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MICROEXTRACTION FOR THE EXTRACTION OF ORGANIC
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OPTIMIZATION OF HEADSPACE SOLID PHASE MICROEXTRACTION FOR THE EXTRACTION OF ORGANIC IMPURITIES IN SEIZED ECSTASY TABLETS

By

Heather Marie Dotzauer

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ABSTRACT

OPTIMIZATION OF HEADSPACE SOLID PHASE MICROEXTRACTION FOR THE EXTRACTION OF ORGANIC IMPURITIES IN SEIZED ECSTASY TABLETS

By

Heather Marie Dotzauer

Chemical profiling of ecstasy tablets can be used to link tablets to common production methods and batches based on the impurities present. Headspace solid phase microextraction (HS-SPME) is a promising alternative to the liquid-liquid extraction (LLE) procedures that are conventionally used to extract organic impurities from ecstasy tablets. HS-SPME uses a thin polymeric fiber to absorb and adsorb impurities from the headspace above a sample. Impurities are then desorbed from the fiber and analyzed by gas chromatography-mass spectrometry (GC-MS). HS-SPME decreases sample preparation time and solvent use, while preconcentrating analytes on the fiber.

In this work, an experimental design was used to optimize HS-SPME for the extraction of organic impurities from illicit ecstasy tablets. The optimized HS-SPME method was compared to LLE in terms of the number of impurities extracted. Impurity profiles of different ecstasy tablets were compared and the extraction capabilities of different SPME fibers were assessed. Fiber degradation was also monitored.

For my grandma, JoAnn, and my mother, Brenda—
two women who taught me to never give up,
especially when things get tough.

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KEY TO ABBREVIATIONS

amu	atomic mass unit
ANOVA	analysis of variance
CAR	Carboxen^{TM}
CCC	central composite circumscribed
CL	confidence level
CW-DVB	Carbowax$^{®}$-divinylbenzene
CW	Carbowax$^{®}$
DEA	Drug Enforcement Agency
d_f	degrees of freedom
DVB	divinylbenzene
DVB-CAR-PDMS	divinylbenzene/Carboxen^{TM}/polydimethylsiloxane
FDA	Food and Drug Administration
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
H_o	null hypothesis
HS-SPME	headspace solid phase microextraction
LLE	liquid-liquid extraction
MDA	3,4-methylenedioxyamphetamine
MDEA	3,4-methylenedioxy-N-ethylamphetamine
MDMA	3,4-methylenedioxymethamphetamine
MDP-2-P	methylenedioxyphenyl-2-propanone
MS	mass spectrometry
MSP	Michigan State Police
NFLIS	National Forensic Laboratory Information System
NMR	nuclear magnetic resonance
P12P	1-phenyl-1-propanone
P2P	1-phenyl-2-propanone
PA	polyacrylate
PDMS	polydimethylsiloxane
PDMS-DVB	polydimethylsiloxane-divinylbenzene
PMA	paramethoxyamphetamine
RT	retention time
SPME	solid phase microextraction
UN	United Nations

CHAPTER 1: Introduction

1.1 MDMA Use and Abuse

3,4-Methylenedioxymethamphetamine (MDMA) is a stimulant and psychoactive drug that is commonly known as 'ecstasy'. The drug is a member of the phenethylamine class, but it has different properties than other phenethylamines, such as methamphetamine, because of the addition of the methylenedioxy group on the benzene ring. MDMA was originally synthesized by Merck, and patented in 1914 as a precursor compound for the synthesis of styptic compounds, not as an appetite suppressant as some publications state [1]. The drug re-emerged in the underground scene in the 1970s. Since this time, MDMA has been illicitly available, but has undergone periods of fluctuating popularity.

1.1.1 Street Usage

In addition to ecstasy, tablets containing MDMA are known by street names such as XTC, E, Love Drug, and Adam, among others. Tablets have a street value of $25 to $45 each [2]. Nowadays, tablets sold on the street as ecstasy may have additional active components, such as caffeine, amphetamine, methamphetamine, or ephedrine, and cutting agents such as powdered milk or sugar [3,4]. The term 'ecstasy' has broadened to include club drugs other than MDMA; in fact, tablets marketed as ecstasy may contain no MDMA at all [5,6]. In illicit tablets, the content of MDMA ranges from 50 to 200 mg, typically of the hydrochloride salt [7]. Total tablet weight is in the range of 200 to 400 mg because of the addition of the cutting agents and additional active ingredients [8].

1.1.2 History and Legislation of MDMA

Although literature reports vary on the origin of MDMA's resurgence, it is agreed that it started with chemist Alexander Shulgin [9]. He was given a sample of the drug, and used nuclear magnetic resonance to confirm its structure [10]. Shulgin created a method to synthesize the drug and reintroduced it in the 1970s. He advocated its use in psychotherapy through his friend, psychological therapist Leo Zeff [10,11]. Although it was never approved by the Food and Drug Administration (FDA), MDMA was used by therapists as a psychotherapeutic tool prior to its scheduling as a schedule I illegal drug in the United States in 1988 [12].

Recently, the FDA has approved two U.S. studies involving the therapeutic use of MDMA for psychotherapy and for anxiety relief. Harvard Medical School in Boston is conducting a trial to determine whether MDMA can alleviate anxiety and pain in cancer patients [13]. Clinical trials have also been approved to test MDMA as a psychotherapeutic aid in the treatment of post-traumatic stress disorder [13].

In the early 1980s MDMA leaked out of the psychotherapeutic community and was sold in bars and clubs. The drug gained popularity at all night parties called raves because it enhanced the dancing experience; MDMA's stimulant effects enabled users to dance for long hours. Other effects that were desirous to the rave-goers included euphoria, empathy, and reduced inhibitions [14]. Because of this increasing abuse, MDMA was placed in schedule I of the Controlled Substances Act as an emergency measure [15]. Due to its lack of medical use and high potential for abuse, MDMA was permanently categorized as a schedule I substance in 1988, despite appeals to this ruling from camps advocating the drug's potential therapeutic uses [12,16].

By the 1990s, MDMA use had become an epidemic, in spite of legislation designed to strictly control its use. From 1994 to 1999, ecstasy related emergency room visits increased from 250 to 2,850 per year [17]. MDMA use expanded beyond the rave scene, making its way into schools and homes [6,17]. In 1996, prevalence of ecstasy use by 10th and 12th graders was 4.6%—higher than for college students and young adults in that same time period [18]. Use of MDMA is most prevalent in adults under the age of 25, but is abused by males and females at the same rate [17]. The 2005 National Survey on Drug Use and Health reported that in their lifetime, 13.7% of people aged 18 to 25 had used MDMA, as compared to 4.7% of the overall population [19]. MDMA is now considered an established drug of abuse [17,20].

In part due to increased legislation and public awareness campaigns, MDMA use has leveled off since 2002 [20]. It is estimated that 1.2 million people used MDMA for the first time in 2002, while only 615,000 admitted first time use in 2005, the latest year for which figures are available [19]. This data was extrapolated from personal interviews with randomly selected U.S. residents over the age of 12 [19]. Although every attempt was made to ensure the scientific validity of the study, these numbers are self-reported, and may not accurately reflect the extent of the epidemic.

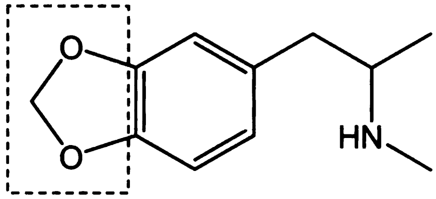
Despite this apparent decrease in the use of MDMA, trafficking of tablets remains a cause for concern; large amounts of tablets continue to be confiscated by the Drug Enforcement Agency (DEA). Although a few clandestine laboratories have been uncovered in the U.S., most MDMA tablets seized are manufactured in the Netherlands and Belgium [2,14]. Detroit, MI, Miami, FL, New York, NY, and Los Angeles, CA are common ports of entry for MDMA coming into the U.S. from these countries [14]. The DEA estimated that in the year 2000, two million MDMA tablets were smuggled into the U.S. each week [21]. Targeted initiatives by the DEA and Canadian law enforcement have identified trafficking organizations. One such initiative, Operation Candy Box, resulted in the seizure of $87 million and 407,000 MDMA tablets from 2001 to 2004 [22].

Trends of MDMA use are reflected in our justice system. Effective November 2001, the Ecstasy Anti-Proliferation Act of 2000 increased the sentence for trafficking MDMA [2]. Trafficking of more than 800 tablets now carries a five year sentence, as opposed to the previous 15 month sentence. The penalty for trafficking more than 8,000 tablets was increased from 41 months to ten years in order to target upper-level distributors [2]. Although a direct correlation cannot be shown, MDMA related arrests and crime laboratory submission of ecstasy tablets for analysis has decreased since this legislation was enacted. In 2000, there were 1,533 MDMA related arrests, which decreased to 936 in 2004 [23]. Crime laboratories across the country reported submission of 13,241,796 tablets suspected to contain MDMA in 2001 and 8,592,376 in 2005 [23].

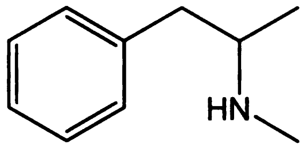
Despite this drastic decrease, MDMA is still prevalent. A 2005 report by the National Forensic Laboratory Information System (NFLIS) showed that MDMA is one of the 25 drugs most frequently identified by regional, state, and national laboratories [24]. According to the NFLIS, MDMA constitutes 0.71% of drug cases analyzed each year. The majority of cases seen by laboratories are cannabis and cocaine, which respectively comprise 33.5% and 32.5% of analyzed drug items [24].

1.1.3 Chemistry and Pharmacology of MDMA

Structural and pharmacological data can be used to explain MDMA's effects and popularity as a club drug. As seen in Figure 1.1, MDMA differs from methamphetamine only in that MDMA contains a methylenedioxy (-O-CH_3-O-) group on the aromatic ring. The presence of methoxy substituents imparts hallucinogenic effects to the compound, while the presence of a methyl group on the α-carbon imparts stimulant effects. Hence, while both methamphetamine and MDMA have stimulant effects, MDMA also has hallucinogenic effects.



MDMA



Methamphetamine

Figure 1.1. Chemical structures of MDMA and methamphetamine. MDMA has the same structure as methamphetamine, except it contains a methylenedioxy (-O-CH_3-O-) group on the aromatic ring, which imparts its hallucinogenic properties.

Unlike its counterpart methamphetamine, MDMA is not sufficiently volatile to be inhaled as a free base [25]. Since MDMA is mostly seen in tablet form, oral ingestion is the most common route of administration, but snorting and injecting are other potential administration routes [14].

After oral ingestion, MDMA reaches peak plasma levels in approximately two hours [26], with effects lasting 3 to 6 hours [12]. Physical effects of MDMA include nausea, blurred vision, faintness, tremors, sweating, chills, muscle tension, and teeth clenching [20]. Because of its slow onset, users often take another tablet before the effects of the first one begin; a practice that contributes to the likelihood of overdose. As tablets are typically synthesized in clandestine laboratories, the purity of the tablets is largely unknown; a factor that also increases the risk of overdose. Deaths from toxic overdose of MDMA have been attributed to toxicity in the brainstem causing central nervous system impairment [9].

Onset of hyperthermia is another serious risk of MDMA consumption that is exacerbated by typical use in crowded rave settings. The inability to regulate the body's temperature, combined with the physical activity of dancing can cause profuse sweating. Users may drink excessive amounts of water in an attempt to avoid overheating. This causes the blood to become diluted, and water enters the brain. The consequences of water swelling the brain are seizures and disruption of respiration and circulation [27]. These effects are intensified when MDMA is used in combination with alcohol or other drugs, a likely scenario at clubs and raves.

1.2 Profiling of Illicit Drugs

The prevalence and danger of MDMA abuse underlines the importance of analysis and detection of the drug. The goal of crime laboratories is to identify the controlled substance(s) in the seized material. Profiling of drugs takes this initiative one step further; drug profiles attempt to identify the source of the tablets by linking those with common traits. Drug profiling is not intended to be conducted in a crime laboratory along with identification tests. More likely, profiling would be conducted by a regional or national laboratory.

Current profiling of illicit ecstasy tablets is based on physical and chemical profiling of tablets. Ecstasy tablets are often colored and imprinted with a motif that is used as an identifying factor. This motif can represent a popular trend such as a smiley face, a Harry Potter logo, or a cellular phone. Users may associate a certain logo with a particular manufacturer and hence, quality of the drug. In reality, color and motif alone do not reflect purity and are not enough to link tablets to a common source [4].

MDMA is synthesized in large batches. Most illicit laboratories are capable of cooking 20-30 kg of MDMA at a time; some super-labs can make up to 100 kg at a time [28]. After synthesis, cutting agents such as starch, lactose, and caffeine are mixed in so that the MDMA can be divided into more tablets [4]. Tablets from the same batch would be expected to have similar additives.

The resulting mixture may be colored with dye, and is then pressed into tablets. Palmitic and stearic acids are used as tableting agents, which help form the tablets and hold them together [4]. At this stage, logos are imprinted on the tablets. Multiple manufacturers may use the same logo, or one manufacture may imprint one batch of MDMA with several different logos.

Due to increased awareness of attempts by law enforcement to profile tablets using logos, illicit manufacturers now frequently change the tablet logo, in an attempt to avoid being linked to seized tablets [29]. Some seized clandestine laboratories have been found to have a variety of metal taps and dyes in order to vary the physical appearance of tablets produced from a common batch of MDMA [30]. A study conducted in Israel over a two year period from 2001 to 2003 concluded that the average life expectancy of a tablet logo is now approximately nine months [29]. During the length of the study, over 300 different logos were recorded.

Tablets with the same physical characteristics, such as dimension, mass, color, and motif, do not necessarily have the same chemical composition, as illustrated by Cheng *et al.* [30]. Three tablets with similar physical characteristics (orange, rectangular tablets with a CC motif) were analyzed. One contained MDMA, while the other two contained methamphetamine. The tablets ranged in purity from 4.7-55%, and contained additives such as ketamine, diazepam, and phenobarbital.

A study by Teng *et al.* further illustrates this point. One-hundred and eighty one MDMA containing tablets were confiscated by the Taiwan National Bureau of Controlled Drugs between 2002 and February 2005. Within this sample set, there were 24 groupings of tablets, with each grouping having the same logo but different colors. The content of MDMA in these tablets differed by as much as 90 mg among tablets with the same logo [5]. The confiscated tablets also contained different additives, including ephedrine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-N-ethylamphetamine (MDEA) and caffeine [5]. Even though the tablets possessed the same logo, they were most likely not from the same batch or the same manufacturer, based on differences in purity and additives.

Tablets with similar physical characteristics have been shown to have differing chemical impurities. Also, common batches of MDMA can be divided to be made into tablets with different colors and motifs. For these reasons, physical characterization is not sufficient to link tablets.

