Optimization of Headspace Solid Phase Microextraction (HS-SPME) for Organic Impurity Profiling of Illicit MDMA tablets

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Abstract

Organic impurity profiling of illicit synthetic drug tablets aims to identify similarities among tablets. Similar impurity profiles indicate a common production method, and similar levels of the same impurities potentially indicate common production laboratories. In this research, headspace solid phase microextraction (HS-SPME) procedures for the extraction of organic impurities from illicit MDMA ('ecstasy') tablets were developed, using a divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber and analyzing the extract using gas chromatography-mass spectrometry (GC-MS). Two sampling methods were investigated; in one, the SPME fiber was exposed to the headspace of a solid sample of MDMA while in the second procedure, the MDMA was dissolved in an aqueous buffer and the fiber was subsequently exposed to the headspace of the buffer solution.

For both sampling methods, HS-SPME extraction time and temperature were optimized using a statistical design of experiments procedure in order to account for the interdependence of these two parameters. Repeatability and reproducibility of the extraction was poor for the solid sampling procedure, potentially due to the inhomogeneous headspace. With the aqueous buffer solution of MDMA, relative standard deviations (RSDs) were typically less than 15% for the early eluting impurities (retention time less than 14 minutes).

The optimized HS-SPME procedure for impurity extraction from an aqueous buffer solution of MDMA was compared to a conventional liquid-liquid extraction procedure in terms of the number of impurities extracted as well as the repeatability and reproducibility of the extraction. The liquid-liquid extracts were dominated by 3,4-methylenedioxyphenyl-2-propanol, MDMA, and 3,4-methylenedioxyethylamphetamine. In general, only 12 impurities were extracted using the liquid-liquid extraction procedure, compared to 16-22 impurities using the HS-SPME procedures, depending on extraction time and extraction temperature. Additionally, impurities in the liquid-liquid extracts were present at levels just above background, making an assessment of precision and repeatability difficult.

Finally, impurity profiles of MDMA tablets from five different exhibits were obtained using HS-SPME for extraction of impurities from aqueous buffer solutions, with analysis by GC-MS. All five exhibits were differentiated based on the impurity profiles obtained using the optimized HS-SPME procedure for impurity extraction.

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Project Description

Although first synthesized in 1912 and never marketed, 3,4-methylenedioxymethamphetamine (MDMA) re-emerged into society in the late 1970s [1, 2]. Now controlled in Schedule I of the Controlled Substances Act, MDMA is probably better known as the club drug 'ecstasy'. This term is now somewhat generic since tablets sold as ecstasy may or may not contain MDMA[3]. However, in North America, there is a trend toward high purity ecstasy tablets that contain only MDMA[3].

MDMA is typically synthesized in clandestine laboratories in Europe and trafficked into the United States[4]. There are numerous documented methods for MDMA synthesis including the Leuckart synthesis and variations of the reductive amination synthesis[5]. Due to lack of purification and quality control procedures in clandestine laboratories, the MDMA produced contains impurities that result not only from the synthesis but also from the starting materials and reagents used. Hence, different batches of MDMA synthesized following the same method are expected to contain different levels of the same impurities[6]. In contrast, MDMA synthesized by different routes would be expected to contain different impurities[6].

Impurity profiling aims to associate and discriminate MDMA tablets based on similarities and differences in the impurities present. The identity and level of the impurities present can indicate the synthetic method used and potentially point to common production sources (*i.e.* clandestine laboratories). Currently, research focuses on liquid-liquid extraction of the organic impurities and subsequent analysis of the extract by gas chromatography-mass spectrometry (GC-MS) to generate the impurity profile[7-9]. However, liquid-liquid extraction requires a relatively large sample mass (100-200 mg, often equivalent to a full tablet)[6, 7, 9], along with the use and subsequent disposal of organic solvents. Furthermore, MDMA typically dominates the extract and the resulting impurity profile to the detriment of impurities present at trace levels.

Solid phase microextraction (SPME) offers an alternative to liquid-liquid extraction procedures for impurity extraction[10]. In SPME, a polymeric coated fiber is introduced into the sample vial; for direct immersion SPME, the fiber is positioned in a liquid sample while in headspace (HS)-SPME, the fiber is positioned in the headspace above the sample, which can be solid or liquid. The sample may be heated and the fiber is left exposed for a specified time, during which analytes absorb and/or adsorb onto the fiber. After the designated extraction time, the fiber is retracted, removed from the sample, and inserted into the heated injection port of the GC. Here, impurities desorb from the fiber and are transferred onto the column for separation and subsequent detection, typically by MS.

HS-SPME offers numerous advantages over liquid-liquid extractions, particularly for impurity profiling applications. Depending on the SPME mode (direct immersion or headspace), little to no sample preparation is necessary and the use of organic solvents can be eliminated. Impurities are pre-concentrated on the fiber and hence, lower sample masses are required. The low volatility of the MDMA salt (the form typically present in illicit tablets) prevents efficient partitioning into the headspace; hence, HS-SPME extracts are not dominated by the illicit substance, resulting in more informative impurity profiles.

The success of SPME in impurity profiling is documented in the literature, primarily for methamphetamine profiling[11-13]. So far, only one study by Kongshaug *et al.* demonstrates SPME for the extraction of impurities from MDMA samples[14]. Acetate buffer (5 mL, 0.1 M) was added to the ground tablet, the solution was sonicated, and heated to 90 °C. The SPME fiber was exposed to the headspace in the sample vial for 30 minutes at 90 °C. Two different SPME fiber types were considered—100 µm polydimethylsiloxane (PDMS) and 65 µm PDMS-divinylbenzene (DVB). MDMA extracts from the two SPME fibers as well as a conventional liquid-liquid extraction using ethyl acetate were analyzed by GC with a flame ionization detector to generate impurity profiles.

Six impurities were identified in the impurity profiles obtained using each extraction method: 3,4-(methylenedioxy)benzaldehyde, isosafrole, 3,4-methylenedioxyphenyl-2-propanone, 1-(3,4-methylenedioxy)phenyl-2-propanol, N,N-dimethyl-methylenedioxyamphetamine, and N-formyl MDMA. However, there were clear differences in the levels of impurities extracted. The liquid-liquid extract was dominated by the impurities 3,4-methylenedioxyphenyl-2-propanone (MDP-2-P) and 1-(3,4-methylenedioxy)phenyl-2-propanol, while other impurities were present at low levels. Impurities were also extracted in low concentrations using the PDMS fiber, potentially due to the non-polar nature of the fiber stationary phase. Levels of impurities extracted were significantly higher using the more polar PDMS-DVB fiber, since extraction of more polar impurities was enhanced.

