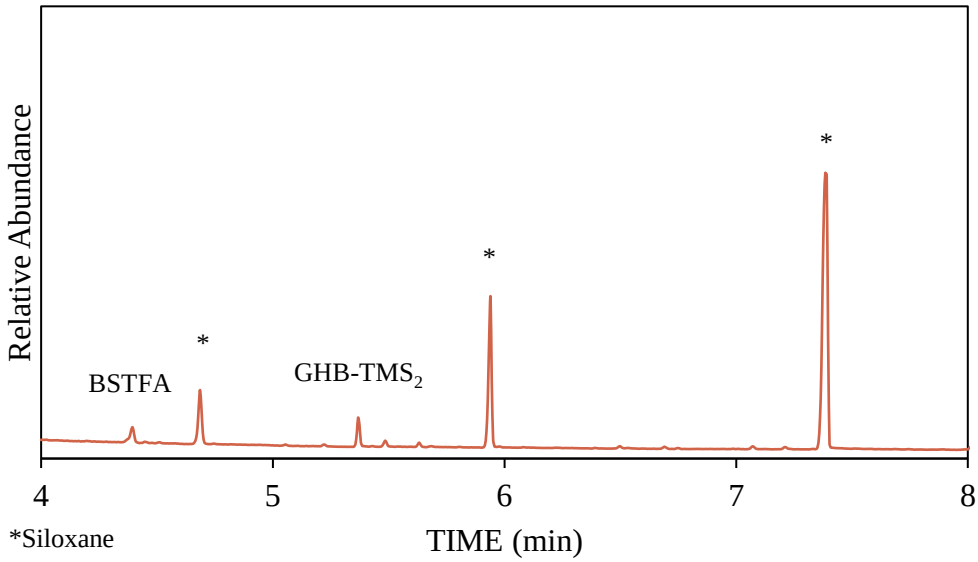
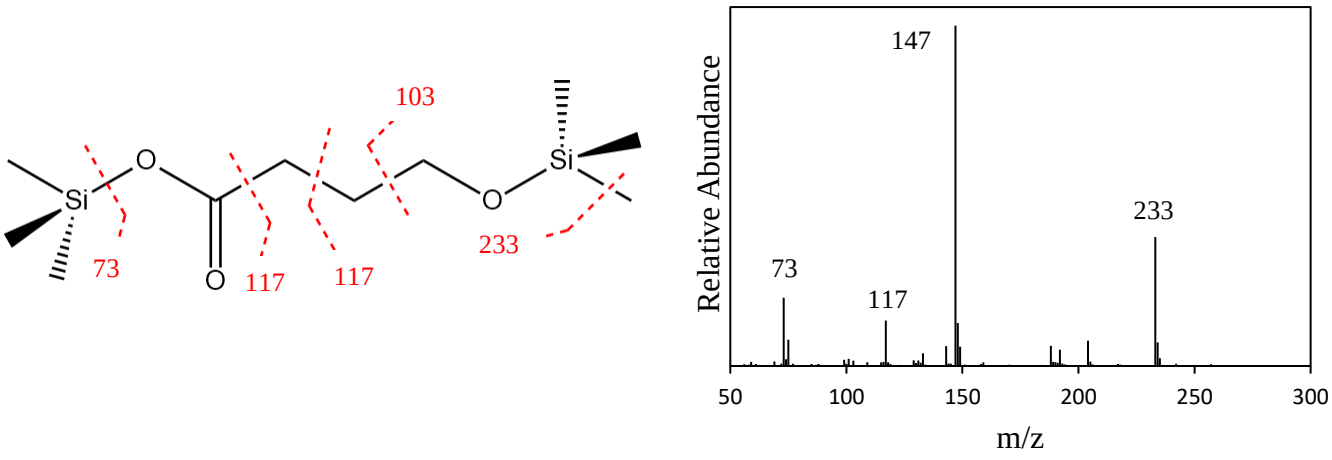
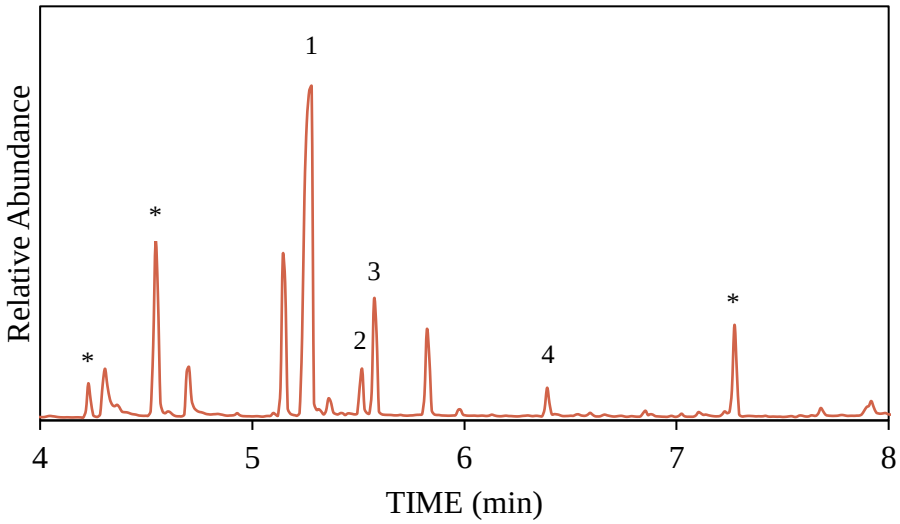


Figure 6. Total Ion Chromatogram (TIC) of GHB-TMS_2 (molecular weight: 248 g/mol) in water with an 8 mg/mL concentration.



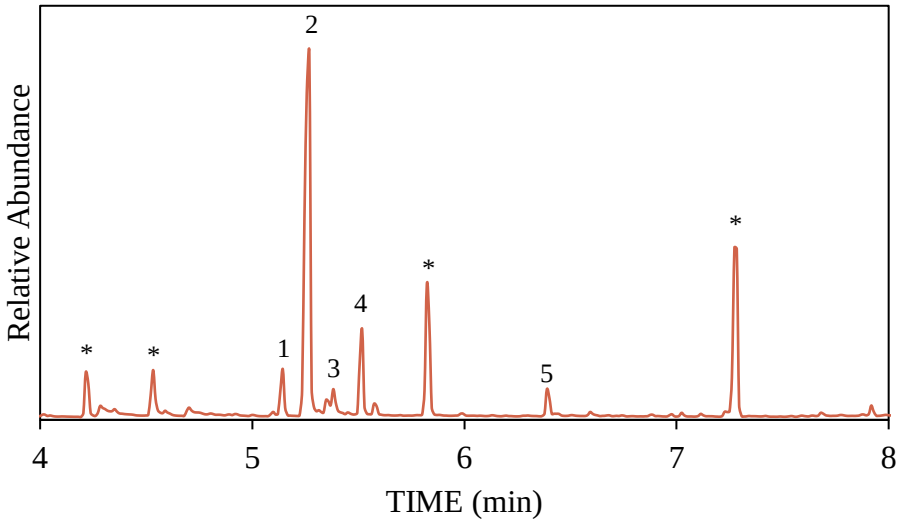
a. b.

Figure 7. Ion fragmentation (a) and mass spectrum (b) for GHB-TMS_2 in water (8 mg/mL).



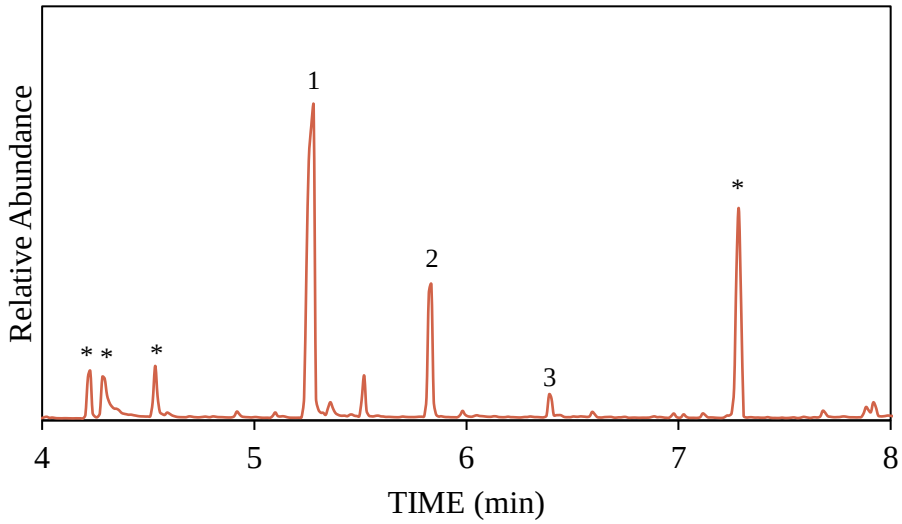
1. GHB-TMS_2
2. Octanoic acid ester-TMS
3. Glycerol-TMS_3
4. Nonanoic acid ester-TMS
* = Siloxane

Figure 8. TIC of GHB-TMS_2 in beer with an 8 mg/mL concentration.



1.Trimethyl(2-phenylethoxy) silane
2. GHB-TMS_2
3. Ethyl succinate-TMS
4. Octanoic acid ester-TMS
5. Nonanoic acid ester-TMS
* = Siloxane

Figure 9. TIC of GHB-TMS_2 in wine with an 8 mg/mL concentration.

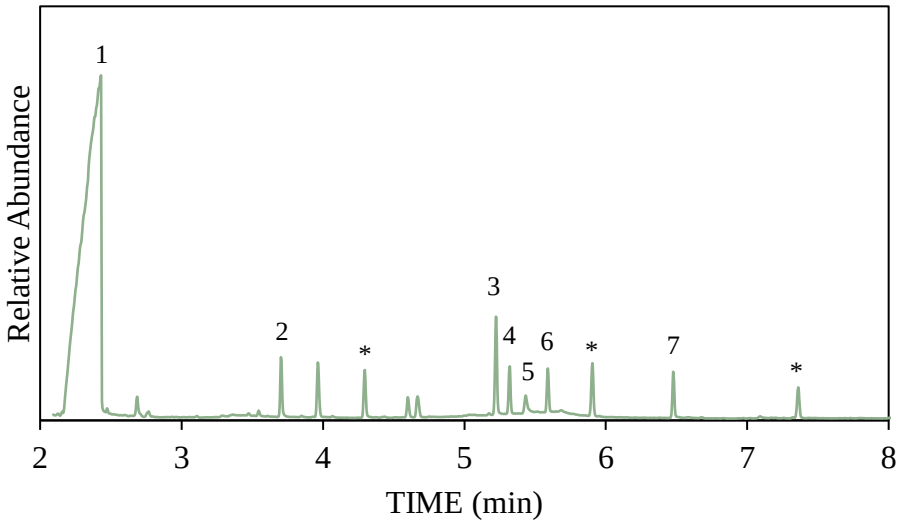


1. GHB-TMS_2
2. Octanoic acid ester-TMS
3. Nonanoic acid ester-TMS
* = Siloxane

Figure 10. TIC of GHB-TMS_2 in rum and Coke© with an 8 mg/mL concentration.

GBL in Beverages

Like GHB, GBL may also be used in DFSA due to its sedative effects. GBL has a short half-life in the body and therefore cannot be detected in a biological sample shortly after ingestion. GBL can, however, be detected in beverages including beer, wine, and rum. The GBL in beer chromatogram is shown in Figure 11 and the GBL mass spectrum and fragmentation are shown in Figures 12 and 13 respectively. GBL was detected in beer, wine, and rum with concentrations of 10 mg/mL. The additional chromatograms for each spiked beverage are shown in Figures 14 and 15.



1. GBL
2. Hexanoic acid-TMS
3. Trimethyl(2-phenylethoxy) silane
4. GHB-TMS_2
5. Benzoic acid ester-TMS
6. Octanoic acid ester-TMS
7. Nonanoic acid ester-TMS
* = Siloxane

Figure 11. TIC of GBL in beer (10 mg/mL).