In addition to physical characterization of tablets, chemical impurity profiling can be used to identify links among tablets. Commonly, chemical impurity profiling involves extraction of impurities from the tablet, typically by liquid-liquid extraction (LLE). Extracts are analyzed using chromatographic techniques, typically gas chromatography (GC). The resulting chromatogram, or impurity profile, can be considered as a chemical fingerprint of the tablet.

Impurity profiling is not a new concept in forensic drug analysis. The DEA has signature analysis programs in place for both heroin and cocaine [31,32]. Because heroin and cocaine are originally extracted from plants, profiling is used to determine geographical and processing origin of the plant. For MDMA and other synthetic drugs, profiling aims to identify the synthetic route used in the synthesis and link batches of common origin by comparing the number and combination of the impurities present in the seized tablet.

1.2.1 Common Synthetic Routes for MDMA Production

Production and preparation for distribution imparts each sample of synthetic drug with its own characteristic composition of impurities [33]. Differences in synthetic methods, initial purity of reagents, subsequent purification steps, by-products, and addition of cutting agents all contribute to the impurity profile of a drug [3]. Batches of MDMA produced using different synthetic methods and/or produced in different laboratories are expected to yield different impurity profiles [4].

Reductive amination of methylenedioxyphenyl-2-propanone (MDP-2-P) is the most common MDMA synthesis route [33]. There are three common reducing agents for the reductive amination synthesis: aluminum/mercury amalgam, cyanoborohydride, and sodium borohydride. In addition to reductive amination, the Leuckart reaction and safrole bromination are other routes for MDMA synthesis [33,34]. Appendix A outlines these common synthesis routes.

Some impurities and by-products are characteristic of a particular route. For example, N-formyl-MDMA is route specific for the Leuckart synthesis and 1,2-(methylenedioxy)-4-(2-N-methyl-iminopropyl) benzene is route specific for reductive amination [33,34]. While some impurities are route specific, different routes can have common impurities. Both the Leuckart and reductive amination routes start with MDP-2-P. Because this precursor is controlled, it needs to be synthesized by the clandestine chemists, typically using piperonal as a precursor [8]. MDP-2-P can itself be synthesized in a variety of ways, which is another source of impurities and by-products.

Because some impurities and by-products are not route specific, the distribution of impurities, rather than specific impurities present, can be most helpful in chemical profiling [4,33]. Tablets with similar levels of the same impurity may even be linked to a common production source [8,30]. This can bolster law enforcement's investigation abilities and link seizures with suppliers [35]. Ultimately, law enforcement agencies can then use the information generated from the impurity profiles to monitor the activities of clandestine laboratories, identify dealer-user networks, and expose drug-trafficking organizations.

1.2.2 Chemical Impurity Profiling

Chemical impurity profiling to identify precursors, impurities, and by-products has been conducted by various researchers [4,5,8,29,30,33,34]. Cheng *et al.* used a LLE method to extract impurities from a variety of seized tablets [8]. Extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) and the resulting chromatograms were compared based on the differences in tablet composition and relative amounts of each impurity. Two differently colored "Armani" tablets seized one year apart were shown to have similar impurity profiles, which indicated they originated from the same illicit manufacturer. Also studied were two physically similar, but differently colored, tablets with a "J" logo. The two tablets had distinct differences in impurity profiles, indicating that they did not originate from the same batch. Similarities in route were not addressed. If the two "J" tablets were synthesized via the same route, the differences in profiles would be attributed to differences in precursors, by-products, cutting agents, or binders. These case samples demonstrate that chemical impurity profiling is essential to discerning linkages between common synthesis routes or common batches; physical characterization is insufficient for profiling efforts.

Like Cheng *et al.*, Palhol *et al.* reported an example of two physically dissimilar seizures that were linked together using an impurity profile generated by LLE and GC-MS [4]. In this case, a seizure of "Euro" tablets and a seizure of "Batman" tablets were seized in the same area within one month of each other. The physical characteristics (mass, diameter, and thickness) and MDMA content of the tablets were not similar, but the impurity profiles were identical in regards to number of impurities and their relative intensities. Palhol *et al.* concluded that these tablets were synthesized by the same route and may even have originated from the same synthetic batch. Since one batch of MDMA can be split into different tablet seizures having different masses, dimensions, colors, and logos, this conclusion is feasible.

Despite being commonly used to extract impurities from illicit tablets for subsequent profiling, LLE has limitations. LLE requires organic solvents, which adds to disposal costs, and is potentially toxic depending on the solvent that is used. Also, formation of emulsions can affect extraction efficiency [36]. High extraction efficiency for the active ingredient in the tablets may mask impurities, which are typically present at trace levels. Fortunately, alternative extraction techniques are available in order to avoid the above-mentioned issues.

Solid phase microextraction (SPME) is one such alternative. The technique uses a thin polymeric fiber to adsorb and/or absorb analytes either directly from a sample solution (immersion SPME) or from the headspace above the solution (headspace (HS) SPME). Analytes are non-exhaustively extracted from the sample and pre-concentrated on the fiber during extraction. The fiber is then placed in a GC inlet to desorb the analytes onto the GC column. Analytes are then analyzed and detected, typically with mass spectrometry (MS). SPME offers numerous advantages over LLE, including minimal sample preparation, relatively non-destructive extraction, reusability of the fiber, and decreased solvent consumption. In regards to profiling, SPME offers greater sensitivity and specificity for extraction of impurities because the low volatility of the MDMA salt suppresses its extraction efficiency [37]. Hence, a SPME extract contains less of the controlled substance which otherwise may mask the presence of impurities, especially at the trace level.

1.3 Research Objectives

The objective of this research was to develop and optimize a HS-SPME procedure for the extraction of organic impurities from seized MDMA tablets. An experimental design was used to assess the interconnection between the HS-SPME variables of extraction time and extraction temperature, both of which have been shown to affect the type and amount of analytes extracted from the sample. GC inlet desorption parameters were also optimized to determine the desorption time and desorption temperature that offered optimum desorption of impurities from the fiber onto the column for analysis. HS-SPME was compared to LLE and the number of impurities extracted by each procedure was tabulated.

SPME fibers with different stationary phases were tested to determine which fiber extracted the greatest number of impurities.

Profiles of the organic impurities in several different ecstasy tablets were generated using HS-SPME and GC-MS to assess differences in the pattern of impurities that may allow differentiation between seizures. These profiles are advantageous to law enforcement because they allow tablets to be grouped according to their similarity in organic impurities, potentially linking seizures to a common batch or synthetic method.

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CHAPTER 2: Theory

2.1 Solid Phase Microextraction (SPME)

SPME was originally developed for environmental applications in order to reduce sample preparation time and minimize solvent consumption during extractions [1], but has become an increasingly used forensic technique, particularly in the analysis of arson debris, explosives, and drugs of abuse [2]. SPME is advantageous for forensic applications because its ability to pre-concentrate analytes increases sensitivity. The method is also beneficial because it can produce clean extracts from complex matrices, such as arson debris or urine [2].

The basic premise of SPME is extraction via a thin fused silica fiber that is coated with an absorbent polymer. The one to two centimeter long fiber is held in a syringe-like metal holder to protect the fiber and prevent exposure to the atmosphere. During extraction, the fiber is exposed in a vial containing the sample. Sample analytes that have an affinity for the fiber's polymeric coating will partition from the sample, absorbing and/or adsorbing onto the fiber coating, thus concentrating the analyte onto the fiber. After a defined extraction time, the fiber is removed from the sample vial and analyzed.

Analytes extracted from the SPME fiber are commonly analyzed by GC-MS, but can be analyzed by liquid chromatography-mass spectrometry as well. For GC-MS analysis, the fiber is inserted into the heated inlet of the gas chromatograph. Upon exposure to the heat of the inlet, analytes are thermally desorbed from the fiber onto the head of the analytical column. The flow of carrier gas moves the analytes through the GC column, where separation occurs. Mass spectrometry is used for detection and identification of the separated impurities.

Two SPME sampling modes exist; immersion SPME, where the fiber is placed directly in the liquid analyte, and HS-SPME, where the fiber is exposed to the headspace above the sample. In general, a longer amount of time is needed for immersion SPME to reach equilibrium, as compared to HS-SPME [3]. In immersion SPME equilibrium is formed between the liquid and the fiber. If the analytes have a greater affinity for the liquid, they will not be extracted well with the fiber. Altering the sample matrix by stirring, changing the pH, or adding salt, can cause a larger mass of sample to partition onto the fiber. Stirring or agitating the sample renews the volume of the sample that is in contact with the fiber, decreasing extraction time [4]. Changing the pH or adding salt can make the analytes less soluble in their liquid matrix and more likely to partition onto the fiber [4]. Because of potential damage to the fiber, changes must be made with constraint. Each fiber has a recommended pH range and solvent compatibility. If optimizing a method for immersion SPME sampling, stirring, pH, and salt addition must all be taken into consideration.

HS-SPME can be applied to sample the headspace above solid or liquid samples. With liquid samples, pH, salt concentration, and stirring would need to be considered as for immersion SPME. HS-SPME of solid samples is least complicated and involves the least sample preparation. For these reasons, this project focuses solely on HS-SPME of solid samples. Figure 2.1 shows the

SPME apparatus during headspace extraction of a solid sample. During this process, equilibrium develops among the analyte in the sample, the headspace, and the fiber. Two of the most important experimental parameters are fiber stationary phase coating and fiber coating thickness, because they directly affect the amount of analyte extracted. A number of experimental parameters, such as vial size, sample size, vial heating, extraction time, and extraction temperature must also be taken into consideration.

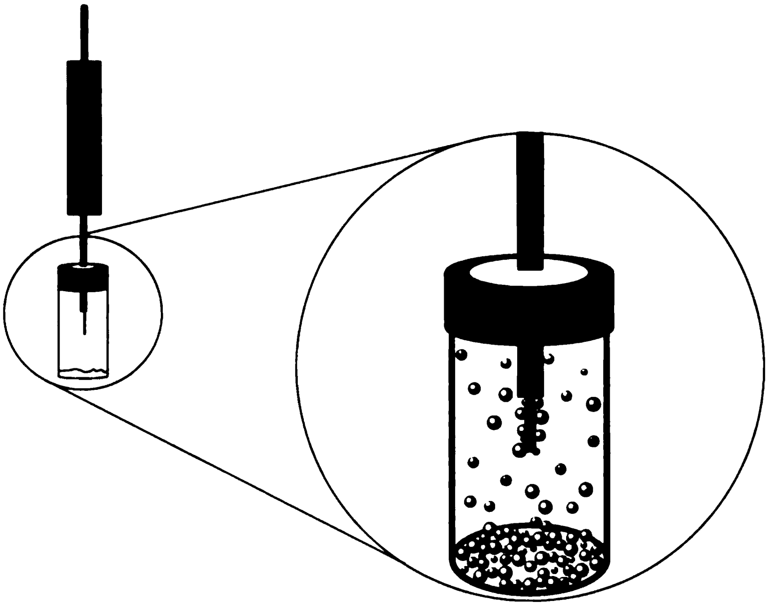

Figure 2.1. SPME apparatus during headspace extraction.

At equilibrium conditions, the mass of analyte extracted by the fiber coating (n) during headspace extraction is given in the following equation [5]:

$$n = \frac{K_{fh}K_{hs}V_fC_0V_s}{K_{fh}K_{hs}V_f + K_{hs}V_h + V_s}$$

where K_{fh} is the ratio of analyte in the fiber coating to analyte in the headspace, K_{hs} is the ratio of analyte in the headspace to analyte in the solid sample, V_f is the volume of the fiber coating, C_0 is the initial concentration of analyte in the sample, V_h is the headspace volume, and V_s is the volume of sample.

High n values are achieved when a larger volume of analyte is in the headspace and the analyte has a high affinity for the fiber. Larger K_{fh} values indicate the analyte has a greater affinity for the fiber than for the headspace, which is preferred for HS-SPME sampling because the mass of analyte extracted by the fiber is increased. Low K_{hs} values indicate that the sample has more affinity for the sample matrix than the headspace and hence, HS-SPME sampling would not be recommended.

The optimum fiber should be selective and have high affinity (large K value) for the analyte(s) of interest; this allows for pre-concentration of the analytes on the fiber, which increases sensitivity of the technique. However, analytes that are attracted too strongly to the fiber are difficult to desorb, and carryover between samples may result. Fibers with different stationary phases can be chosen in regards to the type of analytes being extracted.

Non-polar analytes have a greater affinity for non-polar fiber coatings, *e.g.* polydimethylsiloxane (PDMS), whereas polar analytes are best extracted with polar fiber coatings, *e.g.* polyacrylate (PA) or divinylbenzene (DVB) [3]. Analytes either absorb (become integrated with the polymer) or adsorb (attach to the surface) of the fiber, depending on the stationary phase used. PDMS and PA are absorbent materials, whereas DVB, Carbowax® (CW), and Carboxen™ (CAR) are adsorbent materials. CAR and CW are polymers which aid in the extraction of small molecules. CW increases the surface area of the fiber to aid in the extraction of low molecular weight polar analytes [3]. CAR forms small pores which act as a molecular sieve to trap small molecules [3]. Combination fibers, which combine two or more stationary phases, can be used in order to extract multiple analytes with varying polarities and molecular weights. This is advantageous when working with mixed samples and trying to analyze the variety of analytes in the sample. Single phase fibers work best for targeting specific analytes in a mixture.

Fiber thickness also affects the mass of analyte extracted from the sample, but it is an especially important consideration in the desorption of analytes from the fiber. Increasing fiber coating thickness increases the value for V_f which in turn increases the value for n. Despite this, there are practical limitations to fiber thickness, and a thick fiber is not amenable to all applications. Thin coating layers (7 μm and 30 μm) are more suitable for the extraction of semi-volatile components. Analytes take longer to desorb from thick coatings (100 μm). Therefore, volatile analytes take longer to desorb from these coatings, thus helping to minimize loss of volatile analytes between extraction and desorption [3].

In addition to extraction mode, fiber thickness, and fiber polarity, adsorption of analytes onto the fiber is also affected by vial size, mass of sample, surface area of the sample, sample agitation, extraction time, and sample heating. Each of these parameters can be related back to the equation for HS-SPME extraction. Increasing extraction length ensures enough time for equilibrium to be reached among between the sample, the headspace, and the fiber. Increasing the sample surface area, heating the sample, and agitating the sample allow more sample to move into the headspace in a shorter time frame, thus increasing K_{hs} and increasing the overall amount of analyte extracted in a given time period. Reducing vial size and minimizing the headspace allow the equilibrium to be reached more quickly because V_h is smaller [4,5]. Each of these variables should be optimized in order to achieve reproducibility. Because the factors may be interdependent, the optimization of factors should be conducted concurrently. For example, the use of a small vial may facilitate the attainment of equilibrium to the same extent that applying heat does, but heating the small vial may cause equilibration to occur more rapidly than if the two factors were considered independently.