In this project, HS-SPME methods for the extraction of organic impurities from MDMA tablets were developed and optimized. Two HS-SPME methods were optimized—extraction from the headspace of the powdered tablet and extraction from the headspace of an aqueous buffer solution of the tablet. In both cases, a statistical experimental design procedure was used to optimize the SPME extraction time and extraction temperature in order to account for the interdependence of these two parameters. Fiber extracts were analyzed directly by GC-MS to generate impurity profiles. The optimized extraction procedure was then compared to a conventional liquid-liquid extraction procedure, in terms of the number of impurities extracted as well as the repeatability and reproducibility of the extraction procedure. Finally, the utility of the optimized HS-SPME procedure was demonstrated in the differentiation of five different MDMA exhibits, based on their corresponding impurity profiles.

Project Objectives

1. Optimize HS-SPME methods to extract organic impurities from MDMA tablets.
2. Optimize GC-MS method for extract analysis.
3. Assess variation in impurities among individual tablets.
4. Apply pattern recognition methods to non-subjectively group similar tablets based on the impurities present.

This research is a preliminary assessment of HS-SPME as a viable alternative to conventional liquid-liquid extraction procedures for impurity profiling seized MDMA tablets. It is envisioned that, eventually, a database of MDMA tablets will be created in our laboratory, which will be available to law enforcement agencies. The database will include physical characteristics (tablet dimensions, color, logo) and chemical characteristics (organic impurity

profiles) of each tablet. Local law enforcement agencies will be invited to submit seized MDMA tablets for which impurity profiles will be generated using HS-SPME and GC-MS. The resulting impurity profile will be searched within the database and matched to any tablets with similar physical and chemical characteristics. Similarity in chemical characteristics may be indicative of a common production method and/or source. This intelligence may be used by law enforcement agencies to monitor drug-trafficking networks and distribution chains.

Procedures

Statistical experimental designs were used to optimize extraction time and extraction temperature for HS-SPME of both solid (ground tablet) samples of MDMA and aqueous buffer solutions (ground tablet dissolved in buffer) of MDMA. All SPME extracts were analyzed by GC-MS to generate organic impurity profiles for comparison. The repeatability and reproducibility of the optimized extraction procedure was assessed with replicate extractions performed over a 4 day period. Impurities were also extracted from tablets using a conventional liquid-liquid extraction procedure reported in the literature, which was compared to the optimized HS-SPME method. Finally, organic impurity profiles of tablets from a number of different MDMA exhibits were generated using the optimized HS-SPME procedure as a preliminary assessment of the potential for organic impurity profiling to differentiate MDMA exhibits.

With the exception of tablets from different exhibits, all experiments described below were conducted using a homogenized batch of MDMA tablets from the same exhibit. The use of the homogenate was intended to eliminate variation among individual tablets in an exhibit as a potential source of variation in impurity profiles.

Statistical Experimental Design

A circumscribed central composite (CCC) design was generated using Minitab® Statistical Software (version 15, State College, PA). CCC designs are used to predict optimal system parameters using the minimum number of experiments. Hence, the predicted optimal parameters may not necessarily be tested during the experiments defined in the design.

In essence, the CCC design consists of a factorial design with a star design and center points. Factorial points account for all interactions of the parameters being studied, star points account for squared terms, and the center points allow for an estimation of the overall error. By definition, there are 2^k factorial points in the design, where k is the number of factors. Star points are positioned at (+α, 0), (-α, 0), (0, +α), (0,-α), where $\alpha = [2^k]^{1/4}$ and the center point is located at (0,0).

In this study, HS-SPME extraction time and extraction temperature were optimized. Hence, for two factors, there are four factorial points that define the corners of a square and α = 1.414. True extraction temperatures and times were then determined for the coded values (-1.414, -1, 0, 1, and 1.414). Based on practical limitations and a review of the literature, the upper and lower extraction time limits were 60 minutes and 5 minutes, respectively. For extraction temperature, the upper and lower limits were 85 °C and 23 °C, respectively. In the CCC design, the star points

represent the experimental boundaries; hence, for extraction time, -1.414 is equivalent to 5 minutes and 1.414 is equivalent to 60 minutes. Similarly, for extraction temperature, -1.414 and 1.414 are equivalent to 23 °C and 85 °C, respectively. True extraction times and temperatures corresponding to -1, 0, and 1 were then calculated, generating the experimental design illustrated in Figure 1. Each point in the design represents a single experiment. Each experiment was conducted in triplicate, resulting in a total of 42 experiments, and the design was randomized in order to minimize systematic error.

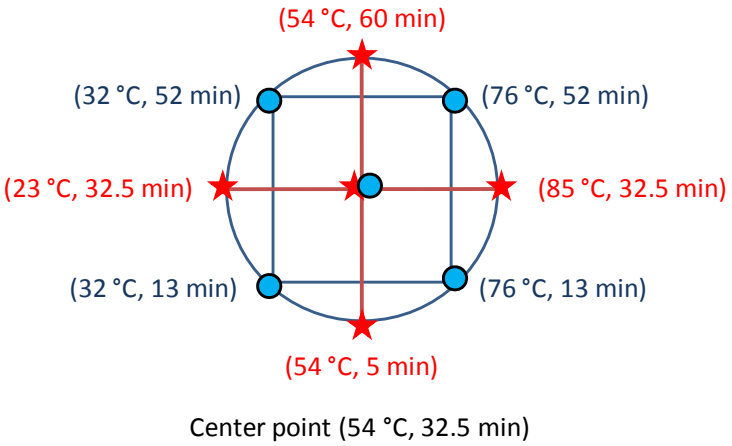


Figure 1 CCC design depicting true experimental conditions

HS-SPME of solid MDMA sample

A 50 mg aliquot of the homogenized MDMA batch was accurately weighed into a 4 mL amber vial and preheated for 5 minutes in a water bath at the extraction temperature defined by the experimental design. A divinylbenzene/carboxen/polydimthylsiloxane (DVB/CAR/PDMS) fiber was then exposed to the sample headspace for the corresponding extraction time specified in the design. After the required extraction time, the fiber was retracted and analyzed by GC-MS.

HS-SPME of aqueous buffer solution of MDMA

A 50 mg aliquot of the homogenized MDMA was weighed into a 10 mL vial and 5 mL carbonate buffer (pH 10, 0.05 M) was added, along with a stir bar. The sample mixture was placed in a water bath positioned on a stir plate and heated to the extraction temperature defined by the experimental design, with stirring. The sample was pre-heated for 10 minutes before the DVB/CAR/PDMS fiber was inserted and exposed to the headspace for the appropriate extraction time. The fiber was then retracted and analyzed by GC-MS.