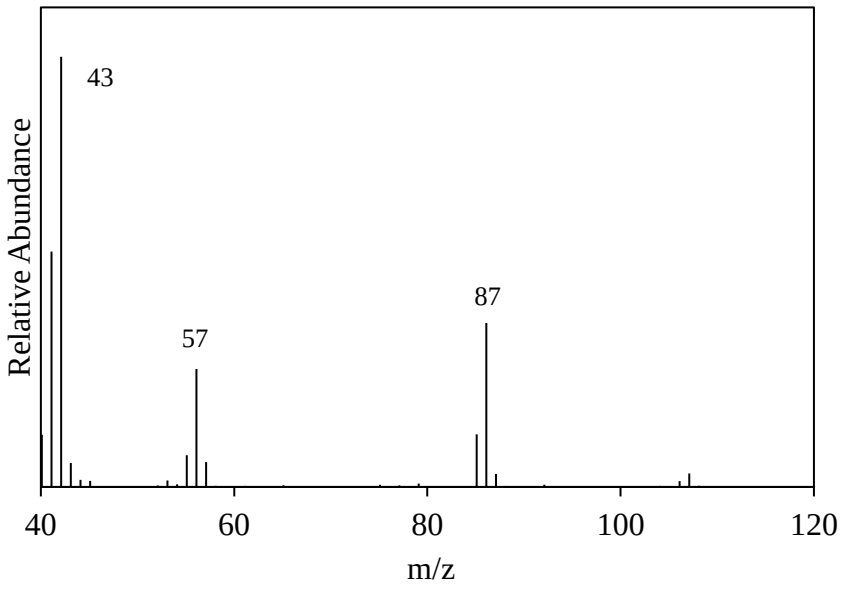
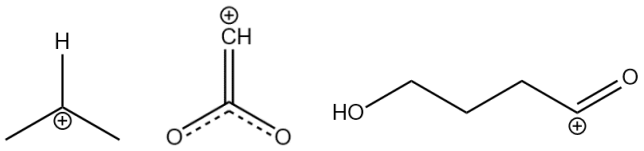
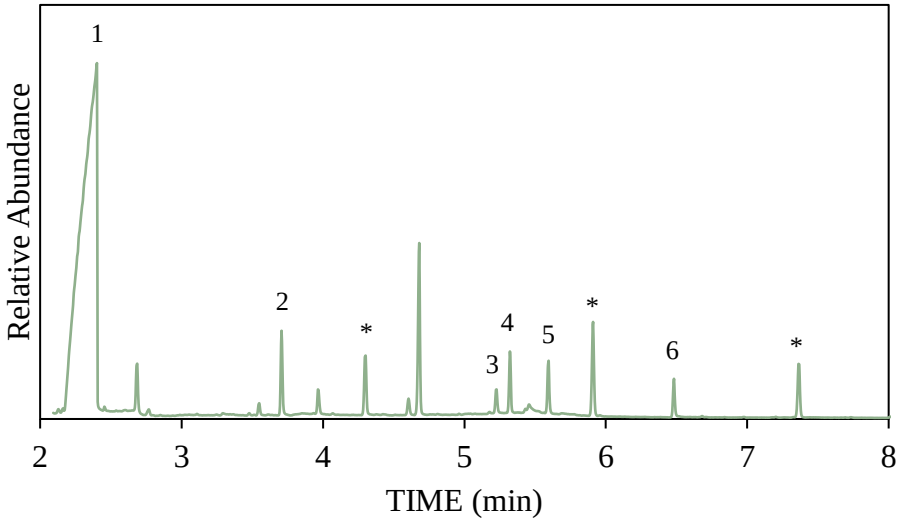


Figure 12. Mass spectrum for GBL in beer.



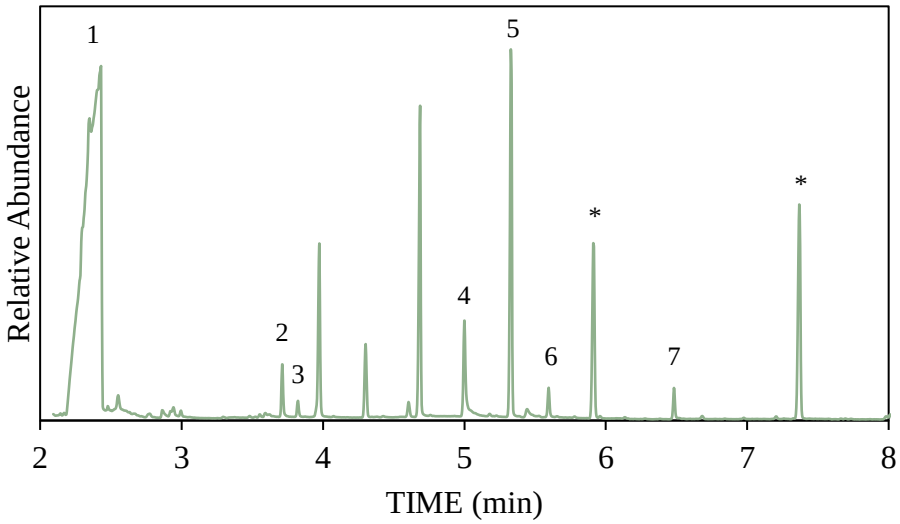
a. b. c.

Figure 13. Ion fragments of GBL a) m/z 42, b) m/z 57, and c) m/z 87.



1. GBL
2. Hexanoic acid ester-TMS
3. Trimethyl(2-phenylethoxy) silane
4. GHB-TMS_2
5. Octanoic acid ester-TMS
6. Nonanoic acid ester-TMS
* = Siloxane

Figure 14. TIC for GBL in wine (10mg/mL).

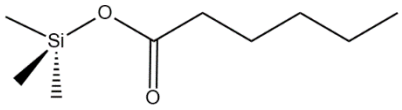
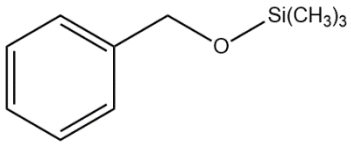
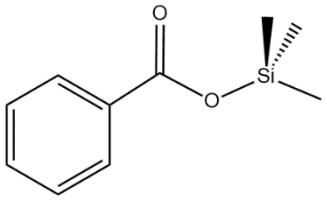
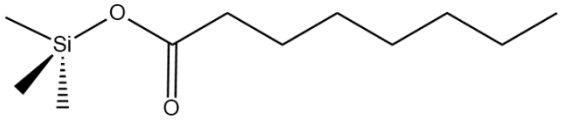
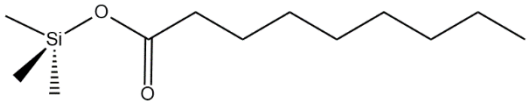
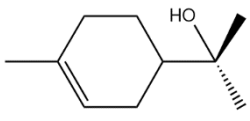


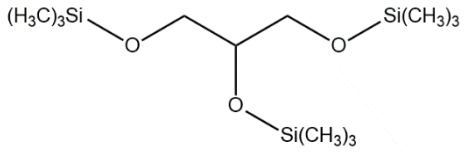
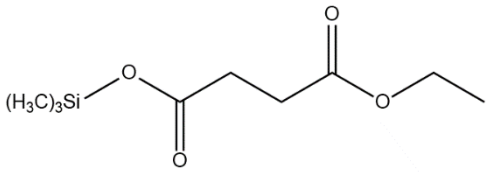
1. GBL
2. Hexanoic acid ester-TMS
3. 2-Propenyl ester hexanoic acid
4. α-Terpineol
5. GHB-TMS_2
6. Octanoic acid ester-TMS
7. Nonanoic acid ester-TMS
* = Siloxane

Figure 15. TIC for GBL in rum (10 mg/mL).

The samples GHB and GBL in beer, wine, and rum contained GBL, GHB-TMS_2, siloxanes, as well as various additional compounds. These additional compounds, along with their structure are shown in Table 1 below.

Table 1. Names and structures for compounds found in the GHB and GBL samples.

Compound	Structure	Derivative of
Hexanoic acid-TMS		Hexanoic acid
Trimethyl(2-phenylethoxy) silane		Phenethyl alcohol
Benzoic acid ester-TMS		Benzoic acid
Octanoic acid ester-TMS		Caprylic acid
Nonanoic acid-TMS		Nonanoic acid
α-Terpineol		NA

Compound	Structure	Derivative of
Glycerol-TMS		Glycerol
Ethyl succinate-TMS		Monoethyl succinate

Hexanoic acid, phenethyl alcohol, benzoic acid, caprylic acid, nonanoic acid, and glycerol are compounds that are found in alcoholic beverages. These compounds are formed during the fermentation process.[18-22] Phenethyl alcohol is an alcohol that comes from the grapes used in wine.[21] Nonanoic acid, 2-propenyl ester hexanoic acid, and α-terpineol are compounds used as flavoring agents.[23-25] Monoethyl succinate is a compound found in wines that is formed during fermentation.[26] These compounds were found in the spiked beverage samples. Many siloxane peaks were also identified. These siloxanes are due to the degradation of the coating on the PDMS/DVB SPME fiber. These compounds tend to become more abundant when high temperatures are used.

Conclusion

GHB may be used to facilitate sexual assault as it causes memory loss and incapacitation. GHB can be a difficult analyte to detect in biological samples due to it being a naturally

occurring compound in humans as well as its short half-life. Therefore, detection in beverages suspected of containing GHB is of high importance. GHB can be detected in beverages including water, beer, wine, and rum and Coke©.

Several of the GHB peaks were “flat-topped” and the GBL peaks exhibited fronting. The poor peak shape could be caused by the high concentration of GHB and GBL in solution. These issues should be considered when real life samples are analyzed. Resolutions for these issues could be diluting the sample, using a split-injection ratio, decreasing the extraction time or temperature, or decreasing the desorption time.

Overall, the performance of this TV-SPME GC-MS method was acceptable for the detection of GHB and GBL in beverages. Sample preparation, aside from possible dilutions, is not needed to perform this method.

CHAPTER 3. DETERMINATION OF AMPHETAMINES, GHB, & GBL IN HUMAN URINE

Introduction

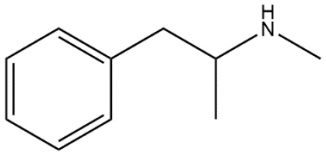
Urine is the most common biological sample used to determine drug use. This is because it is a relatively non-invasive sample to collect and most drugs can be detected in urine.[27,28] For this work, parent drugs as well as their metabolites were screened for in human urine. The drugs of interest were methamphetamine and amphetamine (Figure 16), as well as GHB and GBL (Figure 4).

Methamphetamine & Amphetamine

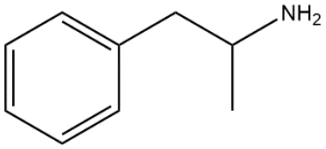
Methamphetamine is a central nervous system (CNS) stimulant that is highly addictive.[3] Methamphetamine is a Schedule II drug that may be prescribed to treat obesity or attention deficit hyperactivity disorder (ADHD).[3] Illegal methamphetamine is supplied to the United States through Mexican drug trafficking organizations as well as through domestic clandestine operations.[3] It is often in the form of a white or off-white powder that may be snorted, injected, or smoked.

Amphetamine may also be used as a CNS stimulant. Amphetamine may be prescribed to treat narcolepsy and ADHD[3]. Amphetamine is a highly addictive Schedule II drug that comes in the form of tablets or powders. It may be taken orally or intravenously.[3] Both methamphetamine and amphetamine are eliminated from the body in urine. In addition, some methamphetamine is metabolized by the user into amphetamine. Methamphetamine is eliminated from urine within 3-6 days and from blood within 24-72 hours.[29]

Although there are many biological samples that may be analyzed for methamphetamine and amphetamine (e.g., hair, nail clippings, urine, plasma, and oral fluid), urine remains a popular, reliable, and convenient biological sample.[30-32]



(a)



(b)

Figure 16. Structures of the phenylethylamines analyzed: a) methamphetamine (149.2 g/mol) and b) amphetamine (135.2 g/mol).

Methamphetamine and amphetamine may be identified using various techniques and methods including numerous SPME techniques. These techniques and methods are shown in Table 2 below.

Table 2. Various techniques used for the detection of methamphetamine and amphetamine.