There are many advantages to HS-SPME that increase its attractiveness as an extraction method. HS-SPME can be conducted using solid samples, thus eliminating additional considerations associated with liquid systems such as stirring, salt concentration, and pH, and minimizing sample preparation [5]. Increased motion of molecules in the gas phase causes reduced equilibration time for HS-SPME as compared to immersion sampling [5]. In addition, HS-SPME of solids lengthens fiber lifetime by slowing degradation of the fiber coating while also eliminating the necessity for solvents, which cuts down on consumable costs and waste generation [6].

2.2 Application of SPME to Drug Profiling

SPME is a valuable tool for the extraction and pre-concentration of analytes from a sample prior to instrumental analysis. To this end, SPME has been used to sample and profile impurities in amphetamine type stimulants; preliminary research into HS-SPME impurity profiling in seized samples of amphetamine and methamphetamine has also been recently reported [7,8,9,10]. The pre-concentration ability of SPME is an obvious advantage when profiling chemical impurities in illicit drug preparations, which are likely to be present only at trace levels. Although SPME can be advantageous, reproducible extractions are imperative and SPME procedures must be fully optimized before chemical profiling is undertaken.

Vu used HS-SPME to characterize volatiles in methamphetamine in order to create a model training material for drug-sniffing canines [7]. Headspace extraction of heated vials of both pharmaceutical grade methamphetamine hydrochloride and illicit methamphetamine samples was performed and the extracts were subsequently analyzed by GC-MS. Optimization of the HS-SPME procedure was limited to determining which fiber type gave the most complete profile under constant extraction conditions using a ten gram sample of pharmaceutical grade methamphetamine. CW-DVB, CAR, and DVB fibers were compared. The CW-DVB fiber detected acetic acid, 1-phenyl-2-propanone (P2P), 1-phenyl-1-propanol, and benzoic acid, whereas the CAR and DVB fibers only detected three of these four impurities. When applied to the analysis of one gram of seized methamphetamine, a more complex profile resulted. The CW-DVB fiber was chosen as the most effective fiber, as it extracted six different impurities, including synthesis byproducts such as phenyl-2-propanone (P2P) and 1-phenyl-1-propanone (P12P) and cutting agents such as dimethyl sulfone.

The fiber was not overloaded by methamphetamine, presumably due to the low volatility of the hydrochloride salt. When testing 12 illicit methamphetamine samples with the optimized extraction method, benzaldehyde and P2P were the only components found to be common among all of the samples tested. For this reason, benzaldehyde and P2P were recommended as the pseudomethamphetamine canine training materials.

Coumbaros *et al.* also used HS-SPME for the analysis of amphetamine-type drugs, but as a means to recover the manufacturing impurities in seized samples of paramethoxyamphetamine (PMA) [8]. Fiber extraction times and temperatures were optimized using headspace extraction of 100 mg of PMA with a PA fiber; extracts were subsequently analyzed by GC-MS. Increasing absorption temperature increased the peak area of the extracted impurities. Tripling the extraction time from 10 minutes to 30 minutes increased the overall amount of impurity extracted, which increased the peak height of the most prevalent impurity two-fold.

Koester *et al.* used HS-SPME for impurity profiling of seized methamphetamine [9]. The SPME fiber was exposed to the headspace of 30 mg samples of methamphetamine at temperatures ranging from 80 °C to 110 °C at a constant extraction time of 25 min.; extracts were subsequently analyzed by GC-MS. The extraction capabilities of HS-SPME were compared to the LLE technique recommended by the United Nations (UN). HS-SPME (at 110 °C) extracted 41 impurities while LLE extracted only eight for a given illicit sample of methamphetamine of the same mass. Again, HS-SPME served to pre-concentrate the impurities and suppress extraction of methamphetamine, thus increasing the sensitivity of the method for the detection of impurities. The greater the number of impurities extracted by HS-SPME, the more detailed the subsequent impurity profile will be, which is beneficial in determining the synthetic route used and linking common batches.

The most recent publication regarding impurity profiling using HS-SPME is that of Kuwayama *et al.* [10]. The HS-SPME extraction method was optimized one parameter at a time. Mass of sample, extraction temperature, extraction time, and fiber type were varied to optimize the HS-SPME procedure.

The DVB-CAR-PDMS fiber extracted more impurities than the PDMS or the CW-DVB fibers. Hence, the DVB-CAR-PDMS fiber was used for all subsequent experiments. Since fibers with mixed stationary phases allow for extraction of impurities with a wide range of polarities, a greater number of impurities could be extracted using the mixed phase fibers, as compared to fibers with a single phase coating. Sample masses of 10, 50, and 100 mg (in a 10 mL vial) were extracted. Little difference was seen in the number of impurities extracted by the DVB-CAR-PDMS fiber due to sample mass, although the larger sample masses (50 mg and 100 mg) increased reproducibility and peak intensity of the compounds. With the 10 mg sample, the smaller impurity peaks were difficult to detect. A 50 mg sample mass produced little chromatographic difference when compared to the 100 mg sample mass, so the smaller mass was chosen for further studies. The method of determining reproducibility and estimating differences between sample masses was not specified.

In regards to sample mass, these conclusions can be rationalized. Samples with a larger surface area should reach equilibrium more quickly because more sample is exposed to the headspace; smaller sample masses may not give a complete impurity profile because the concentration of some analytes may be below the detection limit of the system. Consistent with previous studies, [8,9] higher temperatures (up to the practical limit of 85 °C when using a water bath) increased the number of detected impurities and their intensities. This can be attributed to increased volatility of impurities at increased temperatures.

The amount of time that the fiber was exposed to the headspace was tested at 15, 30, and 60 minutes [10]. The 30 minute extraction produced equal or higher peak intensity for all impurities, as compared with the other extraction times. This would indicate that equilibrium was attained at 30 minutes, so extracting for an additional 30 minutes was not beneficial; no more impurities partitioned onto the fiber. Kuwayama *et al.* also compared the optimized HS-SPME extraction to LLE [10]. This showed that HS-SPME more effectively extracted impurities, while minimizing extraction of the controlled substance, which in this case was methamphetamine.

Despite the wealth of information available on SPME profiling of amphetamine and methamphetamine, so far only one study has focused on MDMA profiling. Kongshaug *et al.* used SPME to analyze both seized amphetamine and seized MDMA [11]. Instead of extracting from the headspace of the powdered solid, the sample was dissolved in a buffer and stirred during the extraction process. Initially, immersion SPME was attempted, but there was an increase in fiber degradation. Kongshaug *et al.* attributed the fiber degradation to contaminants present in the tablet mixture; however, this conclusion was not supported by analytical data. For liquid samples, HS-SPME was the only viable option. The disadvantages of this process are that it destroys the sample and that an optimal buffer system must be chosen. The advantages are that the buffer allows for pH to remain constant between samples (ionization is constant), liquid extraction provides a uniform sample surface area, and stirring allows the portion of sample exposed to the headspace to be renewed. Once optimized, all of these factors could combine to increase the reproducibility of the technique.

PDMS and PDMS-DVB fibers were used for headspace extraction of the MDMA buffer solution. In comparison to the PDMS fiber, the PDMS-DVB fiber extracted and pre-concentrated the impurities more effectively; the combination of stationary phases attracts both polar and non-polar impurities. Along with testing the fiber types, Kongshaug *et al.* estimated fiber lifetime, something that previous researchers excluded from their conclusions. The research suggested that SPME fibers can be used for 50 to 100 headspace extractions without carryover [11].

When HS-SPME was compared to LLE, the MDMA peak was suppressed because a pH 5.0 buffer was used; at this pH, MDMA is ionized, and therefore has a greater affinity for the buffer than for the headspace. The weakness in Kongshaug's study was sample size; only six samples were tested. With this small sample size, repeatability of the extraction for specific impurities could not be reliably assessed. Additionally, only one extraction time and temperature was assessed (30 min. at 90 °C); other time/temperature combinations may have produced greater extraction of impurities since the interdependence of the two factors was not known. Due to the interdependency of SPME factors such as extraction time and extraction temperature, such factors should be optimized concurrently, and not individually, as reported in the literature to date.

2.3 Overview of Experimental Design

In this work, extraction time and extraction temperature for the HS-SPME procedure was optimized concurrently using a statistical design of experiments. Design of experiments allows the importance of different factors in a process and/or the interdependence of such factors to be assessed. Many types of experimental designs exist, and a design that fits the specific goal of the research should be selected. Some goals include screening to identify important factors [12], maximizing an experimental output, or improving the robustness of a process [13]. In this way, designs are specific to the objective of an experiment; one design is not appropriate for all experiments. Furthermore, design options are limited by the number of factors and number of runs that are feasible for the experiment. The advantage of experimental designs is that they maximize the amount of data gained while minimizing the number of experiments, thus saving time and money.

For the HS-SPME extraction optimization, a central composite circumscribed (CCC) response surface design was selected. This design has the ability to assess the relative effect of each factor on the output response and show interdependence between factors [13]. The greatest advantage of a CCC design is that it has a predictive capability. Because CCC designs assess linear and quadratic interactions within factors as well as assessing interactions between factors, a quadratic equation can be generated to model the data. The quadratic equation is then used to predict the optimal parameters within the boundaries of the experiment, thus optimizing the process.

A two factor CCC design will be explained, but CCC designs are not limited to two factors. With a CCC design, data is gathered from five settings of each factor in order to produce a quadratic equation to model the data. The CCC design determines these settings for each factor based on the limitations of the design space. For each factor, center points, factorial points, and star points are assigned. This design is further illustrated in figure 2.2.

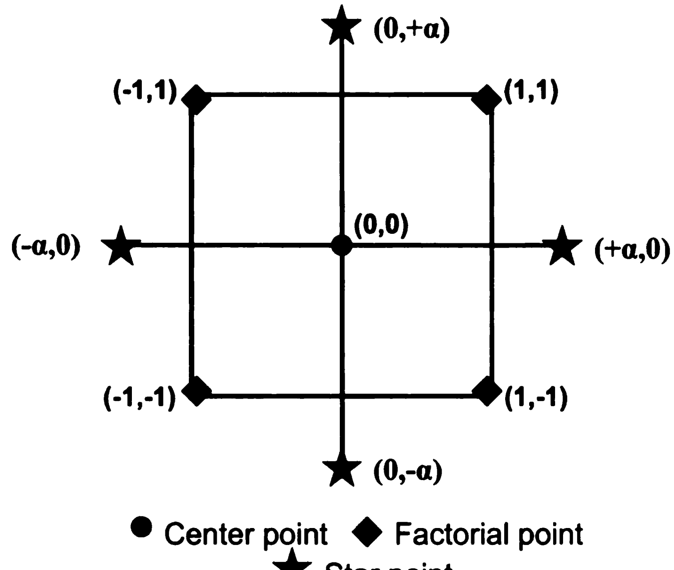

Figure 2.2. Diagram of a Two Factor Central Composite Circumscribed Experimental Design.

Center points are replicates that are taken in the center of the design space (0,0). Factorial points are determined as -1 and +1 for each factor. For the star points, one factor is at the middle setting and the other factor is at $+\alpha$ or $-\alpha$; these are the outer limits of the design space.

The alpha value is dependent on how many factors are in the design, and is determined using the equation:

$$\alpha = [2^k]^{1/4}$$

where k is the number of factors in the design. For a two factor design, the alpha value is 1.414. This value allows the star points to be spaced the same distance from the center as the factorial points, thus allowing for rotatability of the design and preventing the design from being biased in any direction [14].

As figure 2.2 highlights, a CCC experimental design does not test all combinations of the five settings for each factor. In this way, a CCC design minimizes the number of experiments, and the overall time needed to generate the data. The design produces a quadratic equation to model the output data, and optimum settings for each factor can be predicted. However, the optimum factor settings will not necessarily be settings that were analyzed in the experimental design.

2.4 References

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CHAPTER 3: Materials and Experimental Methods

3.1 Sample Preparation

Illicit ecstasy tablets were provided by the Michigan State Police (MSP) Forensic Science Division. Four exhibits were each used in various aspects of this study. For purposes of this research, an exhibit was defined as tablets seized from the same location and at the same time, having similar physical attributes. Each exhibit was given a unique identifier (CJ_FS_01, CJ_FS_02, CJ_FS_03, and CJ_FS_05).

The exhibits were of varying size. CJ_FS_01 contained 3.5 tablets, CJ_FS_02 contained 4 tablets, CJ_FS_03 contained 16 tablets, and CJ_FS_05 contained 100 tablets. Exhibit CJ_FS_04 was not used in this research because it contained only 0.5 tablets.

Figure 3.1 shows one of the seized tablets from exhibit CJ_FS_03. The tablets from this exhibit were purple/pink in color and had a debossed spade logo on one side. The average mass of the tablets (n=16) was 268.0 mg, with masses ranging from 225.9 mg to 276.1 mg. The dimensions of the tablets were constant throughout the batch, with a width of 8 mm and a height of 4 mm.

Figure 3.1. Seized ecstasy tablet from seizure CJ_FS_03.

For the optimization of the HS-SPME procedure, 13 tablets from the same exhibit (CJ_FS_03) were homogenized with a mortar and pestle.

Homogenization eliminates differences in tablet composition as potential sources of variation in the resulting impurity profiles. CJ_FS_03 was used for the study because it was the largest exhibit available at the onset of the experiment. Although it was not a statistically determined sample size, homogenization of 13 tablets provided enough tablet mass so that the same homogenized batch could be used for all studies. This left three full tablets for further analysis. For the duration of the study, the homogenate was stored in the dark in a capped glass vial at room temperature. For the HS-SPME optimization study, appropriate masses of samples were weighed out each day, prior to extraction and analysis, using a Mettler H80 balance (Mettler-Toledo, Inc., Columbus, OH). For subsequent studies, individual tablets were analyzed. The tablets were homogenized with a mortar and pestle directly prior to analysis.

3.2 Experimental Design

A triplicate, two factor CCC experimental design was generated using Minitab® software (version 15, State College, PA). In this experiment, two factors, extraction time and extraction temperature, were optimized for HS-SPME. All other variables were held constant. The limitations for extraction temperature were 23 °C, room temperature, and 85 °C, the highest temperature that the water bath could attain. The limitations for extraction time were 5 min. and 60 min. These times were chosen based on previous literature [1,2]. A table depicting the time and temperature settings for each experiment is shown in Appendix B. Figure 3.1 shows a layout of this design, including values for each point.

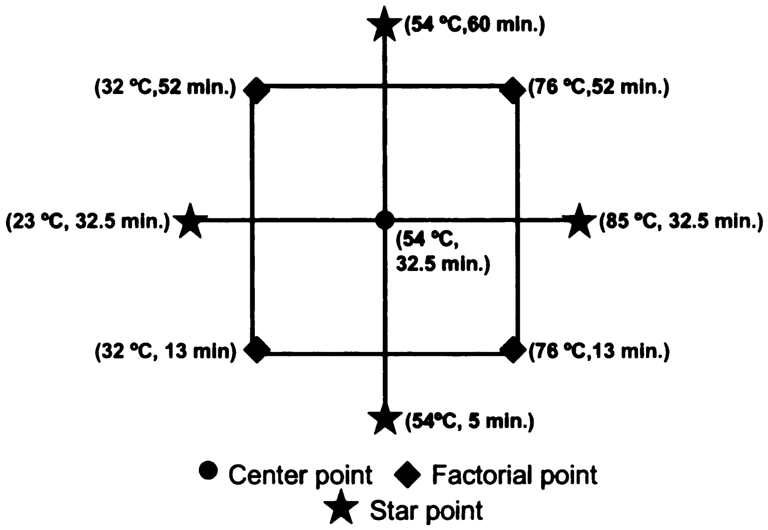

Figure 3.2. Diagram of a CCC Experimental Design Layout for the Optimization of Time and Temperature Settings for HS-SPME.