GC-MS analysis of SPME extracts

All SPME extracts were analyzed using a Focus gas chromatograph coupled to a Polaris ion trap mass spectrometer (Thermo Scientific, Waltham MA), equipped with an Rxi-5ms capillary column (30 m x 0.25 mm x 0.25 µm, Restek, Bellefonte PA). Ultra-high purity helium was used as the carrier gas, with a nominal flow rate of 1 mL min^{-1}. A Merlin Microseal Septum (Supelco,

St Louis MO) was used in place of a traditional septum along with a narrow inlet liner (0.8 mm, SGE Inc., Austin TX), in order to increase carrier gas flow and hence, desorption, of impurities from the SPME fiber.

The fiber was desorbed for 2 minutes at 250 °C in splitless mode before the GC was temperature programmed as follows: 60 °C for 2 minutes, 8 °C min^{-1} to 300 °C, with a final hold of 15 min. During temperature programming, the SPME fiber remained in the heated inlet, with a split ratio of 100:1, in order to clean the fiber. Following analysis of the extract, a blank fiber was run in order to prove the cleanliness of the fiber prior to the next extraction.

Liquid-liquid extraction

The liquid-liquid extraction procedure was based on a previously published and optimized extraction procedure for amphetamine[15]. Briefly, 200 mg of homogenized tablets was accurately weighed into a centrifuge vial, 4 mL 1 M Tris buffer (pH 8.1, Sigma-Aldrich, St Louis MO) was added, and the mixture was sonicated for 30 minutes. A 200 µL aliquot of toluene containing eicosane (Sigma-Aldrich) as internal standard (10 µg eicosane/mL toluene) was added, the mixture was sonicated for a further 20 minutes, and then centrifuged for 10 minutes. The organic layer was transferred into a limited volume insert positioned within a GC vial.

GC-MS analysis of liquid-liquid extract

For analysis of the liquid-liquid extract, the Merlin Microseal septum was replaced with a Thermo-Green septum and a conventional 5 mm inlet liner was used. A 1 µL aliquot of the extract was analyzed by GC-MS, using a 50:1 split ratio, with all other instrumental parameters as described previously.

Repeatability and reproducibility

Samples of MDMA were prepared from the homogenized batch and extracted using the appropriate optimized HS-SPME procedure (solid sample and aqueous buffer solution). Extracts were performed in triplicate each day over a four day period. For each impurity identified, relative standard deviations (RSDs) were calculated based on peak area. The repeatability of the extraction was assessed based on the RSDs of impurities in extracts analyzed on the same day while extraction reproducibility was based on the average RSD of impurities analyzed over the four day period.

For the liquid-liquid extraction procedure, two extracts of homogenized MDMA were prepared and analyzed in triplicate over three consecutive days to assess the repeatability and reproducibility of the extraction procedure in a similar manner.

Analysis of multiple MDMA exhibits using HS-SPME

One tablet from each of five different MDMA exhibits was individually homogenized. Three 50 mg aliquots from each tablet were weighed into separate vials and carbonate buffer (5 mL, pH 10, 0.05 M) was added to the vial along with a magnetic stir bar, enabling triplicate extractions

of each tablet. The vial was pre-heated in a water bath for 10 minutes with stirring at 60 °C. A DVB/CAR/PDMS fiber was then exposed to the headspace for 30 minutes and subsequently analyzed by GC-MS.

Results and Discussion

Optimization of HS-SPME procedure for a solid sample of MDMA

For each extract, the resulting impurity profile was assessed to identify impurities that: (1) were present in all extracts, (2) exhibited good chromatographic peak shape, and (3) were resolved from neighboring impurities. Four impurities met these criteria: benzeneacetamide (retention time 7.84 min); 3,4-methylenedioxy-N,N-dimethylbenzylamine (retention time 12.38 min); 3,4-methylenedioxyamphetamine acetate (retention time 15.05 min); and caffeine (retention time 19.04 min). Peak areas of these four impurities in all 42 extractions included in the experimental design were integrated. Statistical software (Minitab®, version 15, State College PA) was then used to model the data for each of the four impurities.

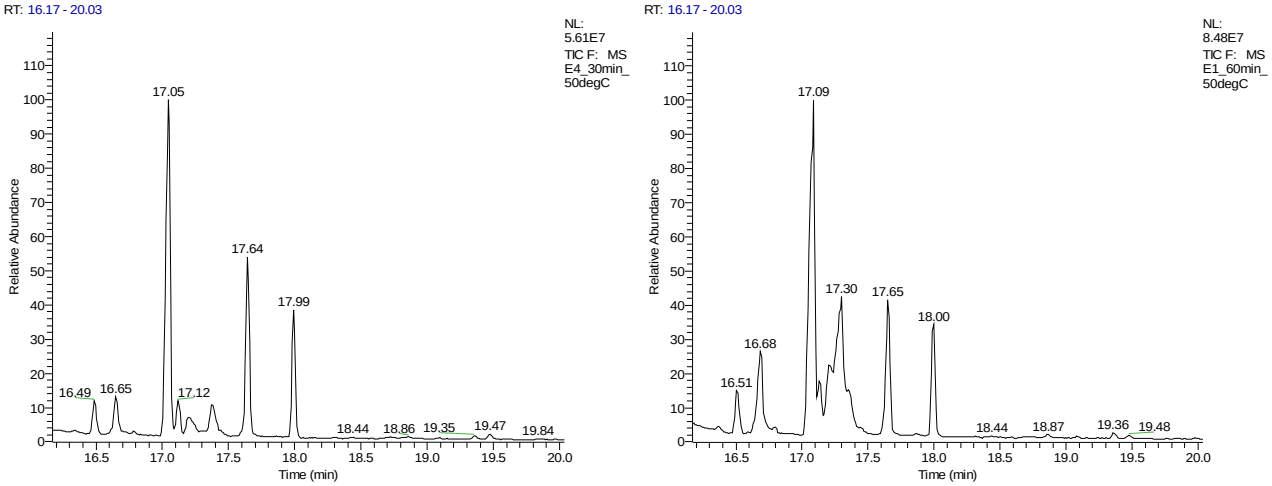
Initially, each impurity was modeled with a full quadratic equation, which included linear terms, squared terms, and interactions. For each impurity, the p-value for lack of fit for each term modeled was assessed. P-values greater than α (in this case, α=0.05) indicated that the term was not significant and hence, the data was remodeled omitting those terms. For the four impurities considered, the time squared term was not important (p value greater than α) and hence, the data for these impurities was remodeled considering linear terms and interactions only. For caffeine, the data was modeled taking into account only the extraction temperature term.