Analyte	Matrix/Solvent	Technique
Methamphetamine	Acidified iodoplatinate solution	Thin-Layer Chromatography (TLC)[33]
Methamphetamine	Chloroform	GC[33]
Methamphetamine	Methanol	HPLC[33]
Amphetamine	1% Ninhydrin in methanol	TLC[34]
Amphetamine	Chloroform:Methanol 4:1	GC[34]
Amphetamine	0.1 Normal hydrochloric acid	HPLC[34]
Methamphetamine	Urine	SPME/GC-MS[35] Electroenhanced (EE) SPME/GC-MS[36] SPME-GC[37, 38]
Methamphetamine/ Amphetamine	Urine	SPME/GC-MS[39-48] SPME/GC-Nitrogen Phosphorous Detector (NPD)[49] Direct-Immersion SPME/GC-NPD[50] SPME/LC-MS[51] SPME/LC[52] SPME/LC-Electrospray Ionization (ESI)-MS[53] SPME-GC[54] SPME/High-Field Asymmetric Waveform Ion Mobility Spectrometry/ESI/MS[55] SPME/GC-Flame Ionization Detection[56] SPME/GC-NPD/GC-MS[57] SPME/Capillary Zone Electrophoresis[58] EE-SPME/GC[59]
Methamphetamine/ Amphetamine	Urine/Hair	SPME/GC-MS[60] SPME/HPLC-ESI-MS[61]
Methamphetamine	Urine/Water	SPME/GC-MS[62]
Methamphetamine/ Amphetamine	Urine/Serum	SPME/GC-MS[63]
Methamphetamine	Urine/Oral Fluid	SPME-Transmission Mode/Direct Analysis in Real Time-MS/MS[64]
Methamphetamine/ Amphetamine	Hair	SPME/GC-MS[65]

Methamphetamine and amphetamine are amines and may be derivatized with trifluoroacetic anhydride (TFAA). Methamphetamine does not have to be derivatized to be detected by the GC, however, derivatization will improve this detection as the volatility of the compound will increase. The reaction of TFAA with methamphetamine is shown in Figure 17 and with amphetamine in Figure 18.

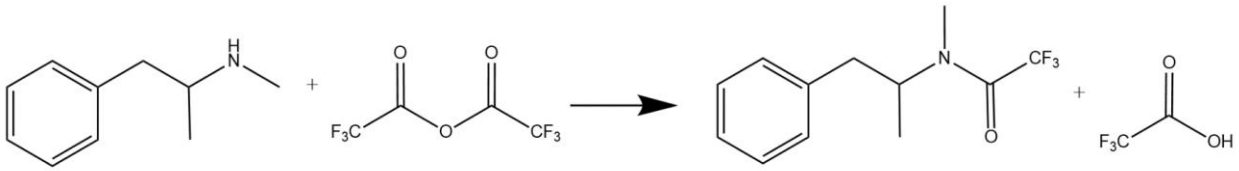


Figure 17. Reaction of methamphetamine with TFAA to form methamphetamine-TFA.

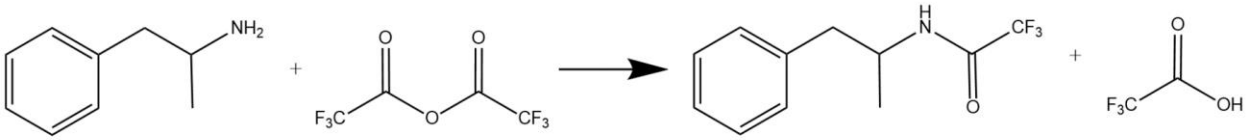


Figure 18. Reaction of amphetamine with TFAA to form amphetamine-TFA.

GHB & GBL

GHB and GBL will interconvert when in solution. Therefore, GHB and GBL are likely consumed together and will both be excreted from the body in urine. GHB is a hydroxylated short chain fatty acid and GBL is its corresponding lactone. GHB is converted to GBL via intramolecular esterification and GBL is converted to GHB via hydrolysis.[66] Fischer esterification is a reaction that occurs between an acid and an alcohol. If a compound contains a carboxylic acid and a hydroxyl group, then intramolecular esterification takes place and a cyclic ester is formed. Hydrolysis is the Fischer esterification reaction in reverse.[67] These reactions are shown in Figure 19 below. GHB and GBL can be detected in urine by GC-FID and GC-MS.[68]

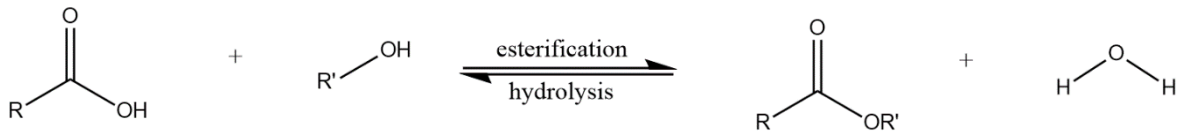


Figure 19. General esterification and hydrolysis reactions.

GHB must be derivatized with BSTFA + 1% TMCS to be detected by the GC. GBL does not need to be derivatized to be detected, but because the matrix is urine and contains water, BSTFA + 1% TMCS will also be used for this analysis to derivatize the water. Drug concentrations in urine are detected as trace amounts, often at ppb levels. Therefore, concentrations in the ng/mL range must be successfully analyzed. Drugs were spiked into a human urine sample known to be free of any illegal substances. The interconversion between GHB and GBL will also be shown because they are metabolites of one another and will appear in a urine sample of a user of either drug.

Experimental

Materials

Methamphetamine sulfate, amphetamine HCl, and GBL were purchased from Sigma-Aldrich (St. Louis, Missouri). GHB was purchased from Cayman Chemical (Ann Arbor, Michigan). All other materials were purchased from suppliers previously referenced in Chapter 2.

Sample Preparation

Methamphetamine & Amphetamine

A stock solution of methamphetamine in water was prepared by placing 1 mg of methamphetamine powder into a vial along with 1 mL of water for a concentration of 1 mg/mL. A stock solution of amphetamine in water was prepared in the same manner. The methamphetamine stock solution and amphetamine stock solution were diluted further by placing 1 µL of each into 1 mL of water. One mL of clean human urine was placed into a vial along with 30 µL of the diluted methamphetamine stock solution and 20 µL of the diluted amphetamine stock solution. The final concentrations were 28.1 ng/mL of methamphetamine and 19.0 ng/mL of amphetamine in water and urine with water making up 2.8% of the solution. This mixture was analyzed by placing 2.4 µL of the sample into a 20 mL headspace vial. TFAA was used as the derivatization agent for this reaction. After a derivatization time of 50 minutes, the SPME fiber was moved to the sample vial where the sample was vaporized by heating to 60℃. The fiber was then placed in the inlet of the GC for desorption at 250℃.

GHB & GBL

A stock solution of GHB in urine was prepared with a concentration of 8 mg/mL by placing 2 mg of GHB powder into a vial along with 250 µL of urine. From this solution, 125 µL were placed into a new vial with 375 µL of urine for a concentration of 2 mg/mL. An additional sample of GHB in water was prepared with a concentration of 1.5 mg/mL. However, due to an instrument failure, this sample stood at room temperature for 72 hours. This sample was then used as an impromptu study of GHB/GBL stability and analyzed.

A stock solution of GBL in urine was prepared with a concentration of 5 µg/mL by placing 2.5 µL of a 1 mg/mL solution of GBL in methanol into a vial. The methanol was evaporated and 500 µL of urine was added to the vial.

GC-MS Parameters

Ten µL of the GBL solution and 2.4 µL of the GHB solution were each placed into a headspace vial for analysis. BSTFA + 1% TMCS was used to derivatize GHB and the water in urine.

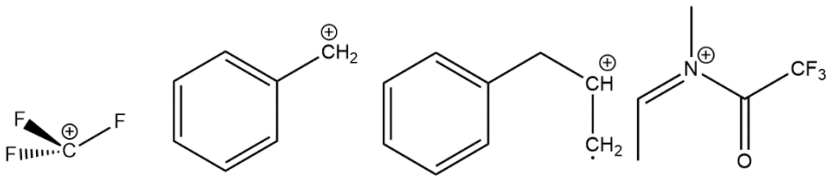
The same instrumentation and column that was used for methods in Chapter 1 were also utilized for these experiments.

The GC inlet was set to 250ºC and was in spitless mode. The initial oven temperature was 60ºC and was held for 1 minute. The temperature was then ramped to 250ºC at 15ºC/min and held for 1 minute at the final temperature. The mass transfer line was set to 250ºC and a flow rate was kept at 2.5 mL/min. The source was held at 230ºC and the quadrupoles were held at 150ºC. The scan parameters were 40-550 m/z. All compounds were identified using the SWGDRUG and/or NIST libraries.

Results and Discussion

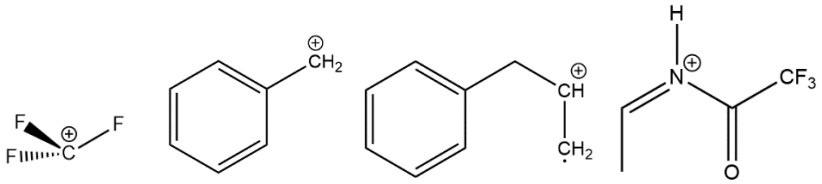
Methamphetamine & Amphetamine in Urine

Methamphetamine and amphetamine were spiked into urine with concentrations of 28.1 and 19.0 ng/mL respectively. The fragment ions for methamphetamine-TFA is shown in Figure 20 and for amphetamine-TFA in Figure 21.



a) b) c) d)

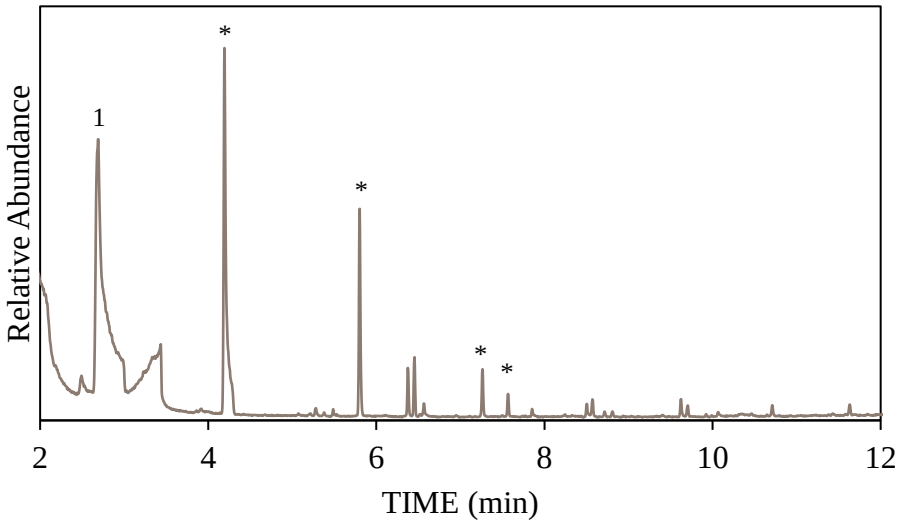
Figure 20. Mass fragmentation for methamphetamine-TFA a) m/z 69, b) m/z 91, c) m/z 118, and d) m/z 154.



a. b. c. d.

Figure 21. Mass fragmentation for amphetamine-TFA a) m/z 69, b) m/z 91, c) m/z 118, and d) m/z 140.

The urine blank analyzed with TFAA is shown in Figure 22 and analyzed with BSTFA + 1% TMCS is shown in in Figure 23. The urine blank analyzed with BSTFA + 1% TMCS displays multiple siloxane peaks. The same sample of urine was used for all analyses.



1. TFA
* = Siloxane

Figure 22. Human urine blank derivatized with TFAA.