With this design, the extreme temperatures and times were set as +α and –α (+1.414 and -1.414 for two factors), and the settings for the factorial and center points were determined from these alpha values by spacing the center point at zero and factorial points at plus and minus one relative to the alpha values. In addition to selecting the appropriate factor settings, Minitab® randomized the run order of the experiments (see Appendix B).

In the design shown in figure 3.2, data for the center point was gathered by exposing the SPME fiber to the vial headspace at 54 °C for 32.5 min. Data for the factorial points was gathered at 32 °C and 76 °C for both 13 and 52 min., and data for the star points was gathered at the extreme value for either desorption time or desorption temperature while the other factor was held at the center value.

3.3 Optimization of HS-SPME Parameters

For the optimization study, a one centimeter, 23 gauge, DVB-CAR on PDMS StableFlex® fiber with a 50/30 μm film thickness (Supelco, Bellefonte, PA) was chosen. This fiber is designed to extract volatile and semi-volatile compounds; a previous study on methamphetamine impurity profiling utilized this fiber [1].

3.3.1 HS-SPME Extraction Protocol

HS-SPME extraction was strictly monitored to ensure that extraction time and temperature were the only two variables; all other controllable factors were held constant. For the optimization study, extraction time and extraction temperature were varied in accordance with the experimental design (see Appendix B).

After microscopic examination and per manufacturer instructions, the fiber was conditioned in a GC inlet at 265 °C for one hour each day. A blank analysis was then conducted to ensure that the fiber was clean before analysis. In preparation for analysis, 30 mg of sample was removed from the homogenized batch and added to a clean 4 mL vial with a septum cap (Supelco). The vial was tapped in an attempt to evenly coat the bottom of the vial with the sample. The vial was immersed 1.5 cm in an Isotemp 102 water bath (ThermoFisher Scientific, Pittsburgh, PA) and pre-heated for 5 min. at the extraction temperature designated by the experimental design. The vial was pierced and the fiber was exposed to the vial headspace for the extraction time designated by the experimental design. The depth of the fiber in the vial was kept constant for all samples; the tip of the fiber was exposed at 1.5 cm above the sample layer.

After extraction, the fiber was retracted into the fiber holder and immediately desorbed in the GC inlet at 250 °C for one min. before the GC temperature program was started. In order to prevent fiber bleed, which can complicate the resulting chromatogram and decrease fiber lifetime, the fiber was removed from the inlet one min. after the GC temperature program was started. The data from the experimental design analyses were used to predict the optimum HS-SPME extraction time and temperature, which were then used in all subsequent analyses.

3.3.2 GC-MS Parameters for HS-SPME

The extracts were analyzed on a Thermo Focus gas chromatograph coupled to a Polaris Q mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA) equipped with a Rxi-5ms capillary column (30 m x 0.25 mm x 0.25 µm, Restek, Bellefonte, PA).

To eliminate fiber bleed, a Merlin Microseal system (Merlin Instrument Company, Half Moon Bay, CA) was used instead of traditional septa for SPME analyses. A narrow inlet liner, (0.8 mm, SGE Inc., Austin, TX) allowed an increased flow of carrier gas around the SPME fiber and increased desorption of the impurities.

The inlet was maintained at 250 °C, using a splitless injection for one min. to maximize sensitivity. The oven temperature began at 60 °C, held for 1 min. and was ramped at 8 °C per min. to 300 °C, with a final hold for 15 min. Helium

(99.999%, AGA Gas, Lansing, MI) was used as the carrier gas at a flow rate of 1 mL/min.

The MS analyses were performed using an ion trap mass analyzer set to the full scan mode (50-650 amu). The MS transfer line and ion source temperatures were set to 275 °C and 225 °C, respectively. Mass spectral data was compared against library spectra to provide presumptive identification of impurity peaks. When possible, standards were analyzed and their mass spectra were compared to unknown analytes in order to provide confirmatory identification.

A shortened GC run was used to clean the fiber. For this step, the fiber was desorbed in a 260 °C inlet for 15 min. at a 50:1 split. The fiber was left in the inlet for the length of the cleaning run. The GC oven parameters were as follows: 60 °C starting temperature and a 20 °C per min. ramp to 300 °C with a 3 min. hold. A further shortened run was used to confirm that no impurities remained on the fiber. The oven temperature program for the shortened run started at 60 °C and ramped at a rate of 40 °C per min. to 300 °C with a 3 min. hold time. If the chromatogram reflected carry-over, this step was repeated until no impurity peaks were detected above the noise level of the chromatogram. Both cleaning runs had flow rate and MS parameters at the same settings as the analytical run.

Xcalibur software (Excalibur Software, Inc., Vienna, VA) was used for data collection and analysis. All peak areas were determined using manual integration.

3.4 Fiber Desorption Time and Temperature Study

A study was conducted to determine the time and temperature at which desorption of impurities from the fiber was optimized. This was assessed based on the chromatographic peak areas of the extracted impurities. For the study, inlet temperatures of 240 °C, 250 °C, and 260 °C were tested. These temperatures were chosen based on literature values and temperature limitations of the fibers [1,3,4]. Desorption times of one and two min. were tested; these settings were also based on literature values [1,5]. Triplicate analyses were conducted at each time and temperature combination using the homogenized batch of tablets and a DVB-CAR-PDMS fiber.

3.5 Comparison of HS-SPME to LLE

The optimized HS-SPME extraction was compared to a conventional LLE procedure [6]. Comparison was based on the type and number of impurities extracted with each technique. For LLE, 1M Tris buffer pH 8.1 was prepared from stock solid (88.84%, Mallinckrodt Inc., Paris, KY).

150 mg of the homogenized batch was dissolved in 4 mL of 1M Tris buffer pH 8.1 in a 10 mL conical vial with screw cap. The resulting solution was then sonicated with a Bransonic 3 sonicator (Branson Cleaning Equipment Company, Shelton, CT) for 30 min., after which 200 µL of toluene was added. The mixture was sonicated for an additional 30 min. After 10 min. of centrifugation, the organic layer was transferred to a glass limited volume GC vial insert for storage and analysis. A blank extract was also prepared using the above procedure, but omitting the ground tablet.

A 1 µL aliquot of the liquid extract was manually injected into the GC using a 10 µL syringe (Hamilton Co., Reno, NV). A traditional low bleed septum was used instead of the Merlin Microseal system, and a 5 mm inlet liner was used. Analysis of the extract utilized a 20:1 split injection to prevent overloading the column. The inlet temperature, oven temperature program, and MS parameters were identical to that of the HS-SPME analysis.

3.6 Comparison of Other Exhibits

One tablet from each exhibit (CJ_FS_01, CJ_FS_02, CJ_FS_03, and CJ_FS_05) was homogenized, and two 30 mg portions of each homogenate were sampled using the optimized HS-SPME parameters. The resulting chromatograms from the analysis of each exhibit were compared in terms of the number and type of impurities extracted to determine if different exhibits contained different impurities.

3.7 Comparison of Fiber Stationary Phases

For the fiber comparison study, a 23 gauge PDMS-DVB StableFlex® fiber with a 65 µm film thickness and a 24 gauge CAR-PDMS fiber with a 75 µm film thickness (Supelco) were compared to the previously used DVB-CAR-PDMS fiber. The CAR-PDMS fiber is available in the 23 gauge StableFlex® variety, but it was not available for this experiment. Optimized HS-SPME extraction and desorption parameters were applied, and extractions from 30 mg portions of the homogenized batch were analyzed in duplicate for each fiber type. The resulting chromatograms were compared in the same manner as those resulting from section 3.6 in order to determine which fiber type extracted the greatest number of impurities and offered the most acceptable chromatography.

3.8 Assessment of Fiber Degradation

Each day prior to analysis, the fiber was microscopically examined at various magnifications using a SMZ800 stereomicroscope (Nikon, Melville, NY). The fiber was photographed using a Nikon DXM1200F digital camera to assess degradation over time. Nikon ACT1 software version 2.62 was used to visualize the images.

3.9 References

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CHAPTER 4: Results and Discussion

In this chapter, the results of six separate yet interconnected studies are presented and discussed. First, an experimental design was used to optimize HS-SPME extraction time and extraction temperature. Second, a short study determined the desorption time and desorption temperature that maximized the peak areas of selected impurities. This ensured that a range of impurities were being efficiently desorbed from the fiber. Third, the optimized HS-SPME extraction parameters were compared to a commonly used LLE method in order to assess the viability of the HS-SPME method. The optimized HS-SPME method was then used to profile impurities in tablets from different ecstasy exhibits. Degradation of the fiber was assessed throughout the study. A preliminary comparison between the fiber used in the optimization study and other available fibers was also made.

4.1 Experimental Design for the Optimization of HS-SPME Parameters

The chromatographic data generated from the CCC experimental design was analyzed using Minitab® in order to generate quadratic equations to model the data. Microsoft® Excel was used to generate residual plots, which assessed the quality of the model equations. These equations were ultimately combined to predict an optimized extraction time and extraction temperature for the extraction of impurities from ecstasy tablets using HS-SPME. Four impurities, N-formylmethamphetamine (retention time (R.T.) 4.4 min.), N-a,a-trimethylphenethylamine (R.T. 11.1 min.), MDEA (R.T. 16.8 min.), and 4-amino-N,N-dimethylbenzamide (R.T. 22.76 min.) were selected for optimization of the extraction time and temperature. These specific impurities were chosen because their peaks exhibited good chromatography and the retention times of the impurities spanned the length of the analysis, enabling differences in molecular weight, boiling point, and polarity to be taken into account.

The chromatographic peaks from the chosen impurities were manually integrated using Xcalibur computer software (Excalibur Software, Inc., Vienna, VA). The mass spectral data was used to define the width of the peak. For each impurity, the peak areas for all extraction time and temperature combinations tested in the experimental design were used to generate quadratic equations that modeled the experimental data for that impurity. This resulted in four quadratic equations, one for each impurity considered in the analysis. Because a quadratic model was used, the model equation was able to take into account interactions between extraction time and extraction temperature.

In order to assess how well the equations modeled the experimental data for the four impurities, residual plots were plotted using Microsoft® Excel. Theoretical peak areas for each impurity were determined using the predicted equations and substituting in the extraction times and temperatures from the experimental design. The theoretical peak areas were plotted against the experimental peak areas generated from analysis of the CCC design. For normally distributed data sets the residual plot should be linear.

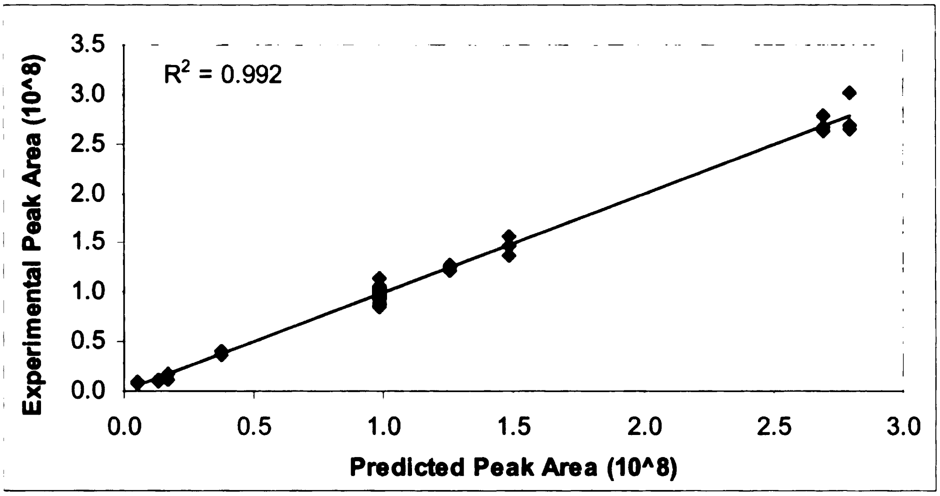


Figure 4.1. Residual Plot of MDEA.

Figure 4.1 shows the residual analysis for MDEA. There was a linear correlation between the experimental peak areas and the predicted peak areas. The R^2 value of 0.992 confirmed the linearity, but took into account slight deviations from linearity that were observed for replicates at the same extraction time and temperature. This linear pattern indicated that the equation generated model from Minitab® was appropriate for MDEA.

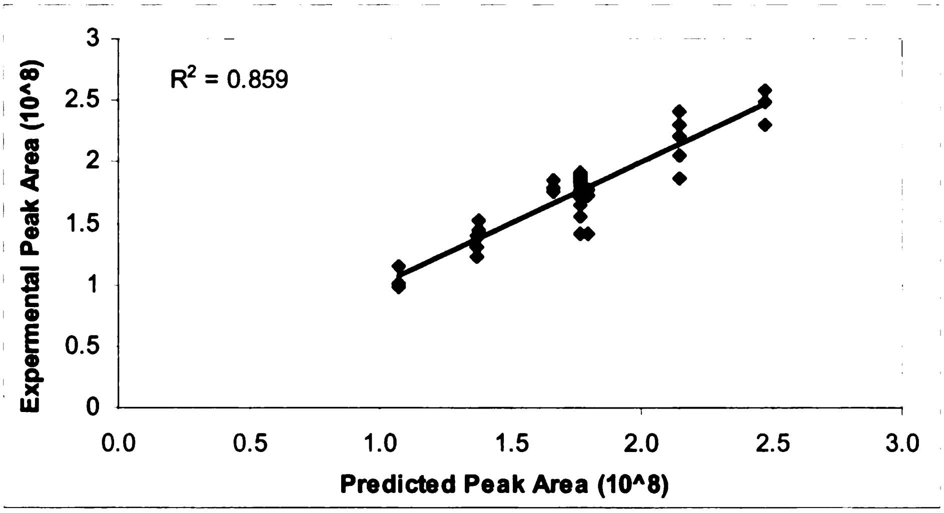


Figure 4.2. Residual Plot of N-formylmethamphetamine.

The residual analysis for N-formylmethamphetamine also showed a distinct linear relationship, although the R^2 value was 0.859. The R^2 value was less than one because the replicate experimental data for a given extraction time and temperature value had a range of peak areas observed. This range was more spread out with the N-formylmethamphetamine data than with the MDEA data, causing a lower R^2 value, although the data remained linear (see Figure 4.2).