Contour plots were then prepared for each impurity (except caffeine) in order to determine the optimum extraction time and extraction temperature as determined from the CCC design. Higher responses (peak areas of impurities) were observed at extraction temperatures above 80 °C with extraction times greater than 55 minutes. However, visual assessment of impurity profiles obtained at longer extraction temperatures and times (*e.g.* 76 °C for 52 minutes) exhibited poorer chromatography, most likely due to overloading the fiber and hence, the GC column. Broad chromatographic peaks are not desirable due to the potential masking of impurities present at lower levels.

An empirical approach was then employed in order to reach a compromise in extraction time and temperature that allowed extraction of impurities without overloading the fiber. A series of extractions were performed at 50 °C with extraction time varying from 10 to 60 minutes in increments of 10 minutes. At the shorter extraction times (10-30 minutes), chromatography was noticeably improved compared to longer extraction times, with narrower peaks and less co-elution. However, for 10 and 20 minute extractions, a number of impurities, although still present, were present at levels close to the baseline. Hence, an extraction time of 30 minutes was considered sufficient in order to extract all impurities extracted at longer times, but with the advantage of improved chromatography (Figure 2).

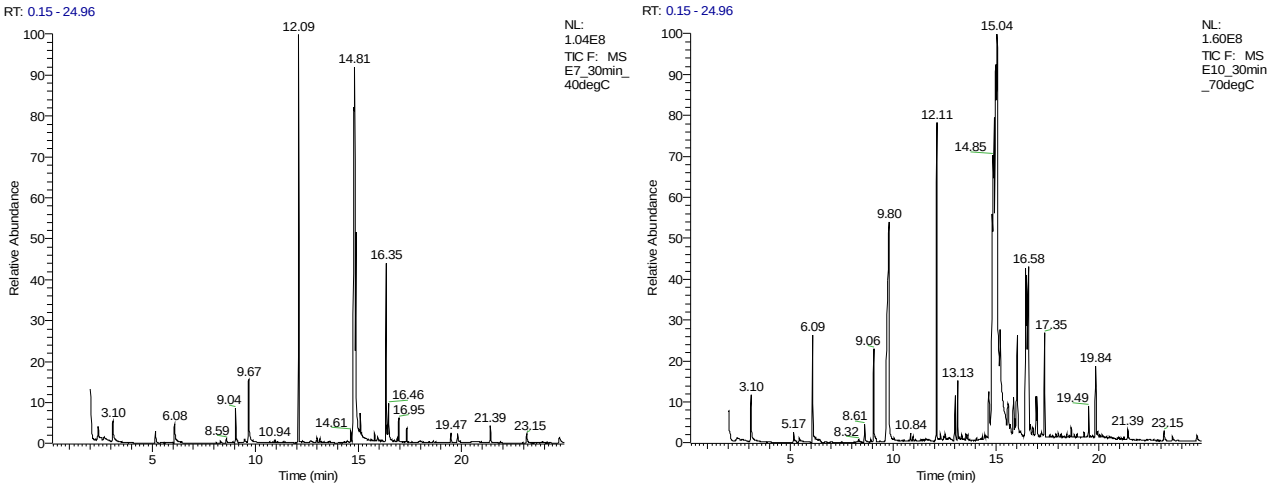
A second series of extractions was performed with the 30 minute extraction time and extraction temperatures ranging from 40 to 80 °C in increments of 10 °C. Resolution and chromatographic peak shape were improved at the lower extraction temperatures (40-60 °C); for

example, N-methylephedrine co-eluted with dibutyl phthalate at extraction temperatures of 70 and 80 °C but was close to baseline resolution at 40, 50, and 60 °C (Figure 3). However, piperonal and 2-(1-methyl-2-pyrrolidinyl)-pyridine were only observed using higher extraction temperatures of 70 °C and 80 °C. In addition, at the higher extraction temperatures, levels of impurities were distinct from the baseline, making identification simpler. At the highest extraction temperature considered (80 °C), a number of impurities exhibited poor chromatographic peak shape (*e.g.* methamphetamine and MDP-2P), potentially due to overloading the fiber.



(a) Extracted at 50 °C for 30 minutes (b) Extracted at 50 °C for 60 minutes

Figure 2 Effect of extraction time on chromatography. Expanded region of chromatogram shown for clarity.



(a) Extracted at 40 °C for 30 minutes (b) Extracted at 70 °C for 30 minutes

Figure 3 Effect of extraction temperature on impurities extracted. The number of impurities extracted clearly increases with temperature although a compromise must be reached to maintain acceptable chromatography.

Hence, for HS-SPME extractions from a solid sample of MDMA, an extraction time of 30 minutes at an extraction temperature of 70 °C (Figure 3b) was deemed a suitable compromise that allowed extraction of all impurities while maintaining acceptable chromatographic peak shape and resolution.

Optimization of HS-SPME procedure for an aqueous buffer sample of MDMA

Due to poor repeatability and reproducibility of HS-SPME extractions from a solid sample of MDMA, a HS-SPME procedure for extractions from an aqueous buffer solution of MDMA was investigated and optimized. With this sampling mode, the sample can be agitated, which serves to increase partitioning of semivolatile analytes into the headspace. A more concentrated and homogenous headspace could potentially improve the repeatability and reproducibility of subsequent extractions.

Following preliminary experiments to investigate the effect of buffer selection on the number of impurities extracted, a carbonate buffer (pH 10, 0.05 M) was chosen for further investigation. Similar to optimization of HS-SPME with solid sampling, a CCC experimental design procedure was employed, resulting in a total of 42 experiments, with extraction time ranging from 5 minutes to 60 minutes and extraction temperature ranging from 23 °C to 85 °C. The experimental design data was analyzed using Minitab® statistical software, following similar procedures as described previously.