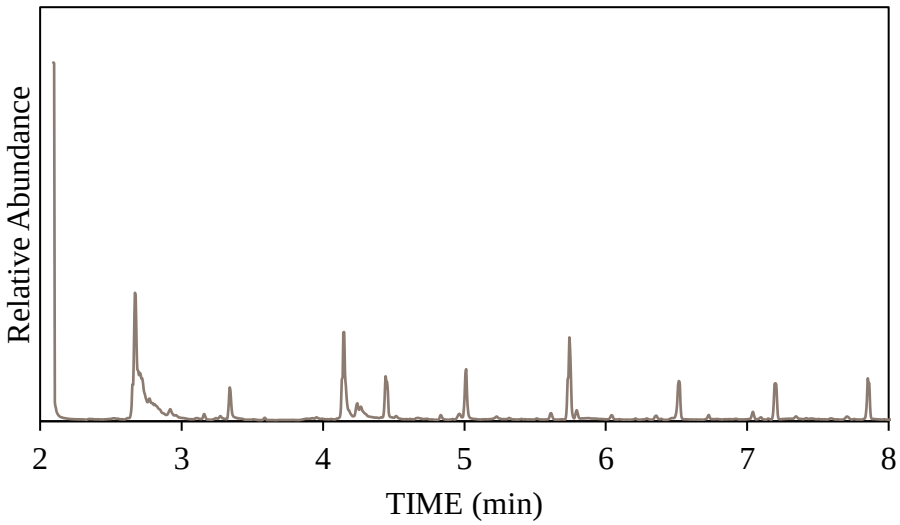
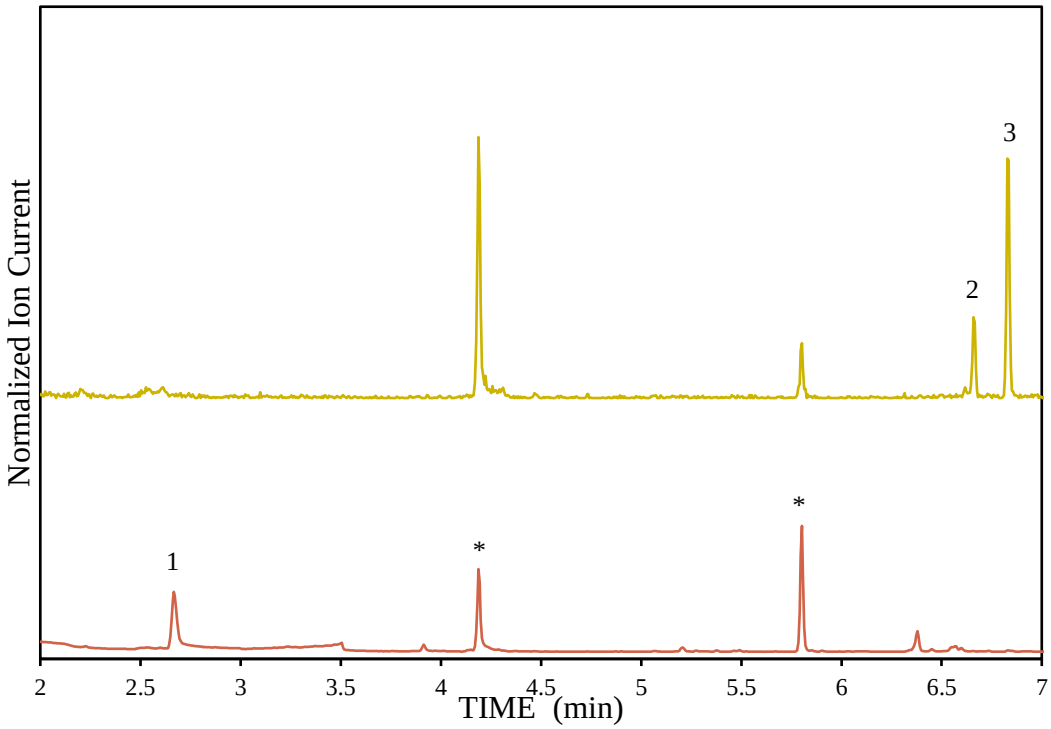


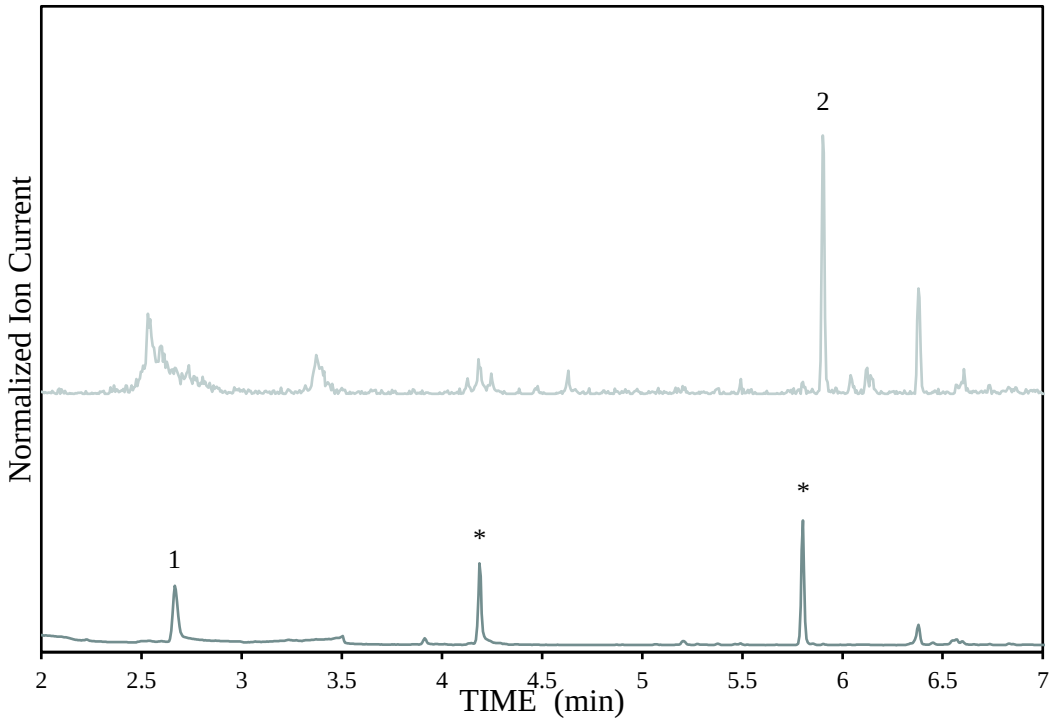
Figure 23. Human urine blank derivatized with BSTFA + 1% TMCS.

The total ion chromatogram along with the extraction ion profiles for methamphetamine-TFA and amphetamine-TFA in urine is shown in Figures 24 and 25.



1. TFA
2. Unknown Compound
3. Methamphetamine-TFA
* = Siloxane

Figure 24. Stacked chromatograms of methamphetamine in urine TIC (bottom) and methamphetamine-TFA EIC (m/z 154, top).



1. TFA
2. Amphetamine-TFA
* = Siloxane

Figure 25. Stacked chromatograms of amphetamine in urine TIC (bottom) and amphetamine-TFA EIC (m/z 140, top).

The mass spectrum for methamphetamine-TFA (m/z 154) is shown in Figure 26 and the extracted ion mass spectrum for amphetamine-TFA (140) is shown in Figure 27.

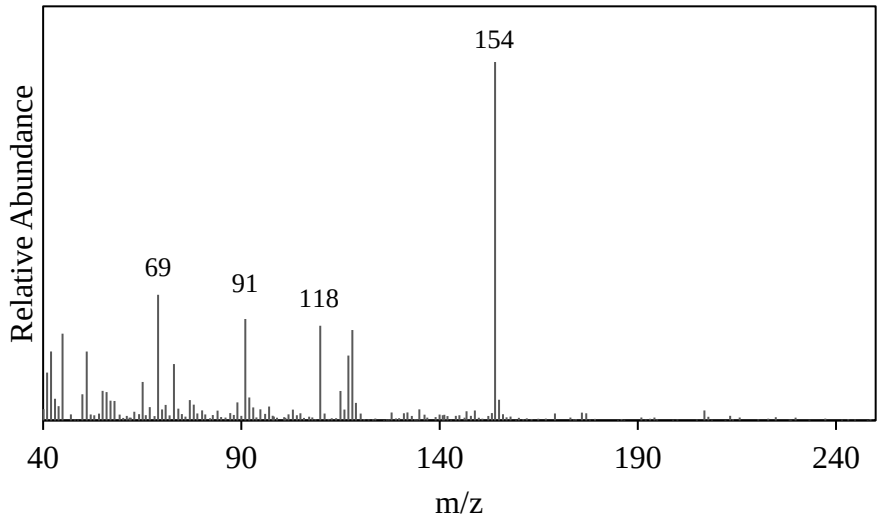


Figure 26. Mass spectrum of methamphetamine-TFA (m/z 154) in urine (28.1 ng/mL).

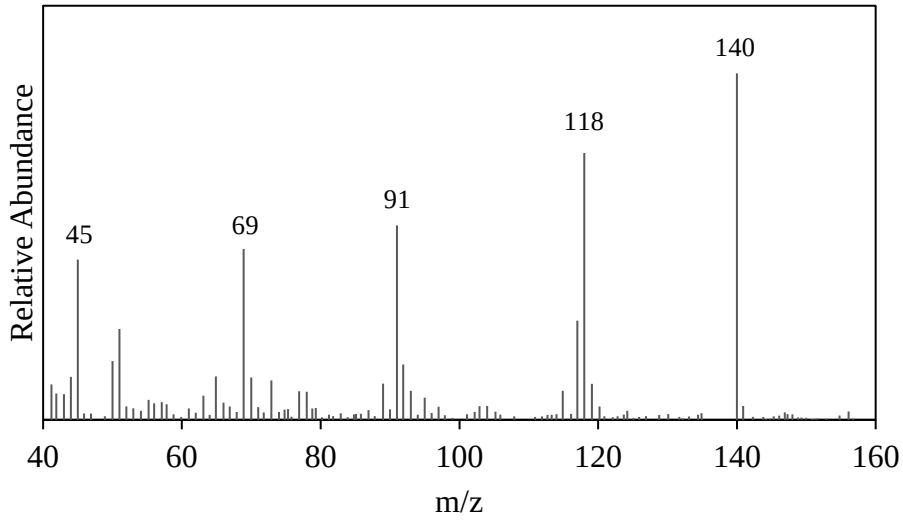
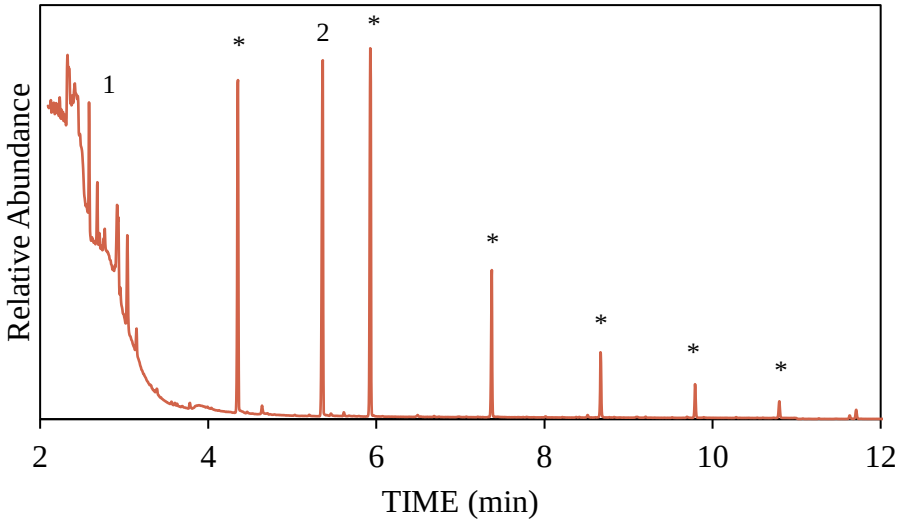


Figure 27. Mass spectrum of amphetamine-TFA (m/z 140) in urine (19.0 ng/mL).

GHB & GBL in Urine

GHB and GBL were spiked into urine with concentrations of 2 mg/mL and 5 µg/mL respectively. The mass spectra for GHB-TMS_2 and GBL are shown in Figures 7 and 13, respectively, in Chapter 1. The TIC and EIC of GHB-TMS_2 in urine are shown in Figure 28

along with its corresponding mass spectrum in Figure 29. Figure 28 displays a BSTFA peak that is likely caused by oversaturated of the fiber with BSTFA. The TIC and EIC of GBL in urine are shown in Figure 30 and 31 along with its corresponding mass spectrum in Figure 32.



1. BSTFA
2. GHB-TMS_2
* = Siloxane

Figure 28. TIC of GHB-TMS_2 in urine (2 mg/mL).

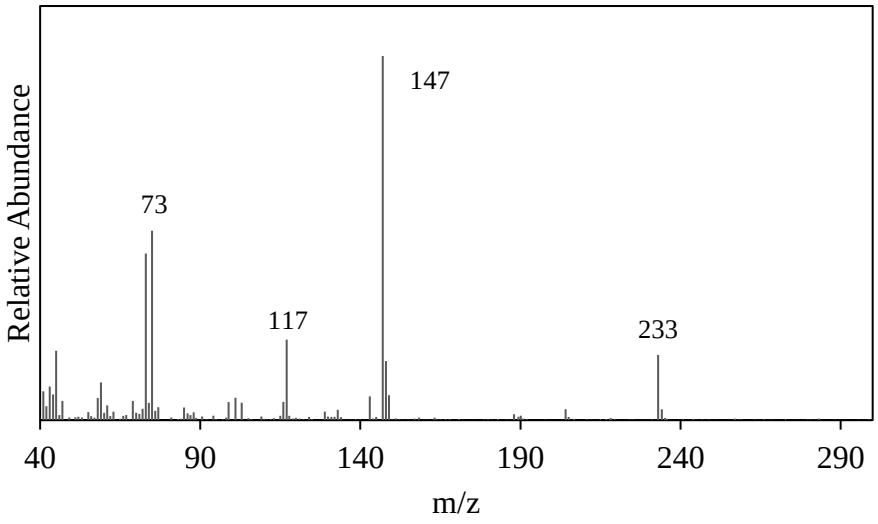


Figure 29. Mass spectrum of GHB-TMS_2 in urine.