In contrast, the residual plot for 4-amino-N,N-dimethylbenzamide showed that the equation generated for the data did not accurately model the experimental data (Figure 4.3).

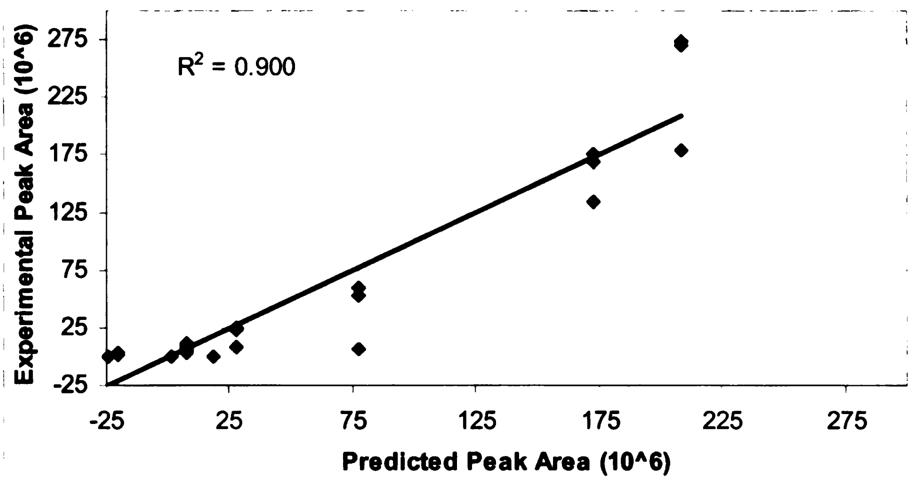


Figure 4.3. Residual Plot of 4-amino-N,N-dimethylbenzamide.

The data for 4-amino-N,N-dimethylbenzamide was not linearly distributed, although the R^2 value (0.900) was better than the R^2 value obtained for N-formylmethamphetamine, an impurity that was included in the optimization. The data for 4-amino-N,N-dimethylbenzamide appeared to have a logarithmic shape;

however, this was not an option because all of the data needed to be treated consistently.

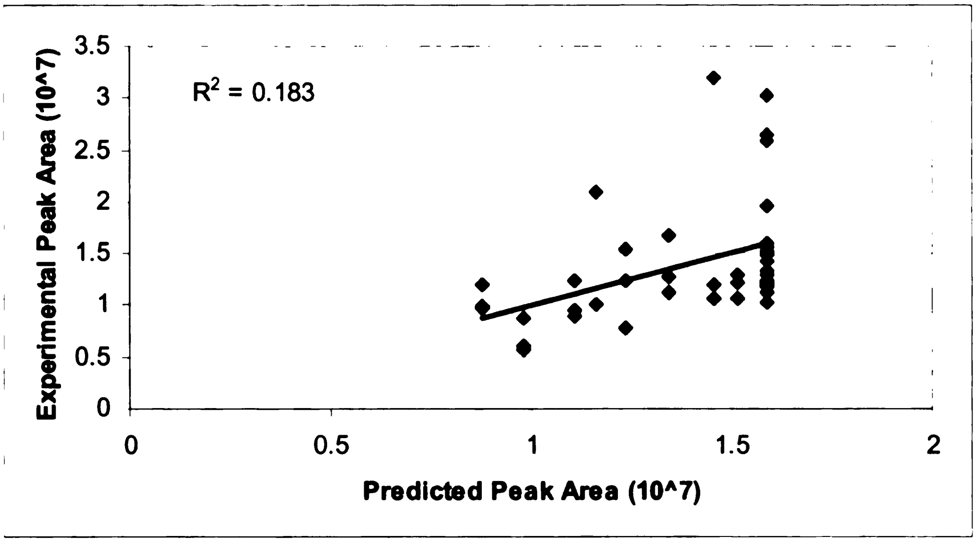


Figure 4.4. Residual Plot of N-a,a-trimethylphenethylamine.

The residual plot for N-a,a-trimethylphenethylamine was also non-linear, with an R^2 value of 0.183 (see Figure 4.4). This impurity did not have an overall shape that was linear or logarithmic; the data points were widely dispersed in a cone shape.

Experimental peak areas for N-a,a-trimethylphenethylamine and 4-amino-N,N-dimethylbenzamide did not fit the quadratic model predicted by Minitab®, meaning that either the extraction of these impurities was independent of time and temperature, or other factors, such as sample surface area or sampling depth affected the extraction. The model equation did not consistently under-predict or over-predict the peak area values for either of the impurities. Because these equations did not accurately model the data, they were not included in the prediction of the optimized extraction parameters, as the poorly fitting equations would cause the predicted optimum extraction time and temperature to be inaccurate. For this reason, only the equations generated from the data for N-formylmethamphetamine and MDEA were used to predict optimal extraction parameters for HS-SPME.

By finding the extraction time and extraction temperature that maximized the response of the quadratic equation, individual impurities could separately predict optimal extraction settings, but these settings would differ for each impurity. In order to predict the optimal time and temperature for the HS-SPME extraction, it was necessary to find combined extraction time and temperature settings that provided a compromise maximum peak area when the equations for the two impurities were considered jointly. Considering multiple impurities allowed for compromise settings that were appropriate for the extraction of both impurities. These settings were computed by finding the extraction time and temperature that gave a compromise maximum peak area for both N-formylmethamphetamine and MDEA.

When the equations for the N-formylmethamphetamine and MDEA were maximized as a composite using Minitab®, the predicted optimum settings for the HS-SPME method were an extraction temperature of 73 °C and an extraction time of 60 min. Ultimately, a 75 °C extraction temperature was chosen instead of 73 °C because 75 °C was easier to maintain consistently with the water bath. Theoretically, these optimized parameters allowed for increased extraction of impurities without limiting the range of impurities that was extracted.

This prediction of an optimum extraction time and temperature only utilized two impurities. The informative area of the chromatogram spans from 3.5

min. to 33.5 min. By analyzing N-formylmethamphetamine and MDEA, the optimization encompasses roughly half of the relevant portion of the chromatogram. Adding more impurities within this time range would increase the quality of the prediction. Adding additional impurities to the optimization, especially those with longer retention times, would increase the likelihood that the prediction holds true for impurities throughout a range of boiling points.

4.2 Optimization of Desorption Parameters for HS-SPME

Once the parameters for HS-SPME were optimized, desorption time (the amount of time that the fiber is exposed to the inlet prior to the start of the run) and desorption temperature (GC inlet temperature) were investigated in order to optimize desorption of impurities from the fiber. The same homogenized batch of ecstasy tablets that was used for the extraction optimization was also used for this study. The impurities were extracted from the homogenate using the optimized HS-SPME extraction time and temperature from section 4.1. Literature references and practical considerations, such as maximum fiber temperature, dictated the study parameters [1,2,3,4]. Desorption times of one min. and two min., and desorption temperatures of 240 °C, 250 °C, and 260 °C were studied. Randomized, triplicate analyses were conducted at each desorption time and temperature combination, resulting in 18 analyses. The peak areas for each selected impurity were used as an indicator of optimum settings. Larger peak areas showed that the analyte was being efficiently desorbed from the fiber.

Assessing multiple peak areas determines if the different impurities are affected differently by changes in desorption temperature. From the resulting chromatograms, three impurities, N-a,a-trimethylphenethylamine (10.8 min.), MDEA (16.6 min.), and 4-amino-N,N-dimethylbenzamide (22.7 min.) were chosen for data analysis because they spanned the chromatogram and had good chromatographic shape. The impurities were manually integrated and peak areas were recorded.

This data was sorted into two data sets for each impurity. The first data set consisted of peak areas for different desorption temperatures when desorption time was held constant. The second data set consisted of peak areas for the different desorption times when temperature was held constant. One way analysis of variance (ANOVA) was conducted on the resulting peak areas to determine if there was a significant difference in peak areas between chromatograms generated from different desorption times and different desorption temperatures.

One way ANOVA assesses the variance between different settings of the same factor. Variance is assessed using F values and a null hypothesis (H_o), *e.g.* the means of the data sets are the same, is generated. The sample size, number of replicates, and chosen confidence level determine the F critical value. If F is greater than F-critical, H_o is rejected and the alternate hypothesis is accepted, *i.e.* the means are not the same [5].

ANOVA was used to determine statistical differences in peak area for each impurity at different desorption temperatures with a desorption time of one min. based on three replicates. The analysis was repeated, this time with a desorption time of 2 min. In each case, the null hypothesis was that there was no difference in peak areas at different desorption temperatures. The analysis was conducted with a confidence level (CL) of 95% and eight degrees of freedom (d_f), resulting in the F values shown in Table 4.1.

Table 4.1. ANOVA values for the comparison of desorption temperature.

	F-value (F-critical =5.14) 95% CL, d_f=8
N-a,a-trimethyphenethylamine	
1 min. desorb	3.64
2 min. desorb	1.07
MDEA	
1 min. desorb	0.15
2 min. desorb	0.49
4-amino-N,N-dimethylbenzamide	
1 min. desorb	0.61
2 min. desorb	2.33

The F values for each selected peak at one and two min. desorption times were all less than the F critical value. This means that the null hypothesis can be accepted; there was not a significant change in the amount of impurity desorbed from the fiber within the tested desorption temperature range. These conclusions are valid only for the impurities considered in this study. Since a range of molecular weights was encompassed by testing peaks at varying retention times, it could be deduced that impurities in a wide range of boiling points were effectively desorbed from the fiber at all of the desorption temperatures tested in this study.

Ultimately, a desorption temperature of 250 °C was chosen in order to decrease wear on the fiber. Also, an inlet temperature of 250 °C is common for LLE, so the two extraction methods could be directly compared without introducing the potential for differential degradation in the injection port [6].

One-way ANOVA was also performed on the desorption time data to determine if there was a significant increase in the peak areas of the impurities studied when the desorption time was varied from one to two min. with a desorption temperature of 250 °C. In this study, the mean areas from three impurity peaks were separately analyzed at both one min. and two min. desorption times. The null hypothesis was that there was no difference in peak area between the tested desorption times of one min. and two min. The confidence level was 95%, and there were five degrees of freedom. Table 4.2 gives the ANOVA values for this comparison.

Table 4.2. ANOVA values for the comparison of desorption time.

	F-value (F-critical =7.709) 95% CL, d_f=5
N-a,a-trimethyphenethylamine	0.74
MDEA	3.59
4-amino-N,N-dimethylbenzamide	0.02

F-values less than the F-critical value showed that the null hypothesis can be accepted for all tested impurities. In regards to peak area of the extracted impurities, it made no difference whether the fiber was desorbed for one min. or two min. A desorption time of one min. was chosen in order to reduce overall analysis time.

From this study, it was determined that there was no significant difference between the desorption temperatures tested or the desorption times tested. A one min. desorption time at a desorption temperature of 250 °C was chosen as the optimized settings for the remainder of the experiments.

4.3 Comparison of LLE and HS-SPME for Extraction of Impurities from Homogenized Ecstasy

In order to make a valid case for the use of HS-SPME as the optimal extraction method for MDMA impurity profiling, it must be compared to a commonly used LLE procedure. The study compared the optimized HS-SPME method to a conventional LLE method using the sample mass deemed to be optimal for LLE (a full tablet) [7]. Previous research conducted in this lab found that it was not advantageous to compare equal sample sizes using the two extraction procedures. With this process, the LLE sample exhibited very broad peaks with poor chromatography throughout the chromatogram, which was caused by the column being overloaded by the splitless injection.

The optimized HS-SPME extraction was analyzed with a splitless injection, while the LLE extract was analyzed using a split injection to improve the chromatography and prevent overloading the column. The mass of sample used to represent a full tablet, 150 mg, was on the low end of average tablet mass because there was not sufficient sample to allow analysis of a larger mass. Figure 4.5 shows the resulting chromatograms from each analysis.

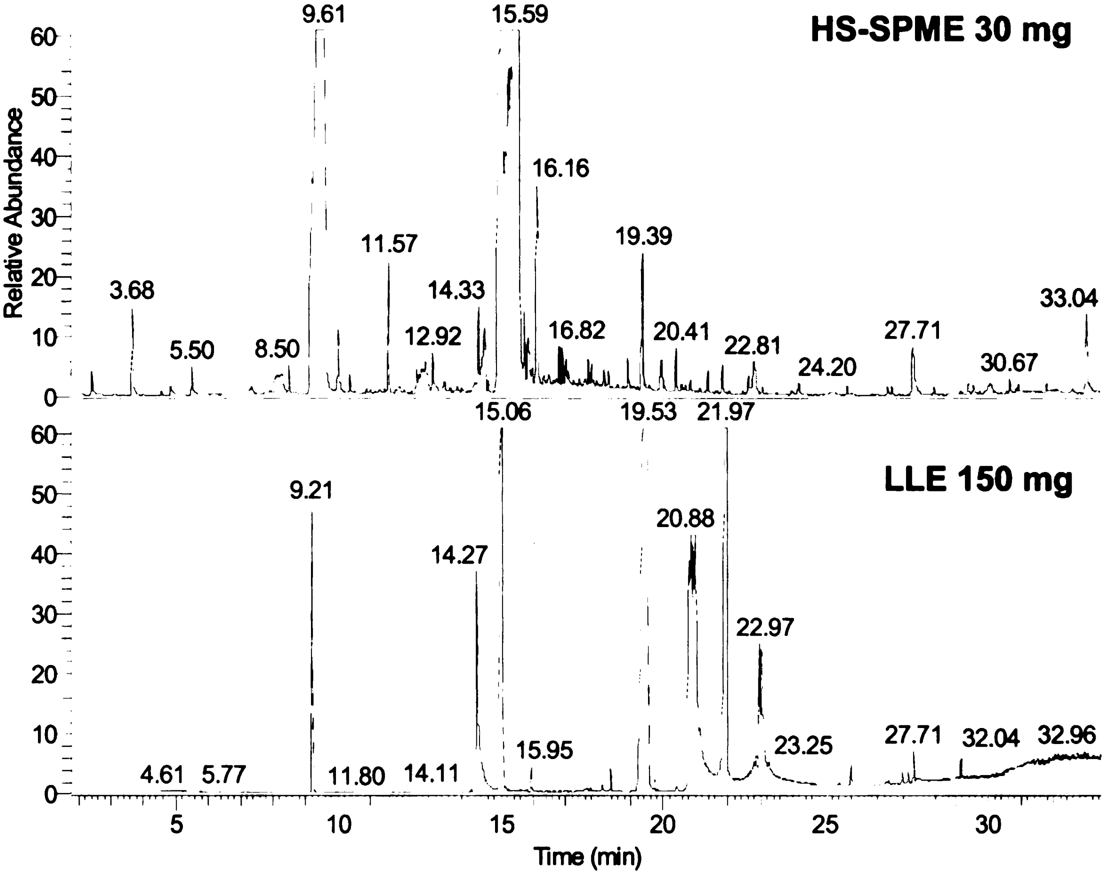


Figure 4.5. Chromatograms generated by HS-SPME and LLE at optimal sample masses.