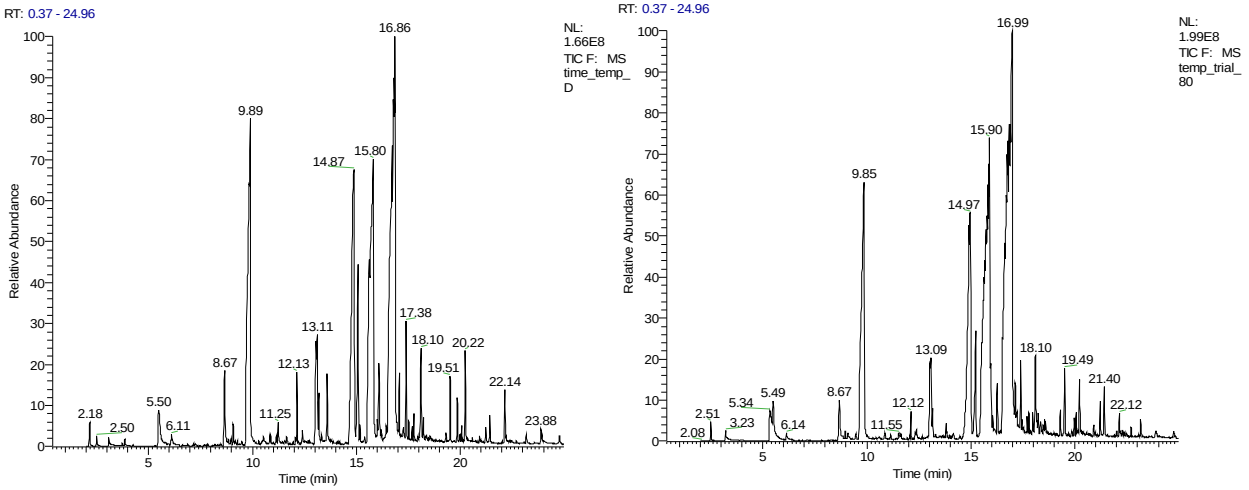
Four impurities were present in all extractions conducted in the design with acceptable chromatography: benzeneacetamide (retention time 8.6 min); methamphetamine (retention time 9.9 min); 3,4-methylenedioxyphenyl-2-propanone (MDP-2-P, retention time 14.8 min); and 3,4-methylenedioxyphenyl-2-propanol (MDP-2-propanol, retention time 15.0 min). Peak areas of each impurity were firstly modeled using full quadratic equations to take into account linear terms, squared terms, and interactions. As described previously, the data was remodeled omitting any terms that were considered to be non-significant (*i.e.* $\alpha > 0.05$). Extraction time and extraction temperature were not significant factors for the extraction of benzeneacteamide and MDP-2-P from the headspace of the carbonate buffer solution of MDMA. However, both extraction time and extraction temperature were significant for the extraction of methamphetamine and only extraction temperature was significant for MDP-2-propanol extraction.

A contour plot was only generated for methamphetamine as this was the only impurity for which extraction time and extraction temperature were both significant. From the contour plot, the optimal extraction time for methamphetamine ranged from 20 minutes to 40 minutes, with an extraction temperature ranging from 55 °C to 70 °C. Visual assessment of impurity profiles generated during the experimental design indicated that while some impurities were only observed with higher extraction temperatures or longer extraction times, chromatographic peak shapes were detrimentally affected as time and temperature increased.

As previously, an empirical approach was adopted to determine a compromise in extraction time and temperature that allowed extraction of all impurities while maintaining acceptable peak shape. A series of extractions was performed holding the extraction temperature constant at 60

°C while varying the extraction time from 10 to 60 minutes in increments of 10 minutes. As expected from previous results, when extraction temperature was held constant (60 °C) chromatography was improved with better resolution as extraction time decreased (20 and 30 minutes compared to 60 minutes). Extraction time appeared to have little effect on the number of impurities extracted as the majority of impurities were extracted at all extraction times. The major exceptions were N-ethylamphetamine (only present albeit at very low levels with 50 minute and 60 minute extraction times) and 1,2-dimethyl-3-phenyl-aziridine, which was not present at extraction times less than 30 minutes. An extraction time of 30 minutes was subsequently determined to be the extraction time that offered a compromise between the number of impurities extracted and acceptable chromatographic peak shape.

The second series of extractions was performed with an extraction time of 30 minutes and extraction temperatures ranging from 40 to 80 °C in increments of 10 °C. Similar to previous results, although lower extraction times offered better chromatography, a number of impurities were not extracted at the lower temperatures. N,N-diethylamphetamine and N-methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene were extracted at temperatures of 40 °C and higher while piperonal, isosafrole, and caffeine were extracted at temperatures of 50 °C and higher. N-ethylamphetamine was only extracted at extraction temperatures of at least 60 °C. As extraction temperature increased further (up to 80 °C), chromatographic peak shape worsened, potentially due to overloading the fiber during extraction and ultimately, the GC column (Figure 4). As a result, 60 °C was deemed to be the extraction temperature that offered the desired compromise between the number of impurities extracted and acceptable chromatography.



(a) Extracted for 30 minutes at 60 °C

(b) Extracted for 30 minutes at 80 °C

Figure 4 Effect of extraction temperature on impurities extracted. With increasing extraction temperature, higher levels of each impurity are extracted albeit with decreasing quality of chromatography peak shape, potentially due to overloading the fiber.

Based on number of impurities extracted and chromatography for all impurities, an extraction time of 30 minutes and an extraction temperature of 60 °C were deemed suitable compromise conditions for HS-SPME extractions from an aqueous buffer solution of MDMA.

Repeatability of HS-SPME procedure for an aqueous buffer solution of MDMA

Using an extraction time of 30 minutes and an extraction time of 60 °C, the repeatability and reproducibility of the HS-SPME method for the extraction of impurities from an aqueous buffer solution of MDMA was assessed. A new DVB/CAR/PDMS fiber was used for this study, samples were prepared as described previously (5 mL carbonate buffer, pH 10, 0.05 M), and the fibers were analyzed using the GC-MS procedure described previously. For each impurity identified, relative standard deviations (RSDs) were calculated based on peak area. The repeatability of the extraction was assessed based on the RSDs of impurities in extracts analyzed on the same day while extraction reproducibility was based on the average RSD of impurities analyzed over the four day period. The RSD values for the impurities identified are shown in Table 1.

Table 1 Repeatability and reproducibility of impurity peak areas from HS-SPME extracts of homogenized MDMA analyzed over a four day period

Impurity (retention time in minutes)	Relative standard deviation (%) of impurity peak area			
	Day 1	Day 2	Day 3	Day 4*
Benzeneacetamide (8.67)	6.7	12.7	7.1	5.5
Methamphetamine (9.92)	17.0	7.0	7.5	7.3
Piperonal (12.40)	9.1	11.1	7.5	2.5
Ephedrine (13.05)	32.7	16.2	12.7	11.7
4-methylenedioxy-dimethylbenzylamine (13.18)	12.3	5.4	1.8	8.0
MDP-2-P (14.80)	31.9	26.9	20.8	14.3
MDP-2-propanol (15.02)	40.1	18.4	29.5	11.0
MDEA (16.79)	14.8	3.1	13.3	58.3
N,N-diethylamphetamine (17.49)	39.7	54.4	8.0	22.8
Salicylic acid (19.30)	28.7	3.6	16.6	35.7

*On days 1 & 4, n = 4; day 2, n = 3; day 3, n = 5

The initial RSD values are encouraging, although significant improvement in the repeatability and reproducibility of the HS-SPME procedure is necessary. For the early eluting impurities (retention times less than 14 minutes), repeatability and reproducibility were acceptable, with RSDs typically less than 15%. The poor repeatability and reproducibility of the later eluting impurities (MDP-2-P, MDP-2-propanol, MDEA, N,N-diethylamphetamine, and salicylic acid) must be investigated further.