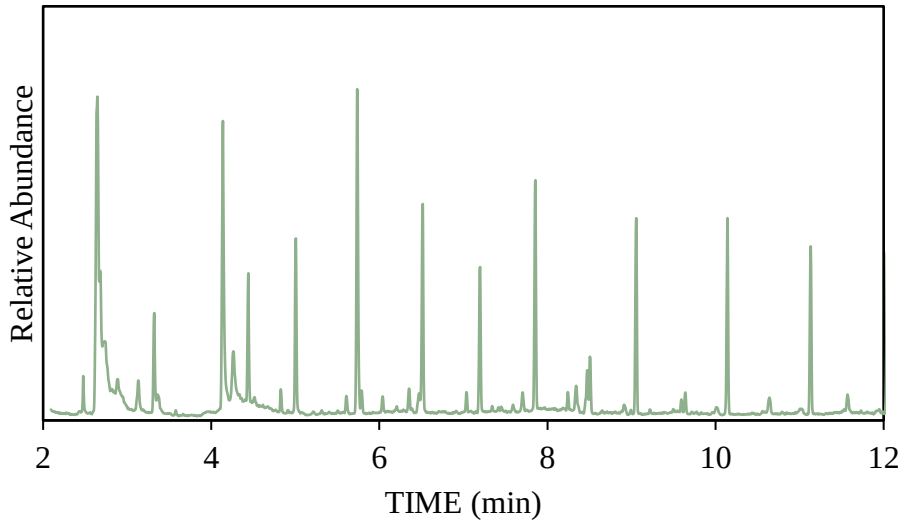


Figure 30. TIC of GBL in urine (5 µg/mL). All major peaks are siloxanes.

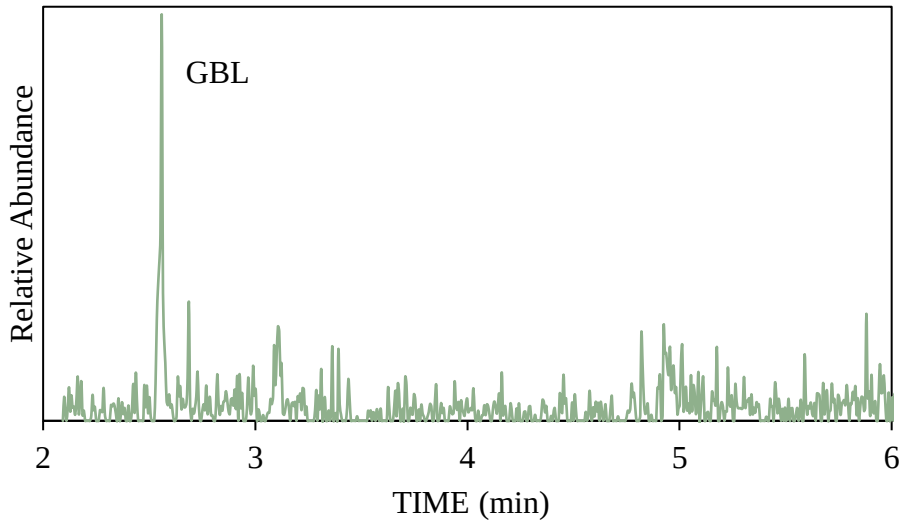


Figure 31. EIC of GBL (m/z 42) in urine (5 µg/mL).

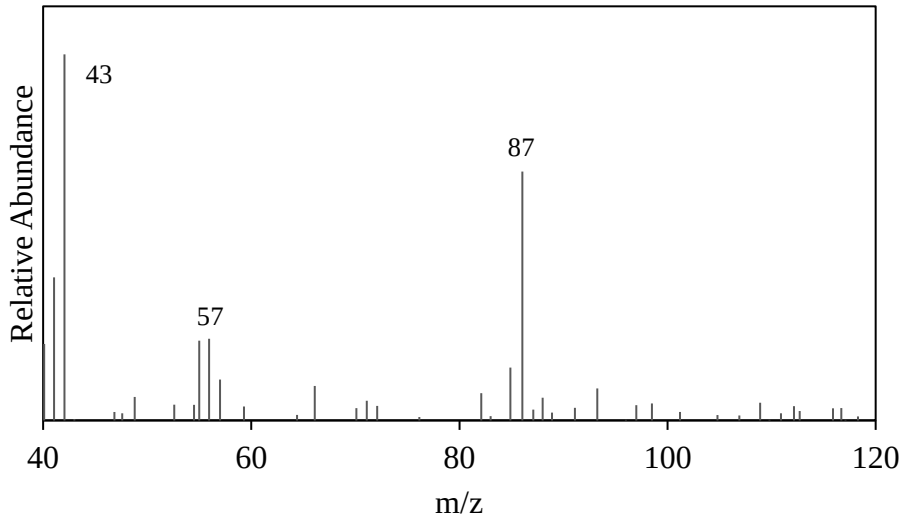


Figure 32. Mass spectrum of GBL in urine.

As previously stated, GHB and GBL interconvert into one another. The reaction for the conversion of GHB to GBL and the reverse is shown in Figure 33. The TIC of the sample of GBL spiked into rum is shown below in Figure 33 The GHB-TMS_2 peak is also shown in Figure 32. The TIC of the sample of GHB in water is shown in Figure 35 along with the GBL ion m/z 42 EIC.

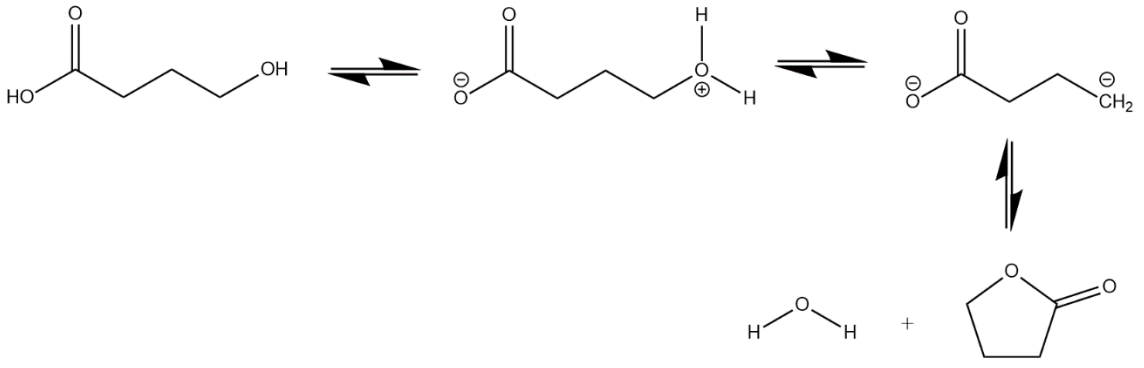


Figure 33. Reaction for the conversion of GHB to GBL (from left to right) and GBL to GHB (from right to left).

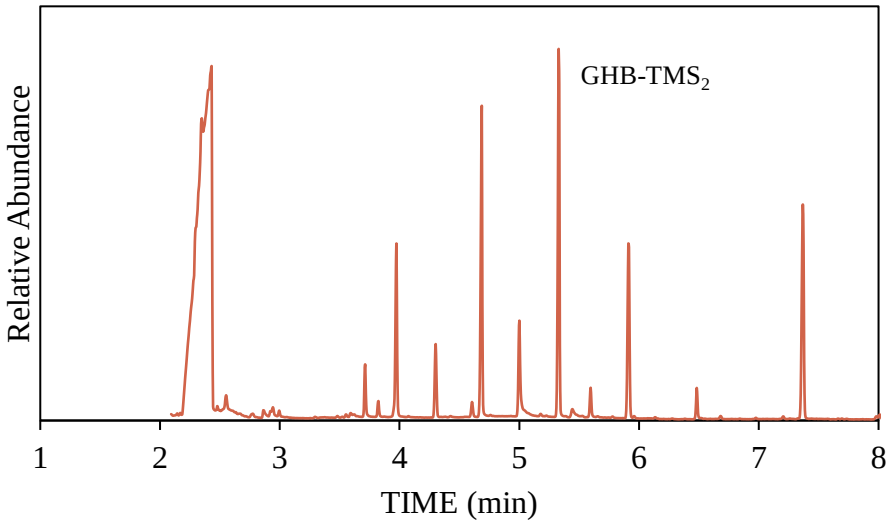


Figure 34. TIC of GBL in rum showing GHB-TMS_2 converted from GBL.

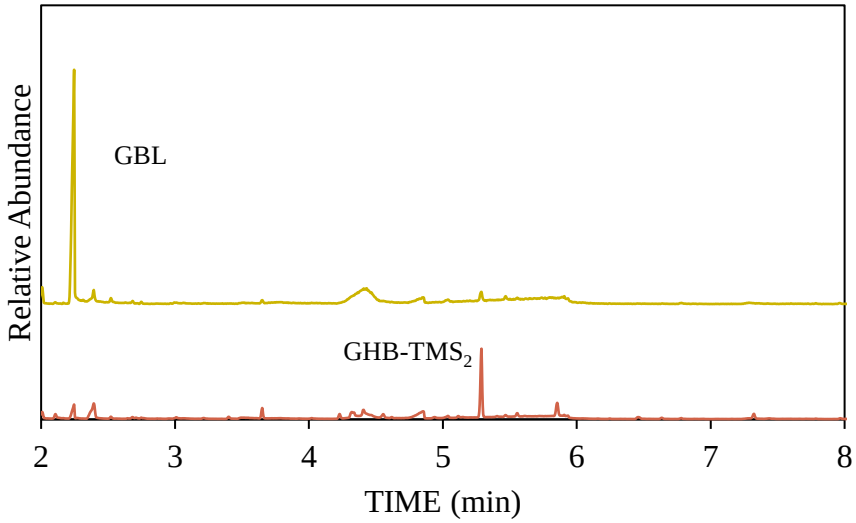


Figure 35. TIC of GHB in water (bottom) and EIC of GBL (m/z 42) (top).

Conclusion

Methamphetamine and amphetamine were spiked into human urine and analyzed using a TV-SPME GC-MS method. Methamphetamine was spiked into urine with a concentration of 28.1 ng/mL along with 19.0 ng/mL of amphetamine (the metabolite of methamphetamine).

These concentrations were in ppb levels, which are realistic concentrations for methamphetamine and amphetamine in the urine of drug users.[69]

Compounds detected in the methamphetamine sample were only those of TFA, methamphetamine-TFA, and siloxanes. Siloxane peaks are caused from degradation of the PDMS/DVB fiber. Methamphetamine-TFA peaks 1 and 2 were both identified as methamphetamine-TFA by the SWGDRUG library, however, based on the retention time of peak 2 and other samples of methamphetamine analyzed using this method, peak 2 is the methamphetamine-TFA peak. Peak 1 is likely a compound with a similar mass spectrum as methamphetamine-TFA and therefore the library could not differentiate the two compounds. The amphetamine in urine sample contained TFA, amphetamine-TFA, and 2,4-Disocyanato-1-methyl-benzene. 2,4-Disocyanato-1-methyl-benzene is likely a contaminate. It is a compound used to synthesize polyurethane foams and was also found in the urine blank sample.[70]

GHB and GBL were also spiked into human urine with concentrations of 2 mg/mL and 5 µg/mL respectively. GHB and GBL are metabolites of one another and should be analyzed for simultaneously. This concentration of GBL in urine was a realistic concentration, however, 2 mg/mL of GHB in urine is not a realistic sample. A realistic concentration of GHB in urine would be around 5 µg/mL, however, this method is not able to detect GHB at these concentrations thus far.

The GHB in urine sample contained BSTFA, siloxanes, and GHB-TMS_2. There were no other compounds detected. Compounds detected in the GBL in urine sample were only those of siloxanes and GBL. The abundance of siloxane peaks in the TIC are likely due to the degradation of the SPME fiber.

CHAPTER 4. METHOD OPTIMIZATION

Introduction

As discussed in Chapter 1, the volume of sample needed to completely vaporize in a TV-SPME experiment may be calculated using Equation 3. For this work, an extraction temperature study was performed for methamphetamine, amphetamine, GHB, and GBL in order to determine the optimal vaporization and extraction temperatures for this method. The samples were heated in the Gerstel agitator at 60, 80, 100, 120, 140, and 160ºC. The initial temperature of 60ºC was chosen because this was the temperature used in prior analyses. As it was also the lowest temperature, 60ºC was used to determine the maximum allowed volume of the samples. Each sample temperature was analyzed in triplicate and the average peak area was calculated and plotted against the extraction temperature. This was done to determine the temperature at which the analyte was best detected by the GC. Methamphetamine and amphetamine were analyzed underivatized in urine and derivatized with TFAA in methanol. GHB was derivatized in water with BSTFA + 1% TMCS.