As seen in figure 4.5, analysis of the LLE produced a chromatogram that was sharper and more defined than the HS-SPME chromatogram, it lacked much of the information contained in the HS-SPME profile. Table 4.3 compares the number of analytes extracted by each extraction method. Some peaks existed in the chromatograms (figure 4.5), but were not identified in table 4.3, since these peaks were a result of fiber bleed and column bleed.

Table 4.3. Comparison of HS-SPME and LLE in terms of the number of analytes detected. The * denotes peaks that elute at different times with the two extraction methods. Tentative identifications were made by comparison to mass spectral library.

Retention Time	Prominent m/z	LLE	HS-SPME	Tentative Peak Identity
3.70	86, 58, 72, 87		X	N-formylmethamphetamine
9.21	58, 91, 115, 148	X	X	Methamphetamine
10.04	135, 108		X	Benzo[d]isothiazole
14.55	135, 178, 77, 51		X	3,4-methylenedioxyphenyl-2-propanone
*14.27/15.09	135, 136, 180, 77, 79	X	X	3,4-methylenedioxyphenyl-2-propanol
*15.06/15.59	58, 77, 135, 194	X	X	MDMA
15.74	194, 58, 86, 118, 91		X	
*15.95/16.16	72, 70, 194, 51, 135	X	X	MDEA
16.92	86, 58, 194, 135, 135		X	
17.02	193, 135, 194, 146, 77		X	
17.71	91, 162, 119, 86, 157	X	X	
17.83	58, 100, 135, 77, 194		X	
18.34	132, 105, 194, 79, 77		X	
19.38	194, 193, 55, 195, 109	X	X	Caffeine
19.96	91, 160, 119, 115, 65		X	
20.41	162, 58, 77, 163, 135		X	MDA Acetate
20.88	87, 55, 73, 60, 171	X		
21.40	227, 165, 196, 182, 228		X	
21.84	86, 120, 56, 58, 99	X	X	
22.64	58, 115, 162, 77, 135		X	
22.81	174, 91, 148, 145, 56		X	
23.04	87, 55, 185, 129, 73	X		
25.79	86, 100, 58, 72	X		
27.69	163, 204, 135, 105, 133		X	
27.72	86, 100, 58, 72	X		
33.02	86, 56, 162, 117, 280		X	
* LLE/SPME	**Total:**	**11**	**22**	

Identification of peaks was based on visual comparison to the National Institute of Standards and Technology mass spectral library, to which standards analyzed in-house were added. In part, lack of identification of peaks can be attributed to differences in mass analyzers. The spectral data for the library was generated using a quadrupole MS, while the instrument used for this research has an ion trap mass analyzer, so spectra cannot be directly compared. To definitively identify all peaks, standards of common impurities from MDMA synthesis should be analyzed in-house, using the same instrument and operating conditions. At the time of this research, only a limited number of standards were available for in-house analysis.

For the homogenized batch, HS-SPME extracted 22 impurities, while LLE extracted 11 impurities (see Table 4.3). These impurities were detected at a wide range of retention times; thus showing that HS-SPME extracted impurities of varying molecular weights and polarities. In most cases, HS-SPME was able to extract all of the impurities that LLE did, except for the impurities at retention times of 20.88, 23.04, 25.79, and 27.72 min. At these times, the temperature of the oven was between 212 °C and 260 °C. Because HS-SPME extracted impurities at 27.69 and 33.02 min. (corresponding to temperatures between 268 °C and 300 °C), this difference in extraction cannot be attributed solely to boiling points. The structure or polarity of these impurities may have prevented them from partitioning into the headspace or onto the fiber. Identification of the impurities extracted only by LLE would allow further investigation into why the impurities were not amenable to HS-SPME because their structures and polarities could be taken into consideration.

However, in the LLE chromatogram MDMA and 3,4-methylenedioxyphenyl-2-propanol were resolved. HS-SPME using the DVB-CAR-PDMS fiber caused co-elution of these two compounds, presumably because they both had a high affinity for the fiber. Since 3,4-methylenedioxyphenyl-2-propanol is an important impurity that is commonly encountered in ecstasy tablets, it is important to be able to resolve this impurity from MDMA for profiling purposes.

In terms of extracting impurities, HS-SPME was more efficient than LLE, using roughly five times less sample and eliminating sample preparation and solvent usage. Based on the number of impurities extracted, HS-SPME was a superior extraction method for impurity profiling. The increased sensitivity of HS-SPME for the extraction of impurities can be attributed to the fiber's ability to pre-concentrate analytes, which was not achieved by LLE.

4.4 Physical and Chemical Comparisons Among Exhibits

4.4.1 Physical Comparison of Tablets

In this study, tablets from different exhibits obtained from MSP were available for analysis. Tablets used in the comparison study were from exhibits CJ_FS_01, CJ_FS_02, CJ_FS_03, and CJ_FS_05. CJ_FS_03 was the exhibit used for the homogenized batch, but for this study, a single tablet was used. Initially, physical characteristics of tablets were compared. Within each exhibit, all tablets has similar physical characteristics. Figure 4.6 shows pictures of each of the tablets.

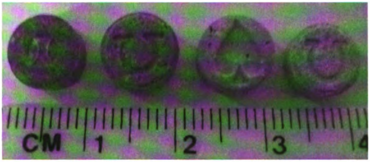


Figure 4.6. Photograph of individual tablets from exhibits (*from left to right*): CJ_FS_01, CJ_FS_02, CJ_FS_03, and CJ_FS_05.

The photograph in figure 4.6 illustrated the variety of characteristics exhibited between the tablets. Tablets from different exhibits had different colors, motifs, edge beveling, consistency, and stability, as indicated in table 4.4. All tablets from all exhibits had similar dimensions.

Table 4.4. Physical characteristics of tablets from different exhibits.

Exhibit CJ_FS_	Color	Logo	Width (mm)	Height (mm)	Edges	Consistency when ground	Stability of Color
01	Dark rose	Baseball	8	4	Beveled on both sides	Powdery	Stable
02	Dark orange	Omega	8	4	Beveled on stamped side	Slightly waxy	Stable
03	Purple/ pink	Spade	8	4	Not beveled	Waxy	Fades in light/heat
05	Blue	Omega	8	4	Beveled on both sides	Extremely powdery	Stable

Exhibits CJ_FS_02 and CJ_FS_05 both had an omega logo, but the proportions of the logo were slightly different (see Figure 4.6); the edges of the tablets also had differences in beveling. The consistency notations in Table 4.4 recorded characteristics that were observed when the tablet was ground in preparation for analysis. The tablets that were considered "powdery" ground up easily without aggregating and did not stick to the mortar or pestle. Tablets that were categorized as "waxy" took more effort to grind, and had to be scraped off the mortar and pestle. The differences in consistency can most likely be attributed to differences in tableting materials. Common tableting materials include stearic and palmitic acids, which are both fatty acids with a waxy consistency [8]. The stability of color notation was included as a discriminating feature because tablets in exhibit CJ_FS_03 faded from purple/pink to purple/gray when exposed to light or heat. The dye may have been light-sensitive. This phenomenon was not observed with the other tablets.

4.4.2 Chemical Impurity Profiling of Tablets using HS-SPME

The main goal of this research was to optimize HS-SPME for the extraction of organic impurities from ecstasy tablets. This organic impurity profile could be used to link tablets to the synthetic method used for manufacturing, or link tablets to a common batch.

One tablet from each of the four exhibits listed above was ground, and 30 mg of the ground tablet was analyzed using the optimized HS-SPME parameters. Lack of sufficient sample size prevented statistical comparisons of this data, so the chromatograms were compared qualitatively. This qualitative comparison is still very beneficial. Statistical comparisons of tablets are not common in the literature, and a qualitative comparison of the pattern of peaks is more common. The overall pattern of the peaks shows the distribution of the impurities present, and can be more informative than identifying individual impurities [8]. Figure 4.7 shows the impurity profiles generated by the analysis of different exhibits.

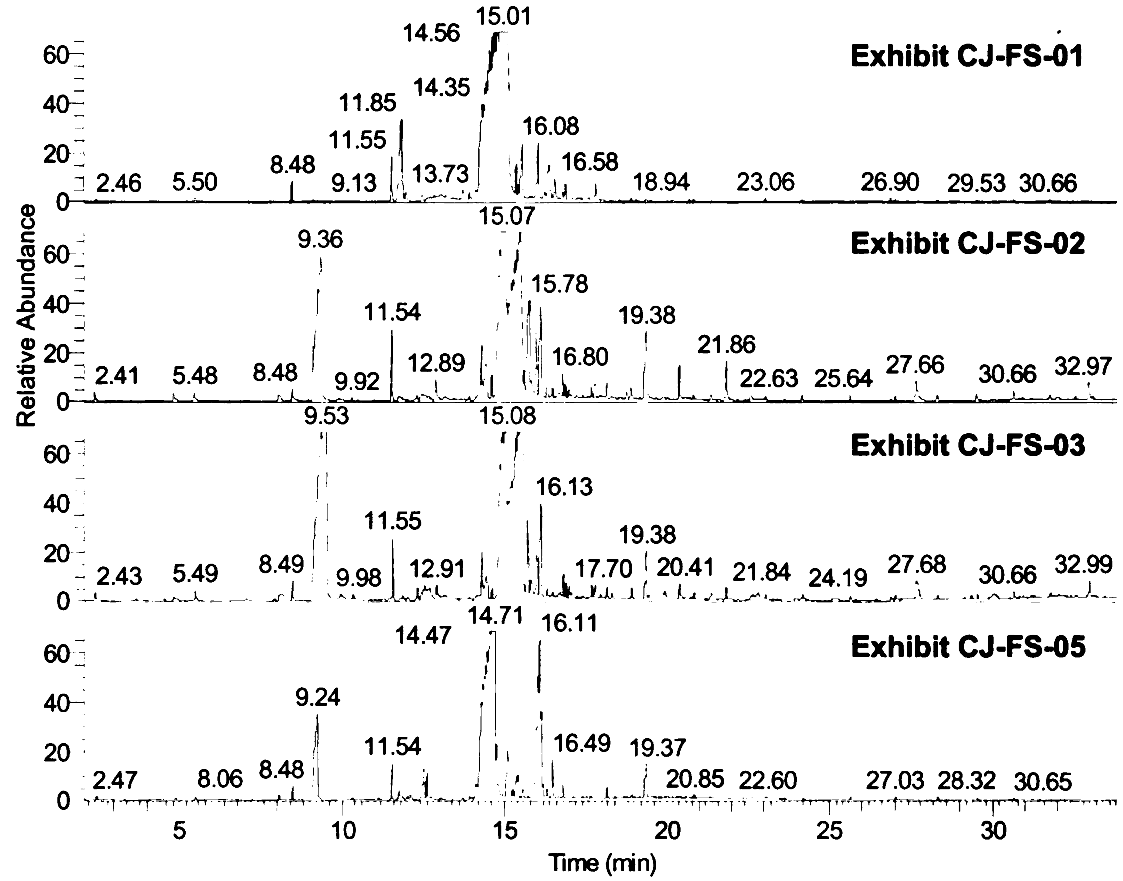


Figure 4.7. Chromatograms comparing the impurities extracted from different exhibits using the optimized extraction procedure with a DVB-CAR-PDMS fiber.

Visual examination of the chromatograms showed that there were different organic impurities present in each of the exhibits, as seen by different retention times. Impurities that were common among the exhibits had varying retention times, *e.g.* methamphetamine, at a retention time of 9.36 min., 9.53 min., and 9.24 min. These differences in retention times were due to differences in peak width.

Appendix C compares and contrasts the impurities extracted from each analyzed tablet; this gives a more detailed view of the similarities and differences between the tablets. Not all impurities were identified; the only impurity present in all tablets analyzed was toluene, a common solvent. The prominent mass to charge ratios at each retention time are given in Appendix C to aid in determining a potential structural identity. No impurities were present in any of the chromatograms after 33 min.

Differences in the type of impurities present may indicate the use of different precursors, or different synthetic routes. For example, piperonal was identified in a tablet from exhibit CJ_FS_01, but not in any of the other tablets analyzed. Piperonal is a common MDMA precursor, and its presence indicated that the synthesis pathway may have started with piperonal. Because the other tablets did not contain piperonal, it does not mean that it was not a precursor to their synthesis. Piperonal could have been consumed in the synthesis, or the final product may have been purified. Hence, analysis of different batches of MDMA with common precursors could result in different impurity profiles. Common impurity profiles can link tablets produced from common synthetic routes, or potentially, common batches. The more common impurities two exhibits share, the more likely it is that they are of a common origin.

Because both MDMA and methamphetamine were identified in three tablets from different exhibits, precursors and by-products from the production of MDMA and methamphetamine were identified in the impurity profile. For example, MDP-2-P and N-formylmethamphetamine were identified in the same impurity profile. While MDP-2-P is most likely a leftover precursor from MDMA synthesis, N-formylmethamphetamine is most likely a by-product from methamphetamine synthesis. These mixed samples may not be able to be attributed to a synthesis method, but are increasingly likely to be related back to a batch. The more impurities that are present in a sample that contains both MDMA and methamphetamine, the less likely it is that the specific combination of multiple impurities will be found anywhere other than that batch. This pattern of peaks can link tablets from a common batch [8].

The analyzed tablets from exhibits CJ_FS_02 and CJ_FS_03 shared 24 common impurities. Since batches of MDMA made by the same synthetic route can have different impurity profiles, this common impurity profile is a strong indication that the two exhibits may share a common source. However, a conclusion cannot be made about whether CJ_FS_02 and CJ_FS_03 originated from the same synthetic route or the same batch until a larger sample set is studied. Larger sample sets will show how common specific impurities are, thus increasing or decreasing their usefulness for profiling.

Slight differences in the abundance of impurities between the homogenized batch of tablets from CJ_FS_03 used in studies 4.1-4.3 and the single tablet from the same exhibit analyzed in this study may be due to variation in composition between tablets in the same seizure. Because the illicitly synthesized batch may not be fully homogenized, individual tablets may have differing ratios of the same impurities. Any residual material on the mixing or tableting equipment may get combined with the first tablets but not the latter tablets produced from the same batch.

For these reasons, more than one tablet from an exhibit should be analyzed when profiling the impurities present. Based on the number of tablets in the seizure, the UN recommends the number of tablets that should be analyzed. If the seizure is less than 10 tablets, all tablets should be analyzed. If the seizure is between 10 and 100 tablets, 10 tablets should be analyzed. When a seizure contains more than 100 tablets, sampling the number of tablets corresponding to the square root of the total number of tablets is recommended [9]. Individual tablets, rather than homogenized batches should be used for analysis. Sampling in this manner would allow inter and intra batch variation to be assessed. This small study does show that exhibits with drastically different physical characteristics can have common impurities and/or overall patterns of impurities, which can potentially indicate a common synthetic route or common batch, thus confirming other studies [8,10].