At this stage, the contribution of the GC method (rather than the HS-SPME procedure) to the poor reproducibility and repeatability is unknown. In some cases, MDP-2-P and MDP-2-propanol were not well-resolved, while for the latter three impurities, broad peak shapes contributed to the high RSD values. Unfortunately, due to time restrictions of this research, a complete optimization of the GC temperature program was not possible. Further optimization of the GC temperature program is warranted to (i) improve resolution between MDP-2-P and MDP-2-propanol and (ii) improve peak shape of MDEA, N,N-diethylamphetamine, and salicylic acid, potentially by increasing the ramp rate in the latter part of the temperature program to minimize

band broadening. Standard mixtures of these impurities should be analyzed to optimize the GC temperature program and hence, improve precision of the instrumental method. Once complete, the reproducibility and repeatability of the HS-SPME method can be more accurately assessed in a similar manner as described above. Further optimization of the HS-SPME procedure may also include the investigation of alternate buffers and fiber coatings for more selective extraction of impurities.

The repeatability and reproducibility data shown in Table 1 are based on peak areas of ten impurities. However, it may be more useful to compare the pattern of impurities (*i.e.* the full chromatogram) rather than selected impurities. Principal components analysis (PCA) is a multivariate statistical procedure used to identify natural clusters in the data set. In this case, chromatograms that are more similar will be clustered together while dissimilar chromatograms will be grouped differently in the sample space. Since the chromatograms in the data set are from replicate extractions, close clustering is expected.

Prior to PCA, chromatograms were retention time aligned, using an algorithm published in the literature[16]. Aligning the chromatograms in this way eliminates slight shifts in retention time as a source of variation in the data. The aligned chromatograms were normalized, mean-centered, and then subjected to PCA using commercially available software (Matlab version 7.4, The Mathworks, Natick MA). The scores plot is shown in Figure 5. The first principal component (PC1) accounts for 28.6% of variance among the replicates while the second PC accounts for a further 24.2% of variance. The scores plot clearly shows a main cluster of replicate impurity profiles with 4 replicates as outliers, isolated from the main cluster. Interestingly, three of the outliers were analyzed on the same day. It should be noted, however, that the sample set is small; with the inclusion of additional samples and the resulting increase in scale, clusters should appear tighter.

Pearson product moment correlation (PPMC) coefficients were also calculated for all pairwise combinations of impurity profiles (full chromatogram) using commercially available software (SAS version 9.1, SAS Institute Inc., Cary NC). Computing the PPMC coefficient between two chromatograms generates a single number (r) that ranges from -1 to 1, allowing a simple assessment of the degree of correlation. PPMC values of 1 indicate identical samples, while values of 0 indicate no correlation between samples. For those replicates that were considered outliers in the PCA scores plot, PPMC coefficients were generally in the range 0.5-0.7, indicating poor correlation. PPMC coefficients for the remaining replicates were generally greater than 0.8, indicating correlation and explaining the PCA clustering of the majority of replicates.

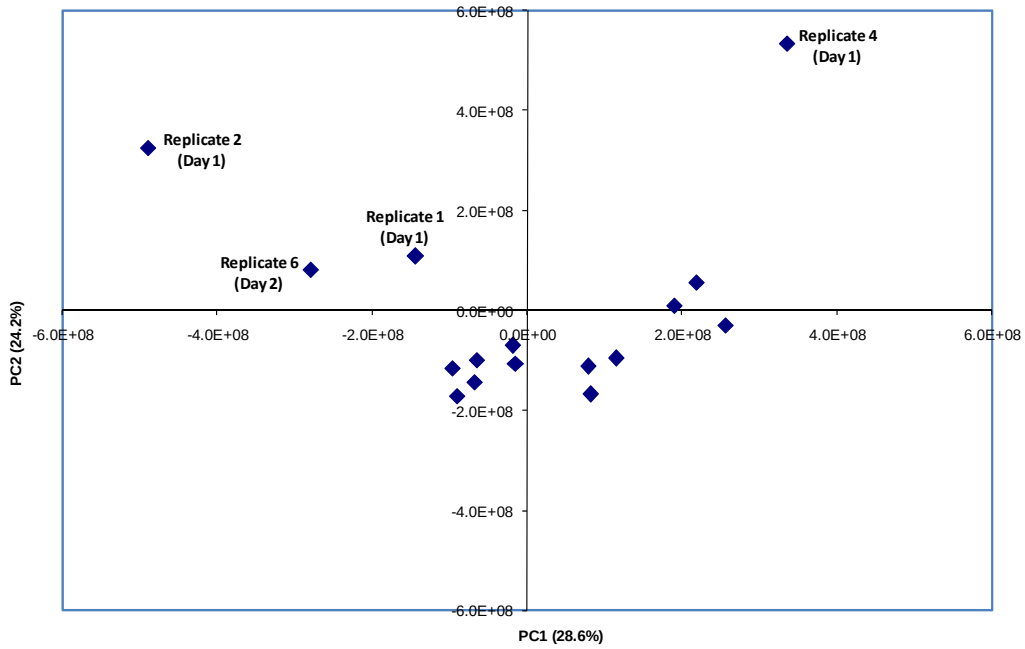
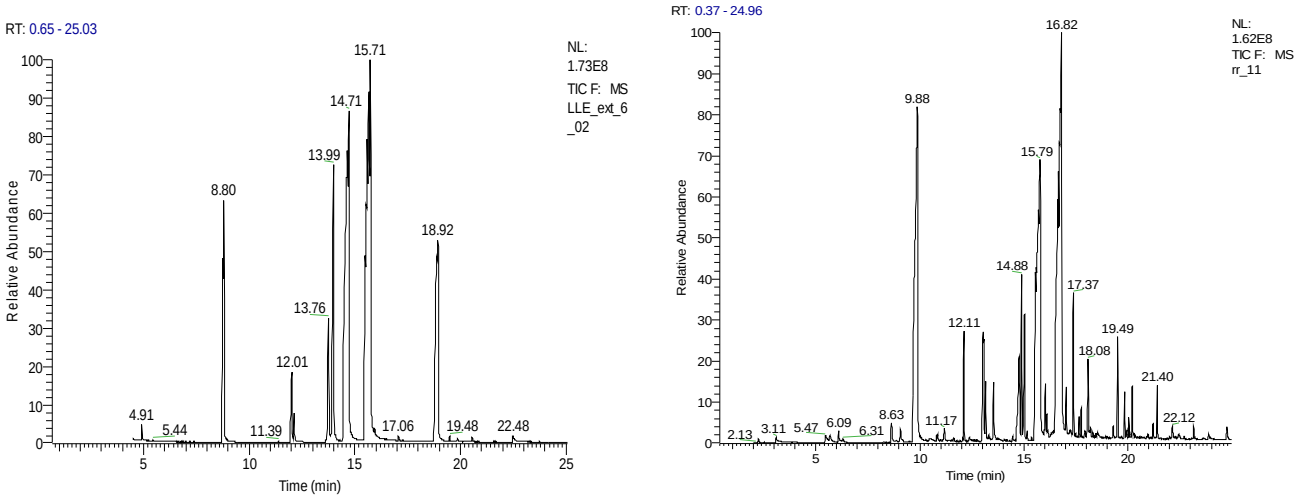