A limit of detection (LOD) study was also performed for methamphetamine and amphetamine with concentrations ranging from 1000 µg/mL to 50 ng/mL in human urine. This range of concentrations was chosen based on realistic concentrations of methamphetamine and amphetamine in urine as well as to increase the likelihood of a successful detection.[69] The extraction temperature used for this study was determined from the previous temperature study of methamphetamine-TFA and amphetamine-TFA.

Experimental

Materials

Methamphetamine sulfate, amphetamine HCl, and GBL were purchased from Sigma-Aldrich (St. Louis, Missouri). GHB was purchased from Cayman Chemical (Ann Arbor, Michigan). TFAA and BSTFA + 1% TMCS were purchased from Thermo Fisher Scientific (Waltham, Massachusetts). Polydimethyl siloxane divinylbenzene (PDMS/DVB) SPME fibers, 65 µm film thickness, were purchased from Supelco (Bellefonte, Pennsylvania).

Sample Preparation

Methamphetamine and amphetamine were dissolved in urine and methanol with a concentration of 1 mg/mL each. GHB and GBL were dissolved in water with a concentration of 6 mg/mL and 10 mg/mL, respectively. All urine and water samples used a sample volume of 2.4 µL and all methanol samples used a sample volume of 24 µL. The PDMS/DVB fiber was first exposed to either a vial containing TFAA (for the phenylethylamines) for 10 minutes or a vial containing BSFTA + 1% TMCS (for GHB and GBL) for 50 minutes The SPME fiber was then inserted into the sample vial for 10 minutes. Sample vials were heated in the agitator at 60, 80, 100, 120, 140, and 160ºC. The fiber was then inserted into the GC inlet for desorption at 250 ºC. A set of vials containing methamphetamine and amphetamine in urine were also analyzed using the same method, but without the derivatization step. All sample temperatures were analyzed in triplicate for all analytes.

We also attempted to determine limits of detection for methamphetamine and amphetamine in urine. Samples of methamphetamine and amphetamine in urine with concentrations of 1000, 500, 100, 10, and 0.05 µg/mL were prepared and analyzed using the same method as the 60°C temperature study for the analytes of interest.

GC-MS Parameters

The same instrumentation and column that were used for methods in Chapter 2 were also utilized for these experiments.

The GC inlet was set to 250ºC and was in spitless mode. The initial oven temperature was 60ºC and was held for 1 minute. The temperature was then ramped to 250ºC at 15ºC/min and held for 1 minute at the final temperature. The mass transfer line was set to 250ºC and the flow rate of hydrogen carrier gas was kept at 2.5 mL/min. The source was held at 230ºC and the quadrupoles were held at 150ºC. The scan parameters were 40-550 m/z. Samples were analyzed in scanning mode and extracted ion profiles were used to find the peak area of each sample. All compounds were identified using the SWGDRUG and/or NIST libraries.

Results and Discussion

Methamphetamine Extraction Temperature Study

Methamphetamine was analyzed with extraction temperatures ranging from 60ºC - 160ºC. The figures below display the average peak area for each temperature underivatized in urine (Figure 36) and with derivatization in methanol (Figure 37). The area for the underivatized compound was found by extracting the m/z 58 ion for methamphetamine for all three samples for each temperature. The area for the derivatized compound was found by extracting the m/z 154 ion for methamphetamine-TFA for all three samples for each temperature.

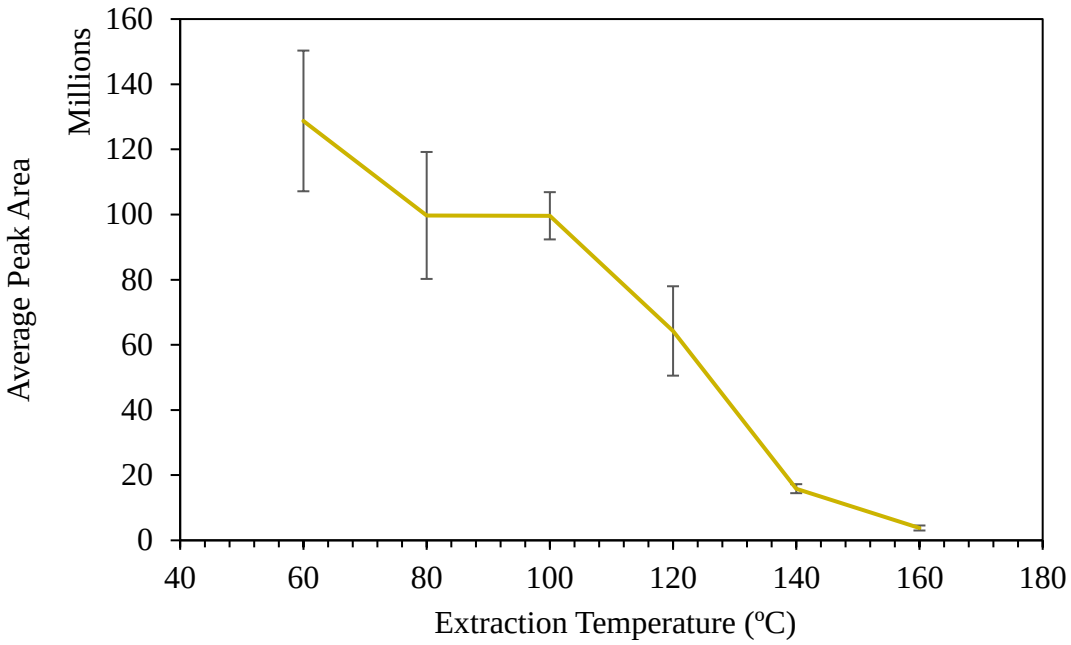


Figure 36. Average Peak Area vs. Extraction Temperature for underivatized methamphetamine in urine (m/z 58).

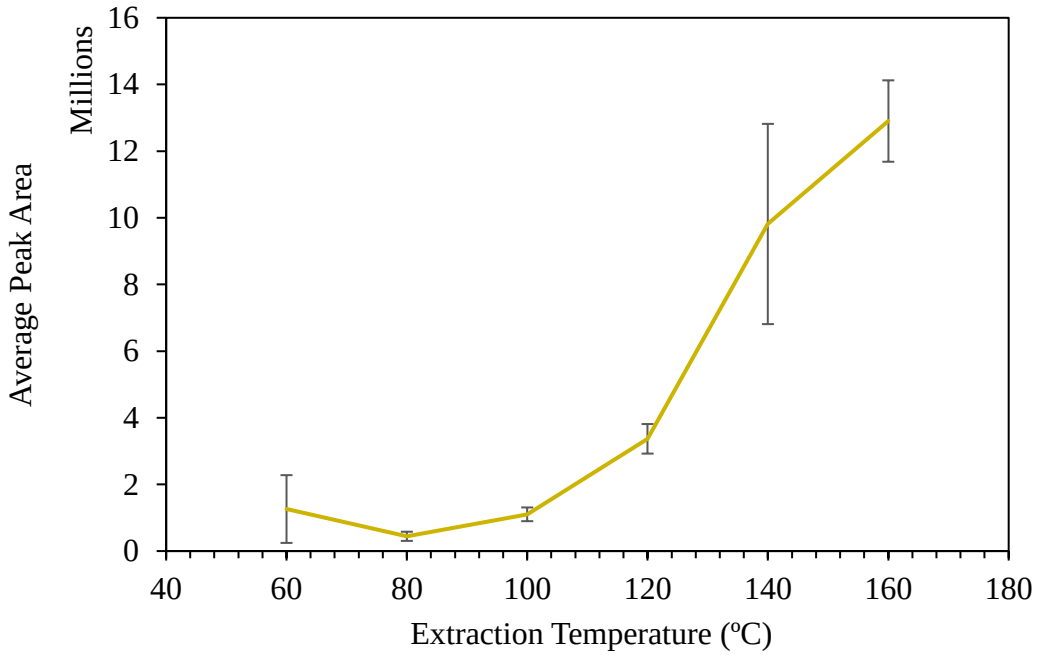


Figure 37. Average Peak Area vs. Extraction Temperature for methamphetamine-TFA in methanol (m/z 154).

Amphetamine Extraction Temperature Study

Amphetamine was analyzed in urine without derivatization and in methanol with derivatization with extraction temperatures ranging from 60ºC - 160ºC. Figure 38 below displays the average peak area for each temperature underivatized in urine. This area was found by extracting the m/z 44 ion for amphetamine for all three samples for each temperature. Figure 39. shows the average peak area for each temperature derivatized in methanol. This area was found by extracting the m/z 140 ion for amphetamine-TFA for all three samples for each temperature.

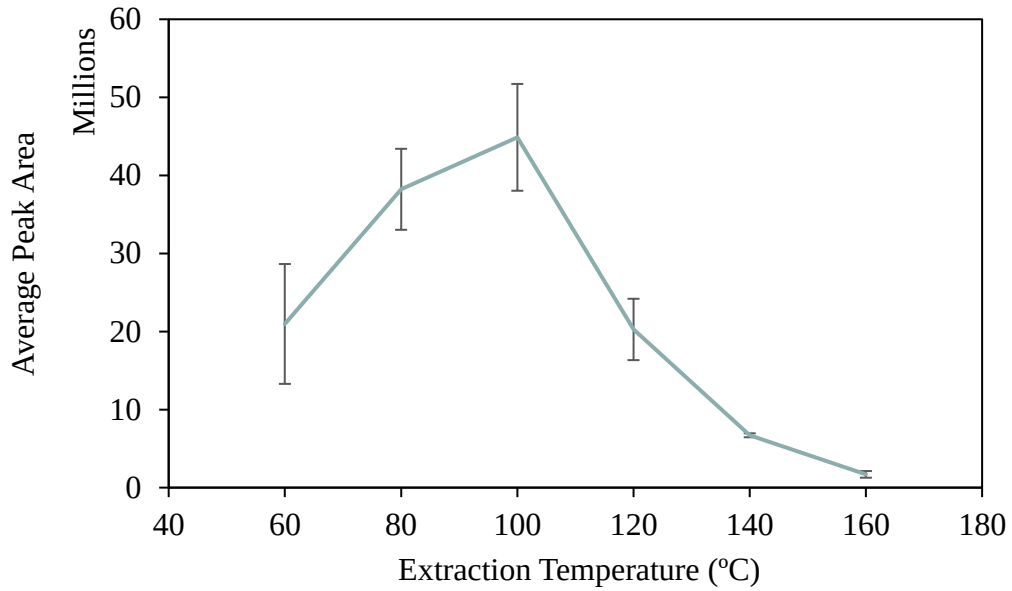


Figure 38. Average Peak Area vs. Extraction Temperature for amphetamine in urine (m/z 44).

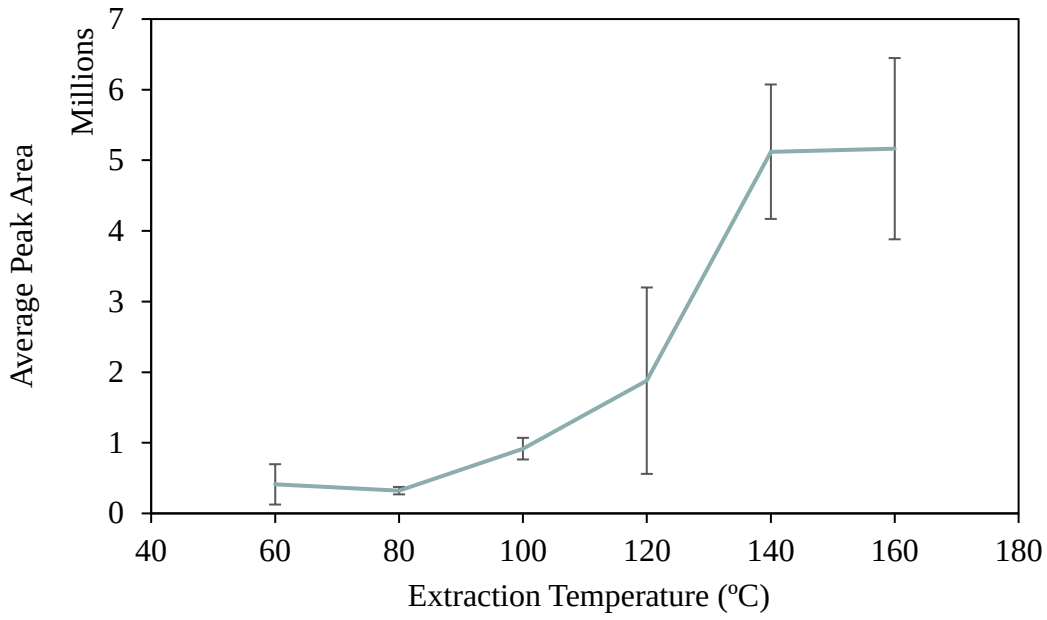


Figure 39. Average Peak Area vs. Extraction Temperature for amphetamine-TFA (m/z 140) in methanol.