4.5 Comparison of Fiber Stationary Phases

Although the HS-SPME extraction parameters were optimized using only one fiber, a DVB-CAR-PDMS fiber, fibers with many different stationary phases are commercially available. This study compared the DVB-CAR-PDMS fiber to a polydimethylsiloxane-divinylbenzene (PDMS-DVB) fiber and a CAR-PDMS (Carboxen™-polydimethylsiloxane) fiber. The PDMS-DVB fiber offers a combination of polar and non-polar stationary phases. The CAR-PDMS fiber combines the non-polar PDMS phase with Carboxen™, which works to trap small polar molecules. The absence of the DVB decreases the fiber's affinity for large polar molecules, which are likely to be present in the sample.

Duplicate extractions of 30 mg of the homogenized batch (CJ_FS_03) were conducted for each fiber. The extraction ability of the three fibers was compared using the HS-SPME parameters optimized for the DVB-CAR-PDMS fiber, although this may not be the optimized parameters for all fiber types. Figure 4.8 shows chromatograms from extractions with each tested fiber type.

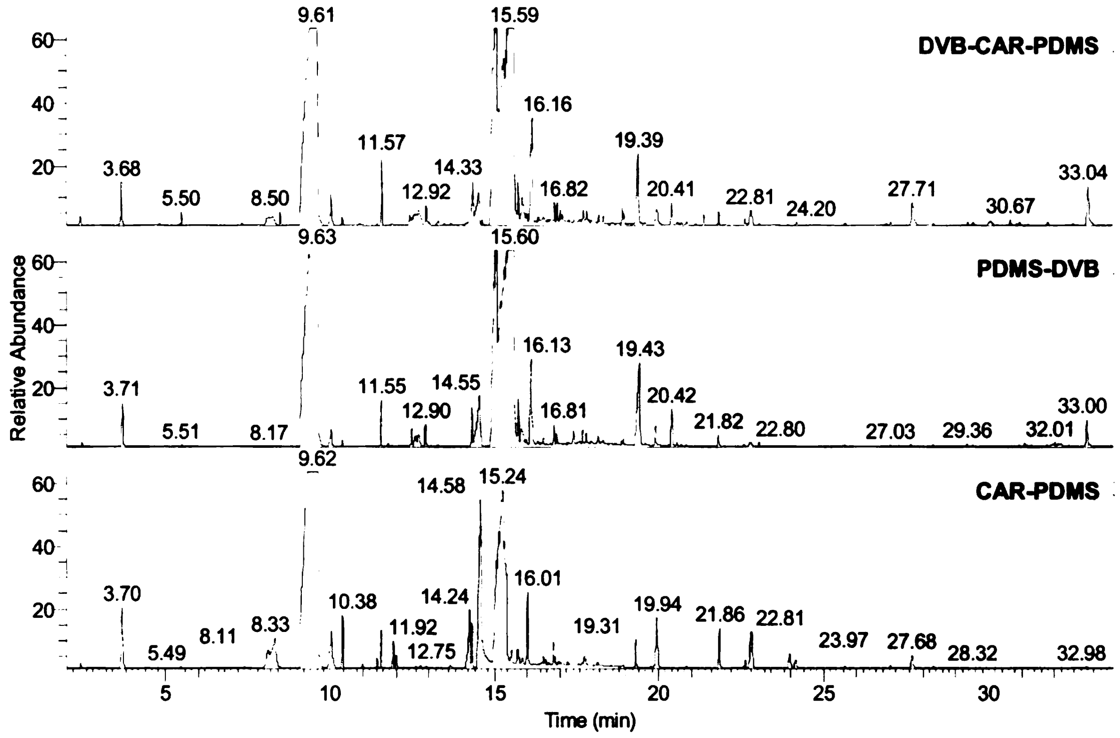


Figure 4.8. Comparison of Different Fiber Stationary Phases.

The chromatograms resulting from the HS-SPME extraction of the homogenized batch using fibers with different stationary phases showed slightly different impurity profiles. Some impurities were not extracted with all fibers, which could potentially be attributed to differences in polarities of the impurities. Differences in polarities cannot be confirmed without identification of the impurities.

The difference in the impurities extracted with each fiber type is further illustrated in Appendix D, which lists the impurities extracted by each fiber. All tested fibers were able to extract a wide range of impurities, as seen by the presence of impurity peaks from 3 to 34 min. Since the GC oven program involved temperature ramps, these times represented a range of boiling points. The DVB-CAR-PDMS fiber extracted the largest number of impurities. This can be attributed to the variety of polymers present on the fiber, as they are designed to extract polar and non-polar compounds of varying sizes. The PDMS-DVB fiber extracted most, but not all, of the same impurities that the DVB-CAR-PDMS fiber did. This may be a result of the lack of CAR, which is meant to retain small polar molecules; it could also be an artifact of the non-optimized extraction time, or a combination of the two factors. Without knowing the identity of all of the impurities and their polarities, it is impossible to determine the cause of the difference in extraction. Non-optimized extraction time would be problematic because if left to equilibrate too long, analytes may begin to migrate off of the fiber back into the headspace. Although the CAR-PDMS fiber extracted the fewest impurities, it extracted different impurities than the other two fibers. The absence of the DVB polymer depletes the fiber of its ability to attract large polar molecules and changes the selectivity of the fiber.

In addition to the presence or absence of a peak, it is important to take into account the peak shape. In general, the CAR-PDMS fiber exhibited the most symmetrical peaks and was able to best separate the methylenedioxyphenyl-2-propanol and the MDMA. Since the co-elution of methylenedioxyphenyl-2-propanol and MDMA was problematic with the DVB-CAR-PDMS fiber, the CAR-PDMS fiber merits further study and optimization.

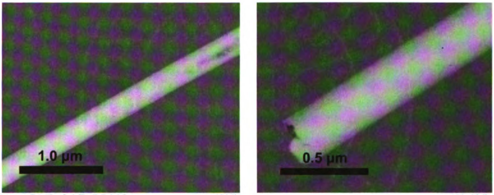
The difference in number of impurities extracted by each fiber may be due to non-optimized extraction parameters for the latter two fibers, or the incapability of those fibers to extract a given impurity. Also, this data was based on a very small sample set, representing only the homogenized batch of tablets from CJ_FS_03. Since ecstasy tablets can have varying impurities that are not all reflected in this batch, the optimum fiber choice predicted through these analyses may not be the overall optimum fiber choice.

In addition to the extraction capabilities of a fiber, the ease of fiber cleaning must be considered before choosing a fiber since long fiber cleaning times decrease the throughput of the analysis. In this study, the fibers were cleaned in the GC inlet at an elevated temperature after the run had finished. The DVB-CAR-PDMS fiber cleaned with about 25 min. of re-conditioning. The PDMS-DVB and CAR-PDMS fibers used in the study were extremely difficult to clean, both needing more than one hour of conditioning between each run in order to eliminate carryover contamination. Again, the difficulty of cleaning may be contributed to the lack of optimization for these fiber types. If the samples were extracted for too long, the fiber would be overloaded and difficult to clean. To limit overall extraction time, the fiber can be left in the inlet during the run to clean, but this results in increased fiber bleed in the resulting chromatogram.

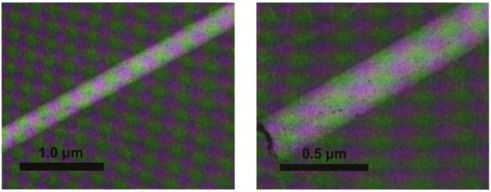
Based on the above considerations, the DVB-CAR-PDMS fiber was the best choice for the extraction of organic impurities from the homogenized batch of ecstasy tablets. This fiber choice took into account both the extraction ability of the fiber and the ease of fiber cleaning.

4.6 Assessment of Fiber Degradation

Prior to extraction each day, the SPME fiber was stereoscopically observed for evidence of fiber wear *e.g.* cracking, pitting, or stripping of the fiber coating and oxidation *e.g.* blackening of the fiber. The fiber shown in figure 4.8, a multiphase DVB-CAR-PDMS fiber, had been subjected to 18 hours of conditioning, 163 cleaning runs, and 63 extractions. Figure 4.9 compares photos of this fiber on the first and last day of use. According to a study by Kongshaug *et al.*, the fibers are re-useable for 50-100 extractions [1]. Since conditioning should not contribute to fiber wear, this fiber was within its expected lifetime.



a) New DVB-CAR-PDMS (3X) (6.3X)



b) DVB-CAR-PDMS fiber after 63 uses. (3X) (6.3X)

Figure 4.9. DVB-CAR-PDMS fiber compared at (a) first day of use and (b) after 63 extractions.

The blackened area on the fiber increased with fiber use, as seen in figure 4.9. Blackening of the fiber surface is caused by oxidation from oxygen contamination in the GC carrier gas or exposure to environmental oxygen [11]. Despite the increase in oxidized areas, the chromatographic data did not show diminished extraction capability. The main reason for retiring the fiber was the increase of fiber bleed in the chromatographic data. Excessive amounts of fiber bleed can co-elute with the analytes of interest; increased bleed is also a sign that the stationary phase of the fiber is breaking down. Fiber breakdown causes a non-uniform fiber surface that changes as the fiber degrades; this may alter the fiber's ability to reproducibly extract compounds. As the fiber was used for more extractions, it became increasingly difficult to clean, which also contributed to the decision to retire the fiber. Difficulty in cleaning is potentially caused by degradation of the fiber coating causing small cracks in the coating that trap analytes. The most difficult impurities to clean off of the fiber were methylenedioxyphenyl-2-propanol and caffeine, which can potentially be related to the polar nature of both compounds.

This observational evidence indicates that fibers are viable for up to 63 extractions. However, the length of the extraction and the type of impurities being analyzed has a large effect on fiber degradation. For future studies, photographic examination should be continued and the decision to discontinue use of a fiber should take into account increasing fiber bleed as well as oxidation and damage to the fiber surface.

4.7 Summary

Extraction time and temperature are interdependent factors that affect the extraction of impurities from illicit ecstasy tablets by HS-SPME. A CCC experimental design was an efficient method of optimizing HS-SPME extraction time and temperature while taking into account their interactions. Optimized HS-SPME extraction of impurities was achieved by exposing the fiber for 60 min. at 75 °C. The impurities were efficiently desorbed from the fiber with a 1 min. desorption at 250 °C. This optimized HS-SPME extraction procedure extracted more analytes than a conventional LLE procedure. When compared to other fiber types, the DVB-CAR-PDMS extracted more impurities and took less time to clean, allowing for the most sensitive extraction in the shortest overall time. Analysis of different exhibits indicated some common impurities, but also different impurities. Further studies are needed to determine how discriminating particular impurities are in terms of organic profiling and relating impurities to specific synthesis routes or common batches.

4.8 References

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CHAPTER 5: Conclusions and Future Work

5.1 Conclusions

In this work, HS-SPME was optimized for the extraction of organic impurities from ecstasy tablets. Experimental design was used to optimize the HS-SPME extraction procedure. This optimized extraction procedure was then used to investigate desorption parameters, compare HS-SPME to LLE, investigate different fiber polarities, and investigate the differences in impurity profiles between different exhibits of ecstasy tablets.

HS-SPME is an effective method for extracting organic impurities from ecstasy tablets, but it must be optimized before being employed. Extraction time and extraction temperature are important, interrelated variables that must be considered together when optimizing a HS-SPME extraction. With the homogenized batch used in this study, an extraction temperature of 75 °C for an extraction time of 60 min. extracted the most impurities and maximized the peak areas of the impurities investigated. A desorption time of one minute at an inlet temperature of 250 °C was sufficient to reproducibly desorb extracted impurities from the SPME fiber. Higher desorption temperatures and longer desorption times were not necessary and did not result in improved desorption within the limitations of the study.

In regards to the homogenized batch, HS-SPME was a more effective extraction technique than LLE. Due to its selectivity and pre-concentration ability, HS-SPME extracted more impurities than LLE, with greater sensitivity. HS-SPME generated a more informative impurity profile, which increased the profile's usefulness as a means to link or discriminate between batches of confiscated ecstasy.

Despite the capabilities of HS-SPME, its limitations must be taken into consideration before it is employed for profiling. With the current method, HS-SPME is a time intensive process; the extraction takes about one hour and samples must be extracted individually. In addition, the fiber must be cleaned between analyses, a process that can add up to 45 min. to the analysis time, resulting in a total analysis time of roughly 2 hours per sample. In contrast, the LLE process takes about 1.5 hours, but many samples can be extracted simultaneously, thus decreasing the overall analysis time. GC-MS analysis of liquid extracts can also be automated, which further increases the throughput of the method in comparison with HS-SPME. Technology for HS-SPME extraction using automated techniques exists as well, although it is a relatively new technology. Adding autosampling capability to HS-SPME would greatly reduce the amount of analyst involvement necessary for the extraction, and increase the feasibility for implementing HS-SPME for impurity profiling, where high throughput would be needed to develop and maintain a database of impurity profiles. In regards to cost effectiveness, the initial expense of a SPME fiber is negated by its ability to increase the sensitivity of the analysis and provide a more discriminatory profile.

Analysis of different exhibits with diverse physical characteristics showed that impurities varied from tablet to tablet. Even though the tablets did not share physical characteristics (except for logo in the case of CJ_FS_02 and

CJ_FS_05), they shared some common impurities. Exhibits CJ_FS_02 and CJ_FS_03 shared many common impurities, but no conclusions could be drawn about the similarity of these two tablets. More samples must be analyzed to give a larger context to the importance of the detected impurities, as some impurities may be more common than others.

Analysis of different fiber stationary phases showed that the DVB-CAR-PDMS fiber extracted more impurities than the DVB-CAR or DVB-PDMS fibers. This preliminary comparison was based on the non-optimized extraction parameters for the latter two fibers. For a true comparison, each fiber should be individually optimized. This may improve the extraction capabilities of the latter two fibers, thus making them viable fiber choices for this extraction.

Stereoscopic examination and photographic documentation were effective ways to assess the degradation of the fiber, specifically the effects of oxidation, which caused the fiber to blacken over time. In terms of fiber degradation, the DVB-CAR-PDMS fiber lasted for 63 extractions, 163 cleaning runs, and 18 hours of conditioning. Fiber use was discontinued based on increased fiber bleed and severe oxidation of the fiber. This fiber lifetime was reasonable, considering published data validating the fiber for 50-100 extractions.

Altogether, this study provided an initial proof of concept for the use of HS-SPME to extract impurities from illicit ecstasy tablets. HS-SPME was more effective than commonly used methods, when comparing the overall number of impurities extracted by each technique. This is important, because larger numbers of detected impurities result in a more characteristic profile. However, these conclusions are limited to the small number of exhibits studied. Further studies are needed to refine the extraction and analysis techniques, and larger sample sizes are needed to apply statistics to this work.