Figure 5 PCA scores plot for replicates analyzed over a four day period

While the application of PCA and PPMC coefficients is limited in the present work due to the small sample population available, the potential of these mathematical procedures for impurity profiling has been demonstrated. PCA provides a procedure by which a single impurity profile can be compared with a large population (*e.g.* a database) while PPMC allows a pairwise comparison of profiles, thus requiring only two samples. Ideally, tablets from the same exhibit would be expected to cluster closely in the PCA scores plots and have high PPMC coefficients. Tablets from different exhibits would be expected to be clearly separated in the PCA scores plot, which would be reflected by the PPMC coefficients. However, clandestine synthesis and tablet production methods may preclude such distinct clusters and this aspect will be further investigated in our future research.

Comparison of liquid-liquid extraction with HS-SPME from aqueous buffer solution of MDMA

Impurity profiles obtained from liquid-liquid extractions of the homogenized MDMA were dominated by MDP-2-propanol (retention time 13.76 minutes), MDMA (14.71 minutes), and MDEA (15.71 minutes) (Figure 6). However, increasing the split ratio is not conducive since impurities present at trace levels are not observed. Caffeine (18.92 minutes) and 3,4-methylenedioxy-N,N-dimethylbenzylamine (12.12 minutes) were also present in the liquid-liquid extracts, similar to the HS-SPME extracts. However, each impurity was present at levels just above background, making an assessment of precision and repeatability difficult.



(a) Liquid-liquid extraction

(b) HS-SPME extraction from carbonate buffer solution

Figure 6 Comparison of liquid-liquid extraction and HS-SPME procedures for extraction of impurities from a homogenized batch of MDMA tablets

The large, broad peaks attributed to MDP-2-propanol, MDMA, and MDEA in the liquid-liquid extraction impurity profiles were potentially masking the trace impurities present in the homogenized batch (*e.g.* MDP-2-P). Since extraction is mainly based on solubility, rather than volatility and polarity as is the case for HS-SPME, the advantage of HS-SPME in suppressing extraction of the controlled substance was apparent.

Impurity profiles obtained following liquid-liquid extraction of the homogenized batch were less informative than the HS-SPME profiles. In general, only 12 impurities were extracted using the liquid-liquid procedure, compared to 16-22 impurities using the HS-SPME procedures, depending on extraction time and extraction temperature.

MDP-2-P was not observed in the liquid-liquid extracts; however, this impurity may be masked by the broad MDP-2-propanol peak. Additionally, impurities such as benzeneacetamide, benzophenone, safrole, piperonal, N-methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene, previously identified in the HS-SPME profiles, were not present in the impurity profiles obtained by liquid-liquid extraction.

Analysis of multiple MDMA exhibits using HS-SPME

The optimized HS-SPME procedure for extraction of impurities from an aqueous buffer solution of MDMA was then applied to obtain impurity profiles of MDMA tablets from a total of five different exhibits. Physical characteristics of tablets from each exhibit are detailed in Table 2.

Due to a restricted supply of tablets, one tablet from each exhibit was homogenized and three 50 mg aliquots from each were weighed into separate vials, allowing triplicate extractions of each tablet. Carbonate buffer (5 mL, pH 10, 0.05 M) was added to the vial along with a magnetic

stir bar and the vial was pre-heated in a water bath for 10 minutes with stirring at 60 °C. A DVB/CAR/PDMS fiber was then exposed to the headspace for 30 minutes and subsequently analyzed by GC-MS. Representative impurity profiles from each exhibit are shown in Figures 7-11.

Table 2 Physical attributes of MDMA tablets from different exhibits*

Exhibit Identifier	Color	Logo	Width /mm	Height /mm	Edges
CJ_FS_01	Dark rose	Baseball	8	4	Beveled (both sides)
CJ_FS_02	Dark orange	Omega	8	4	Beveled (logo side only)
CJ_FS_03	Purple/pink	Spade	8	4	Not beveled
CJ_FS_05	Blue	Omega	8	4	Beveled (both sides)
MSU_900_01	Pale pink/dark pink mottled	Lacoste (crocodile)	8	4	Beveled (logo side only)

* All exhibits obtained from Michigan State Police Forensic Science Division

As expected, there were similarities in impurities present in the extracts of the five different exhibits, but there were sufficient differences to allow distinction among the five. All exhibits contained both piperonal and MDP-2-P. The latter is a common precursor for MDMA synthesis while the former is used as a precursor to synthesize MDP-2-P, which is now a Drug Enforcement Administration List I chemical.

The alcohol MDP-2-propanol is formed during the reductive amination synthesis of MDMA, which commonly uses MDP-2-P and methylamine as the starting reagents. An imine intermediate is formed that is subsequently reduced to yield the final MDMA product. During reduction of the imine a side reaction can occur—reduction of MDP-2-P to the corresponding alcohol, MDP-2-propanol. In order to minimize this side reaction and hence, increase the yield of MDMA, $NaBH_4$ is used as the reducing agent and the reaction vessel is cooled to very low temperatures during the reaction, known as the "cold method". As a result, the reducing agent is more selective, yielding less alcohol. Interestingly, MDP-2-propanol was not present in exhibit CJ_FS_03, which may indicate that the cold method was used during the synthesis of MDMA in this exhibit.