This study showed that for underivatized methamphetamine in urine, 60°C was the optimal extraction temperature. However, for derivatized methamphetamine in methanol, 160°C was the optimal extraction temperature. For underivatized amphetamine in urine, 100°C was the optimal extraction temperature and for amphetamine-TFA in methanol, 140-160°C was the optimal extraction temperature. The peak area for amphetamine-TFA in methanol begins to plateau around 140ºC, so it would be sufficient to use this temperature. However, because methamphetamine is often found with amphetamine, 160ºC would be preferred. Most experiments performed with methamphetamine as the analyte of interest are performed with derivatization.

The difference in the extraction temperatures for underivatized and derivatized methamphetamine and amphetamine is likely due to the higher boiling points of the derivatives. For example, the retention time difference between methamphetamine and methamphetamine-

TFA is more than 2 minutes, corresponding to a difference in elution temperature of 27.7ºC for the methamphetamine samples and 21.9ºC for the amphetamine sample.

Methamphetamine and Amphetamine LOD

An LOD study was attempted for methamphetamine and amphetamine with concentrations ranging from 50 ng/mL to 1000 µg/mL in urine. Each concentration was analyzed in triplicate. TFAA was used as the derivatization agent for these experiments. The SPME fiber stripped on multiple occasions at various temperatures and extraction times. An LOD study was not completed due to the fiber damage. TFAA is an effective reagent for the derivatization of amines, however, when the sample is water based (e.g. urine), trifluoracetic acid (pKa = 0.3) is produced[71]. This reaction is shown in Figure 40. The production of this acid greatly degrades the integrity of the fiber. The harshness of TFAA along with the high temperatures creates a harmful environment for the SPME fibers and therefore an extraction temperature of 60°C was used for all experiments going forward.

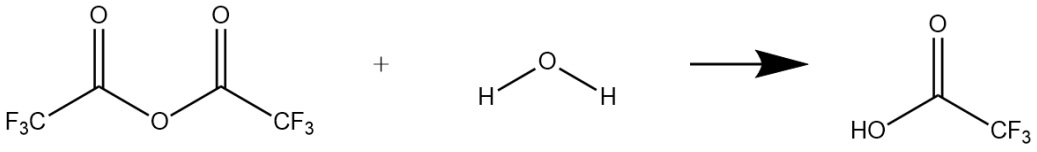


Figure 40. Reaction of TFAA with water to form TFA.

GHB Extraction Temperature Study

GHB was analyzed in water with extraction temperatures ranging from 60ºC - 160ºC. Figure 41 below displays the average peak area for each temperature. This area was found by

extracting the m/z 233 ion for GHB-TMS_2 for all three samples for each temperature. It was determined that 120ºC was the optimal temperature for GHB as it had the largest average peak area. When accounting for the error of this data, it is possible that extraction temperature does not have much effect on GHB.

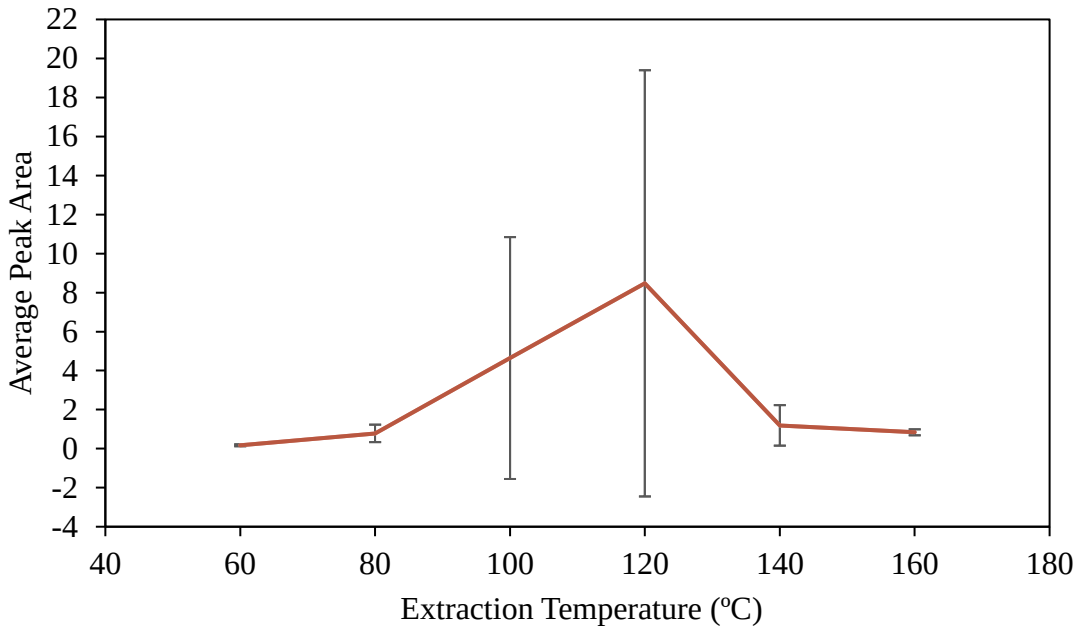


Figure 41. Average Peak Area vs. Extraction Temperature for GHB-TMS_2 (m/z 233).

GBL Extraction Temperature Study

GBL was spiked into water with a concentration of 10 mg/mL and analyzed with extraction temperatures of 60, 80, 100, 120, 140, and 160ºC. Figure 42 below displays average peak area for each temperature. The average peak area value was found by extracting the m/z 42 ion for each sample and taking the average of each temperature triplicate.

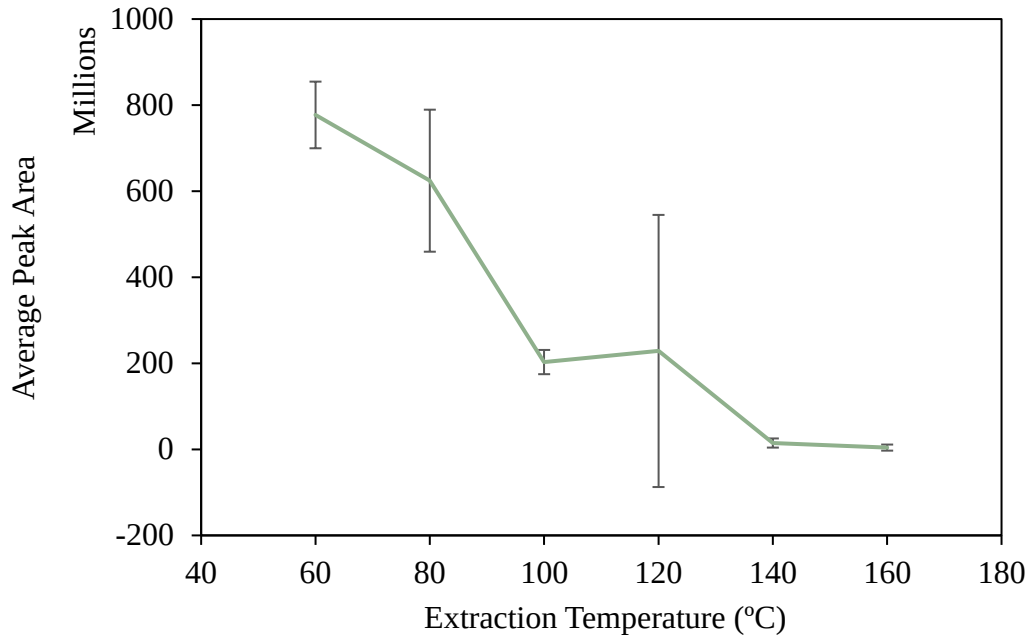


Figure 42. Average Peak Area vs. Extraction Temperature for GBL (m/z 42).

The GHB extraction temperature study showed that 100-120°C was the optimal extraction temperature for GHB-TMS_2 and 60°C for GBL. Similar issues arise with BSTFA as they do with TFAA. While BSTFA is not as harsh for the SPME fibers as TFAA, it still causes them to degrade. In addition, GHB requires a 50-minute derivatization time which causes the fibers to degrade more quickly. The long derivatization time and high temperatures are not ideal conditions for the SPME fiber. Therefore, 60°C was used for future GHB and GBL experiments.

Conclusion

Methamphetamine, amphetamine, GHB, and GBL extraction temperature studies were performed to determine the optimal extraction temperature at which these analytes would be detected by the GC. Extraction temperatures ranging from 60-160°C were utilized. The optimal temperatures were determined to be 60°C for methamphetamine, 160°C for methamphetamine-

TFA, 100°C for amphetamine, 140-160°C for amphetamine-TFA, 100-120°C for GHB, and 60°C for GBL. Due to the wide range in temperature shown by the error bars, these studies should be performed again to verify these optimal temperatures.

An LOD study was attempted for methamphetamine and amphetamine with concentrations ranging from 50 ng/mL to 1000 µg/mL in urine with TFAA as the derivatization agent. As stated, when TFAA reacts with a water-based solvent, trifluoracetic acid is produced. The production of this acid causes the SPME fiber to swell and break or causes the coating to strip away more easily. This study was analyzing 6 varying concentrations in triplicate for a total of 18 samples. Therefore, the fiber could not handle the acid production and broke or stripped during each attempt of this study. Therefore, the LOD study of methamphetamine and amphetamine was not completed.

CHAPTER 5. FUTURE WORK

Powder Samples

It was previously determined that powder samples may also be identified using the TV-SPME method.[1] While the use of the TV-SPME technique to analyze solid samples will not technically be a total vaporization method, the same method parameters used in TV-SPME will be utilized. A sample of a powder or a powder mixture will be placed into a GC headspace vial without any sample preparation. This will be done with pure samples, mixtures, and street samples obtained from a local law enforcement agency. Samples that will be analyzed include:

1) methamphetamine, pseudoephedrine and caffeine

2) cocaine, procaine and inositol

3) heroin, diphenhydramine and fentanyl

4) ethylpentylone and caffeine

5) tablets including hydrocodone, oxycodone, and hydromorphone

These mixtures have been suggested by Donna Roskowski, a former drug chemist for the Indiana State Police.

These samples will be analyzed using variants of the original TV-SPME method. Samples will be analyzed both underivatized and derivatized when necessary. Derivatization will be done by exposing the SPME fiber to the vapor of the derivatization agent as well as immersing the fiber into an organic solution of the derivatization agent. Exposing the SPME fiber to a solvent such as acetonitrile prior to the solid drug sample will also be performed to determine if the solvent on the fiber helps to adsorb the solid sample. A summary of successful methods and the analyte completed thus far are shown in Table 3. To date, methamphetamine and caffeine in powder form have been analyzed utilizing this method. Solid caffeine and solid

methamphetamine were analyzed using a PDMS/DVB fiber. Solid caffeine and solid methamphetamine were each placed into a 20 mL headspace vial and analyzed at 60°C with a 10-minute extraction time. Methamphetamine was analyzed with and without derivatization. Both methamphetamine samples had an extraction temperature of 120°C. Methamphetamine was derivatized with TFAA for 10-minutes to form methamphetamine-TFA. The chromatograms for these compounds are shown in Figures 43-45 below.

Table 3. Summary of successful methods and analytes.