5.2 Future Work

The end goal of this project was to optimize HS-SPME for the extraction of organic impurities from ecstasy tablets. By optimizing HS-SPME to extract the most impurities, the discriminatory ability of the organic profile of the tablet is increased. With further work, these impurity profiles can be used to determine the method used to synthesize the MDMA, and potentially link batches of MDMA to a common clandestine source. In exhibits where more than one active ingredient is identified *e.g.* MDMA and methamphetamine, it may be difficult to determine the synthesis method used to create each drug because of common precursors and by-products. However, the mixtures of impurities in these samples make them more likely to be differentiated from other sources, and increase the likelihood that batches with these complicated impurity profiles can be linked.

Items that need to be addressed before this HS-SPME method can be used for profiling include improving the chromatography of the impurities, improving the fiber cleaning method, settling on an optimized extraction method that includes optimal fiber type, vial size, sample size, and extraction parameters, and confirming the identity of the impurities.

If the chromatography was improved, reproducibility of peak areas would also improve; peaks would be resolved from each other. Sharpening of the peaks would allow the trace impurities present in low levels to be better discriminated from the background. This would allow for the collection of data for more impurities. The more impurities used for the optimization, the more appropriate the optimized parameters would be for a wide range of impurities. The quality of the prediction would increase. A high quality prediction of optimized extraction time and temperature would increase the quality of the profile by optimally extracting impurities with a wide range of characteristics. This would increase the amount of information that the profiles could provide, leading to more accurate profiling.

Reducing fiber cleaning time would reduce the overall analysis time for HS-SPME and increase the viability of the technique. Potentially, the investigation of higher desorption temperatures and longer desorption times could lead to a cleaner fiber, and reduce fiber cleaning time, although it may have a negative impact on the chromatographic quality because fiber bleed peaks would become more prevalent in the chromatogram. Alternatives to cleaning the fiber in the injection port should be investigated. Separate heated cleaning blocks can be purchased. This would allow the fiber to be cleaned during the run without using the injection port, which increases the background noise in the chromatogram; it would also eliminate the time needed to clean the fiber after the run. This would reduce the per sample analysis time and HS-SPME would be better suited for high throughput analyses.

Other avenues to be pursued include testing and optimizing other factors such as sample size, vial size, and the effect of sample pre-heating on the HS-

SPME extraction. These factors have an effect on the attainment of equilibrium. Since reproducibility in extraction is achieved at equilibrium, the more quickly equilibrium can be reached, the shorter the analysis time can be. For example, larger sample size or smaller vial size may increase the speed of equilibration. If pre-heating the vial was not determined to have an effect on equilibrium, the pre-heating step could be eliminated and the overall analysis time could be further decreased.

To measure the variation in extraction from run to run, an internal standard should be used. Because of the complicated nature of fiber-headspace-sample equilibrium, this is not easily implemented. Some options include exposing the fiber to the headspace of a vial containing an internal standard either before or after the extraction. The loss of either internal standard into the sample vial or loss of sample into the internal standard vial would need to be taken into consideration. Alternatively, the vial of sample could be spiked with internal standard, but this would alter the composition of the sample in the vial and the sample could not be re-used.

In addition to HS-SPME of solid samples, HS-SPME of tablet dissolved in liquid systems should be investigated. Liquid samples would overcome the problem of non-reproducible surface area, and modifiers, such as salts, could be added to the liquid phase in order to increase the affinity of the impurities for the headspace. Increasing the number of detected impurities would allow a more detailed profile to be developed.

However, the destructive nature of liquid sampling should be taken into consideration before implementing this extraction scheme. With HS-SPME of solids, the initial material can be recovered in its initial form. With HS-SPME of liquids, the sample is destroyed. Depending on how much sample there is to work with, this may not be an issue. Potentially, if the analysis only used 30 mg of tablet, there would be enough material preserved to conduct additional tests, even if the exhibit contained only one tablet. If the exhibit contained less than one tablet, destruction of the exhibit for HS-SPME of a liquid sample may not be the best option.

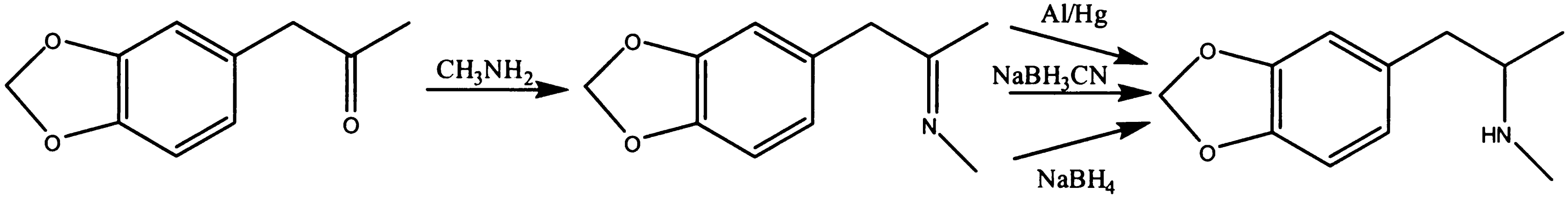
Standards should be run to definitively identify the extracted impurities. The difficulty with this is the expense of standards; in addition, standards are not readily available for all potential impurities. Tandem MS could also be employed for structural determination of the impurities; the ion trap instrument used in this research has that capability.

A larger number of exhibits and a larger number of tablets from each exhibit need to be analyzed so that statistically significant conclusions can be drawn. Once impurities are definitively identified and a statistically sound number of analyses are made, conclusions concerning likely synthetic route can be drawn, which is the first step in linking tablets from common batches.

Eventually, a searchable database of organic profiles could be created which would allow tablets with common impurity profiles to be linked, according to synthetic route. From there, common batches could be potentially linked through similarities in organic profiles, but this is dependent on how common the impurities are. These linkages could be used to trace ecstasy distribution up the supply chain to the manufacturers with an end goal of reducing the overall supply of the drug.

Appendix A: Common Synthetic Routes for MDMA.

Reductive Amination:

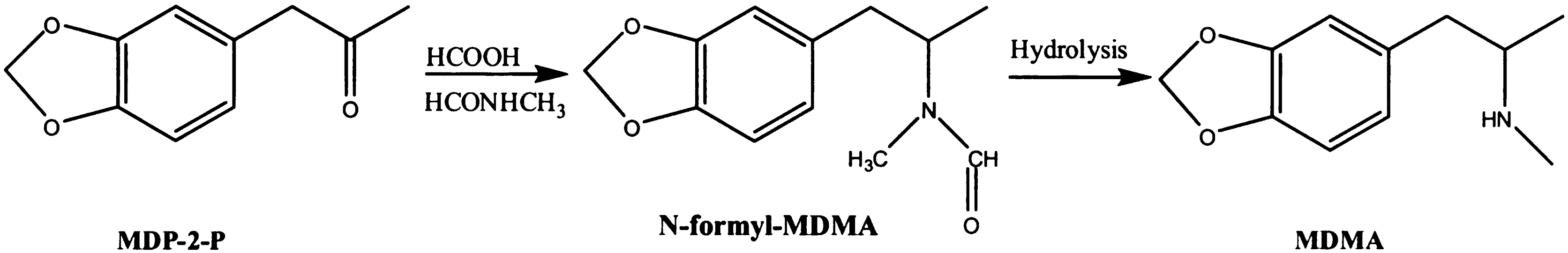


MDP-2-P

1,2-methylenedioxy-4-(2-*N*-methyliminopropyl) benzene

MDMA

Leuckart Method:

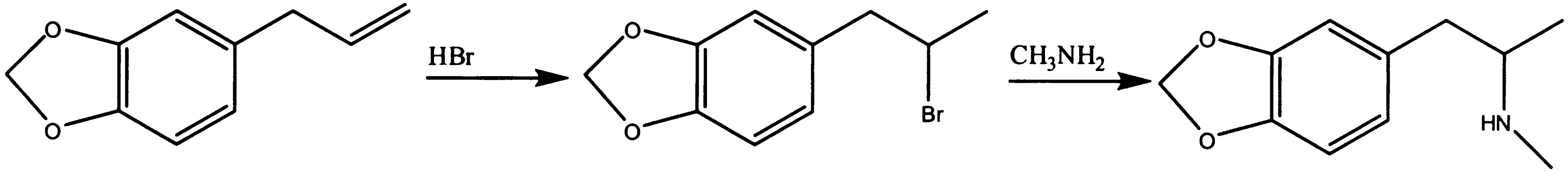


MDP-2-P

N-formyl-MDMA

MDMA

Safrole Bromination:



Safrol

1,2-methylenedioxy-4-(2-bromopropyl) benzene

MDMA

Appendix B: Minitab® Generated Runs for Two Factor CCC Design.

***Point Type:** -1 = star point 0 = center point 1 = factorial point

Run Order	PtType*	Exposure Temp. (ºC ±2º)	Exposure Time (min.)
1	1	76	13.0
2	0	54	32.5
3	0	54	32.5
4	0	54	32.5
5	1	32	13.0
6	1	76	52.0
7	1	32	52.0
8	-1	54	5.0
9	0	54	32.5
10	-1	54	60.0
11	0	54	32.5
12	-1	85	32.5
13	-1	23	32.5
14	0	54	32.5
15	1	76	52.0
16	1	32	52.0
17	0	54	32.5
18	0	54	32.5
19	1	32	13.0
20	0	54	32.5
21	1	76	13.0
22	1	32	52.0
23	0	54	32.5
24	1	76	52.0
25	1	76	13.0
26	0	54	32.5
27	0	54	32.5
28	1	32	13.0
29	-1	54	5.0
30	-1	23	32.5
31	0	54	32.5
32	0	54	32.5
33	0	54	32.5
34	-1	54	60.0
35	-1	85	32.5
36	-1	54	5.0
37	0	54	32.5
38	0	54	32.5
39	-1	85	32.5
40	-1	23	32.5
41	0	54	32.5
42	-1	54	60.0

Appendix C: Comparison of the Impurities Present in the Four Exhibits.

Peaks present in the chromatograms that are not listed below are caused by either fiber bleed or column bleed.

R.T.	Prominent m/z	CJ-FS-01	CJ-FS-02	CJ-FS-03	CJ-FS-05	Tentative Peak Identity
3.66	86, 58, 87, 72	X	X	X		N-formyl methamphetamine
4.85	105, 77, 51, 106		X	X	X	a-oxo-benzene acetaldehyde
8.06	91, 92, 65, 63, 89	X	X	X	X	Toluene
9.21	58, 91, 150, 65, 119		X	X	X	Methamphetamine
10.31	72, 91, 70, 115		X	X	X	N-a-trimethyl benzeneethanamine
11.85	148, 150, 121, 65	X				Piperonal
11.99	146, 147, 63, 62	X				
12.51	56, 71, 91, 58, 117				X	
12.61	135, 136, 179, 180, 77				X	
12.89	135, 136, 164, 77, 162		X	X		
13.73	149, 180, 121, 65	X				Methyl piperonylate
14.44	135, 77, 178, 51		X	X		MDP-2-P
14.66	180, 135, 136, 163, 77	X			X	
14.96	135, 136, 180, 77		X	X		3,4-methylenedioxy phenyl-2-propanol
15.12	58, 77, 135, 136, 194				X	MDMA
15.26	58, 77, 135, 194		X	X		MDMA
15.49	58, 180, 135, 77, 136	X				
15.57	162, 135, 180, 77	X				
15.78	194, 58, 86, 118, 91		X	X		
15.94	149, 194, 177, 176		X	X		
16.13	72, 70, 77		X	X		MDEA
16.11	149, 72, 177				X	
16.38	178, 149, 163, 148, 222	X				
16.49	182, 105, 77, 181				X	
16.58	124, 152, 192, 83, 55	X				
16.88	86, 58, 194, 77		X	X		
16.92	192, 163, 134, 104, 179	X				
17.71	91, 162, 119, 86, 157		X	X		
17.80	58, 100, 135, 77, 194		X	X		
17.83	83, 185, 121, 138, 79	X				
18.18	55, 676, 83, 111, 101		X	X	X	
18.32	132, 105, 79, 91			X		
18.77	120, 92, 138		X			Salicylic acid
19.38	194, 193, 55, 109, 195		X	X	X	Caffeine
19.94	91, 160, 119, 115			X		
20.41	162, 58, 77, 163, 135		X	X		MDA Acetate
21.38	227, 168, 256, 196, 87		X	X		
21.86	86, 120, 56, 58, 99		X	X		
22.63	58, 115, 162, 77, 135		X	X		
23.06	176, 149, 91	X	X	X		
26.90	176, 149, 91	X				

27.68	163, 204, 135, 105, 77	X	X
29.37	86, 236, 56, 117, 58		X
30.05	218, 188, 162		X
32.99	86, 280, 56, 162, 58	X	X

Appendix D: Comparison of Different Fiber Types.

Key to fiber type: 1) DVB-CAR-PDMS 2) PDMS-DVB 3) CAR-PDMS

R.T.	Prominent m/z	1	2	3	Tentative Peak Identity
3.79	86, 58, 87, 72	X	X	X	n-formylmethamphetamine
4.85	105, 77, 51, 106	X			a-oxo-benzeneacetaldehyde
8.13	91, 92, 65, 89	X	X	X	Toluene
9.60	58, 150, 91, 65	X	X	X	Methamphetamine
10.04	135, 108, 58, 56, 91	X	X	X	Benzo[d]isothiazole
10.38	72, 91, 70, 115	X	X	X	N-a-trimethylbenzeneethanamine
11.92	91, 116, 131, 149, 89			X	
12.01	116, 91, 131, 89, 65			X	
12.90	135, 136, 164, 77	X	X		
14.24	135, 178, 77, 79			X	MDP-2-P
14.52	135, 178, 77, 79	X	X		MDP-2-P
14.55	135, 136, 180, 58, 77			X	
15.08	135, 136, 180, 58, 77	X	X		3,4-methylenedioxyphenyl-2-propanol
15.24	58, 194, 135, 77	X	X	X	MDMA
16.01	72, 70, 77, 135			X	MDEA
16.13	72, 70, 77, 135	X	X		MDEA
16.89	86, 58, 194, 77, 135	X	X	X	
17.02	193, 135, 146, 194, 77	X			
17.41	190, 148, 147, 194, 188		X		
17.70	162, 91, 119, 86, 157	X	X		
17.82	58, 100, 135, 194	X	X		
18.17	55, 67, 111, 83, 194	X	X		
18.33	132, 105, 79, 91	X			
19.31	194, 193, 55, 109,195	X	X	X	Caffeine
19.90	135, 162, 77, 91		X		
19.95	91, 160, 119, 115	X			
20.42	162, 58, 163, 77, 135	X	X		MDA Acetate
21.40	227, 168, 196	X			
21.84	86, 120, 99, 58	X	X	X	
22.63	58, 115, 162, 77	X		X	
22.81	174, 91, 148, 145, 115	X	X	X	
23.97	160, 163, 105, 91, 135			X	
24.16	91, 119, 160			X	
27.68	163, 204, 135, 105	X		X	
33.00	86, 280, 162, 56, 99	X	X		
	Total # of Compounds Extracted:	**26**	**21**	**19**	