	Headspace of Solid Sample	**TV-SPME of Sample in Solvent**	**TV-SPME of Sample in Urine**
Underivatized	Caffeine Pseudoephedrine	Amphetamine Methamphetamine GBL	Amphetamine Methamphetamine GBL*
Derivatized		Amphetamine Methamphetamine GHB	Amphetamine* Methamphetamine* GHB

*Trace concentrations

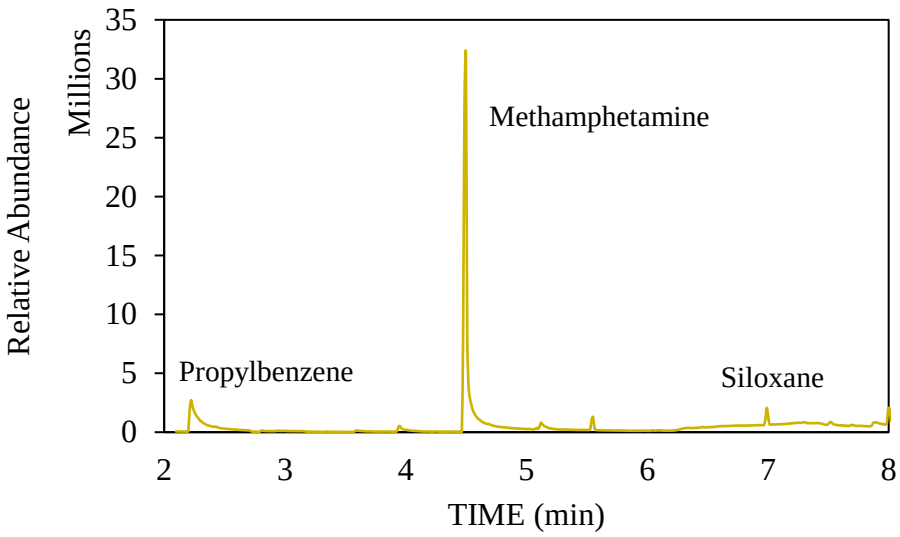
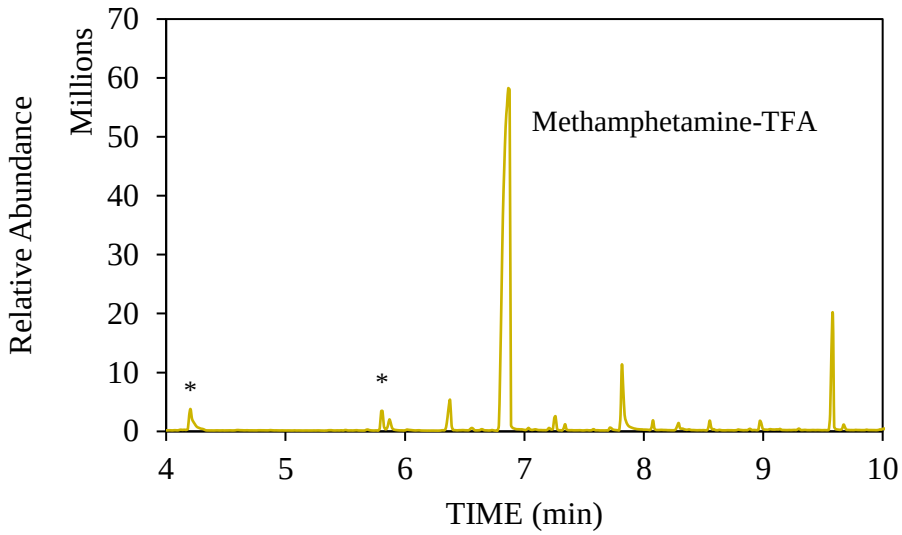
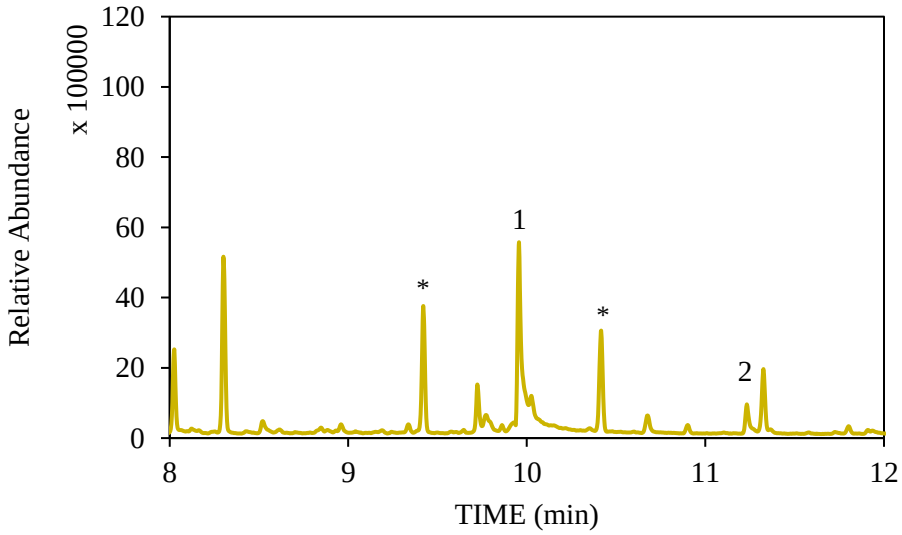


Figure 43. Underivatized solid methamphetamine.



* = Siloxane

Figure 44. Derivatized solid methamphetamine.



1. Caffeine
2. Hexadecanoic acid-TMS

* = Siloxane

Figure 45. TIC of caffeine powder with TV-SPME method.

Plant Material

Green leafy plant material (GLPM) is one of the most common pieces of evidence submitted to crime laboratories.[72] GLPM is often determined to be marijuana through microscopic and macroscopic identification as well as other chemical testing methods that must be performed manually.[73] The identification of these exhibits must be done individually and are therefore time consuming. With this automated SPME method, multiple samples may be analyzed in sequence. For example, a sample of tetrahydrocannabinol and a sample of cannabidiol may be analyzed within the same automated sequence because they require the same method parameters.

Future work will include the analysis of naturally occurring compounds found in marijuana including tetrahydrocannabinol (THC), cannabidiol (CBD), and cannabinol (CBN) “as is” and derivatized with BSTFA + 1% TMCS. Synthetic cannabinoids that will also be analyzed with and without derivatization will include 5-fluoro-ADB, FUB-AMB, and fluoro-MDMB-PICA. These synthetic cannabinoids will be derivatized with BSTFA + 1% TMCS. The analyses of these compound will be performed in the same way as the powder substances.

Toxicological Samples

Previous experiments involving drugs in biological samples included methamphetamine, amphetamine, THC-COOH, benzoylecgonine, GHB, and GBL in urine. THC-COOH is the metabolite of THC and benzoylecgonine is the metabolite of cocaine. Realistic concentrations of these samples are at ppb levels. GBL, methamphetamine, and amphetamine in urine were successfully detected at these concentrations. However, GHB, THC-COOH, and benzoylecgonine could not be detected.

Future work will include making various changes to the methodology to detect these compounds at ppb concentrations. Various temperatures (>100°C) and derivatization agents will be utilized including butyl chloroformate. Butyl chloroformate has successfully derivatized methamphetamine in acetonitrile when a 10-minute derivatization time was used. The resulting chromatogram is shown in Figure 48 and the mass spectrum is shown in Figure 49. Butyl chloroformate is not has harsh on the SPME fibers as TFAA and therefore higher temperatures may be used. The general reaction for butyl chloroformate with an amine is shown in Figure 46. The butyl chloroformate derivative of methamphetamine is shown in Figure 47. This derivatization agent will be used to identify methamphetamine and amphetamine in urine at ppb levels. THC-COOH and benzoylecgonine will be analyzed using BSTFA + 1% TMCS as the derivatization agent. Various derivatization times (5-60 minutes) and temperatures (>100°C) will be used to detect these compounds in urine.

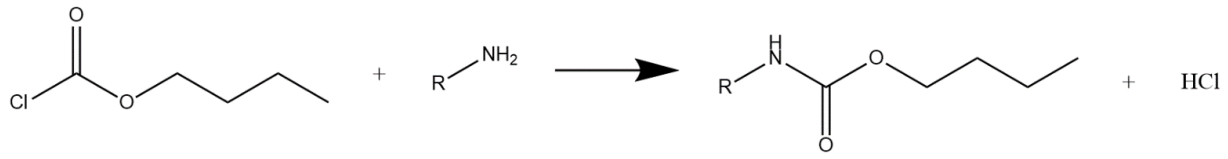


Figure 46. General reaction of an amine with butyl chloroformate.

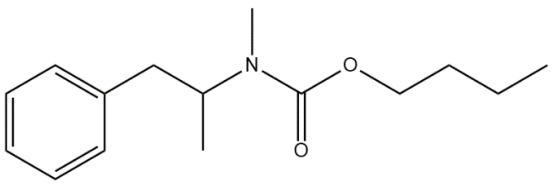


Figure 47. The butyl chloroformate derivative of methamphetamine.

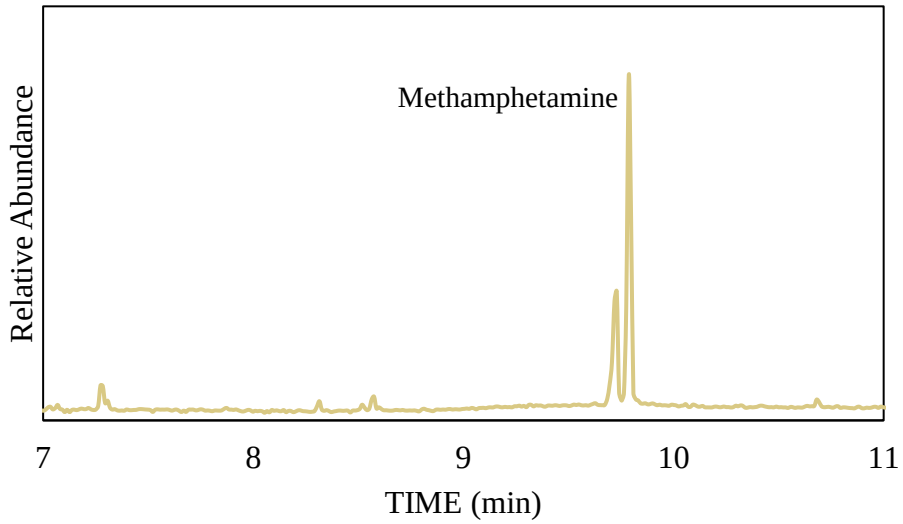


Figure 48. TIC for methamphetamine in acetonitrile derivatized with butyl chloroformate (1 mg/mL).

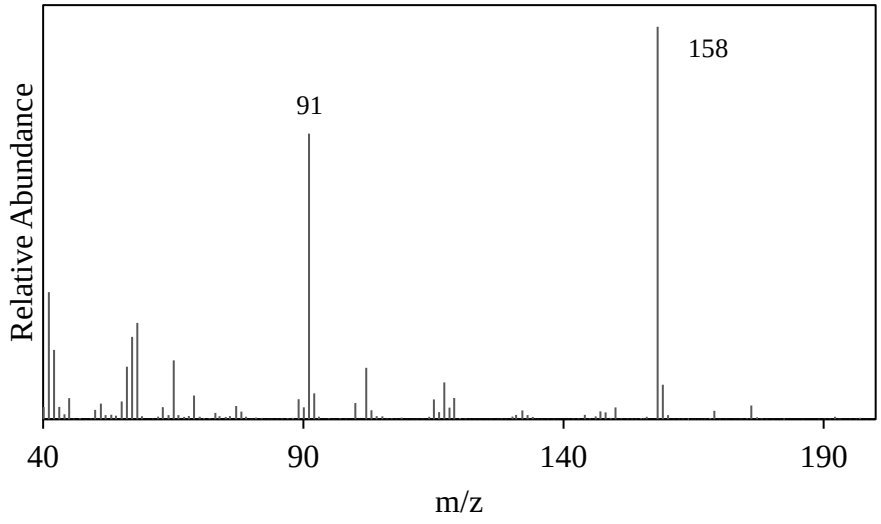
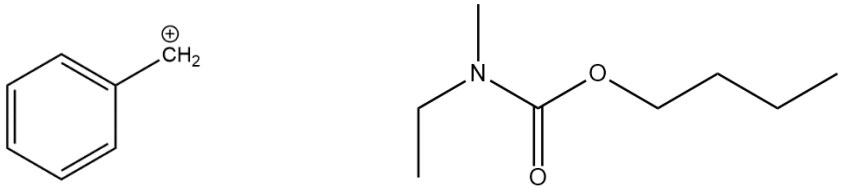


Figure 49. Mass spectrum for methamphetamine in acetonitrile derivatized with butyl chloroformate (1 mg/mL).

The mass fragments for methamphetamine derivatized with butyl chloroformate are shown in Figure 50.



(a) (b)

Figure 50. Mass fragments a) m/z 91 and b) m/z 158 for methamphetamine derivatized with butyl chloroformate.

Once these drugs have successfully been detected in urine, additional biological samples including blood or plasma and saliva will be spiked with realistic concentrations of these drugs and analyzed using the TV-SPME method.

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