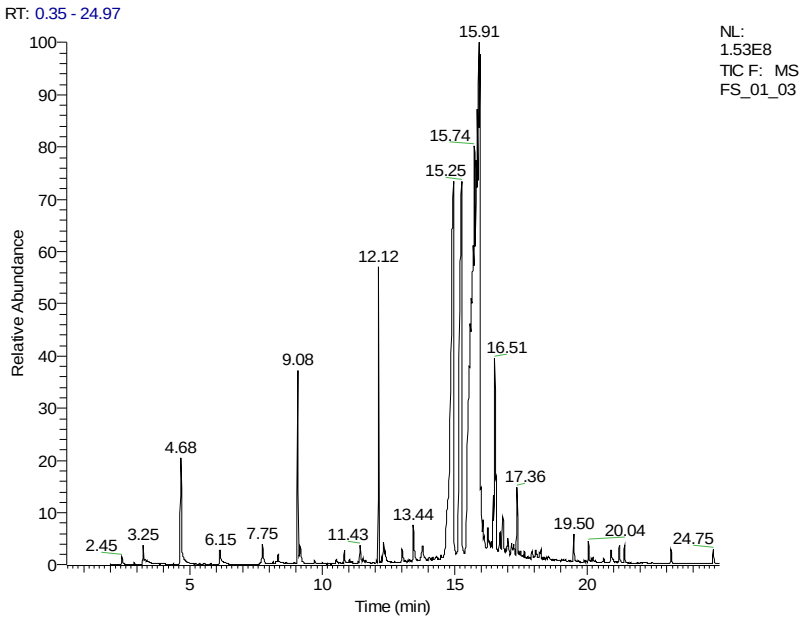


Figure 7 Exemplar impurity profile from exhibit CJ_FS_01

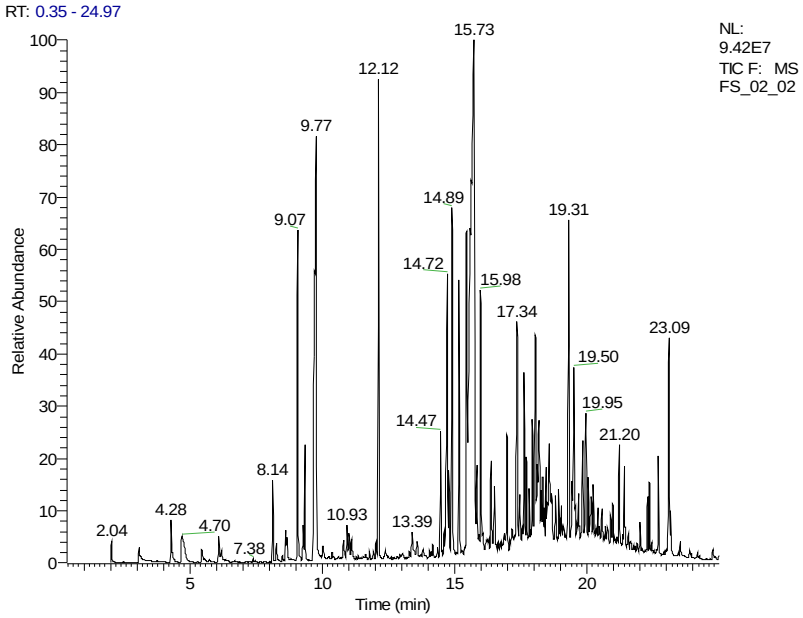


Figure 8 Exemplar impurity profile from exhibit CJ_FS_02

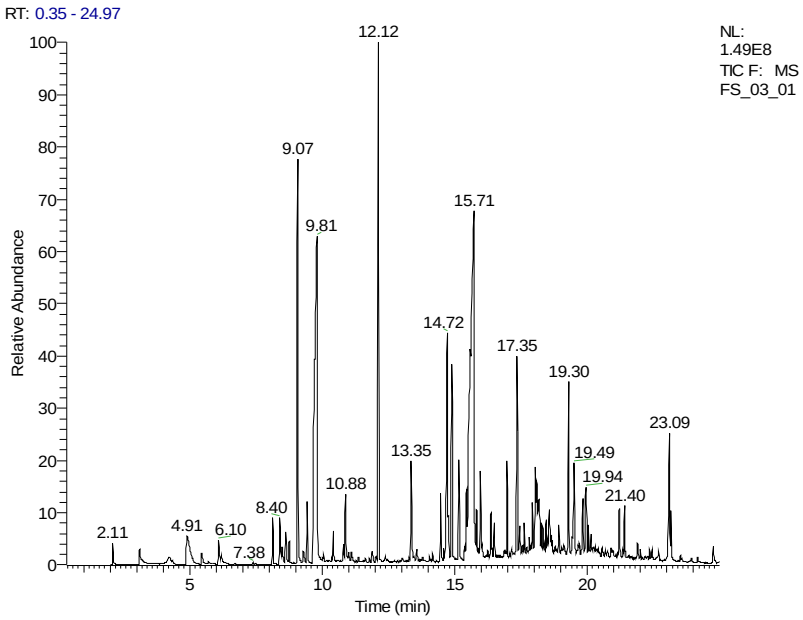


Figure 9 Exemplar impurity profile from exhibit CJ_FS_03

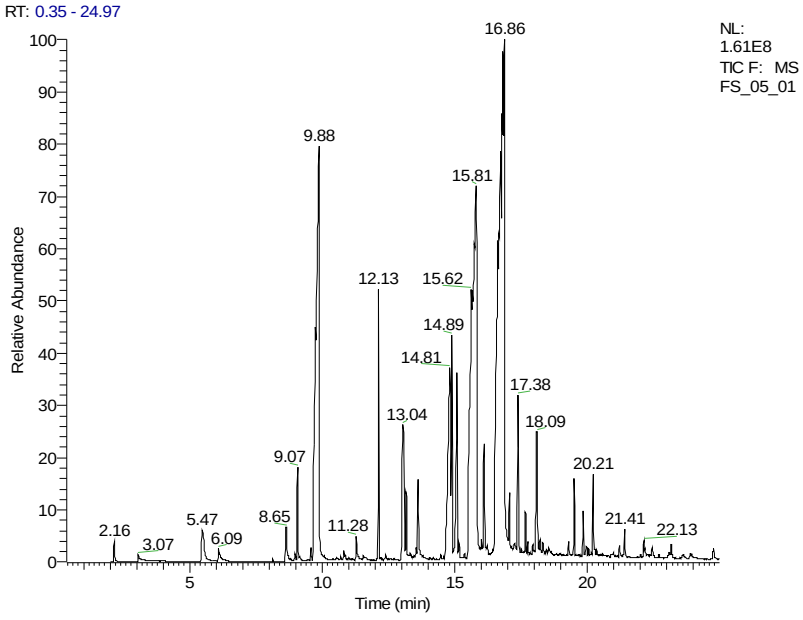


Figure 10 Exemplar impurity profile from exhibit CJ_FS_05

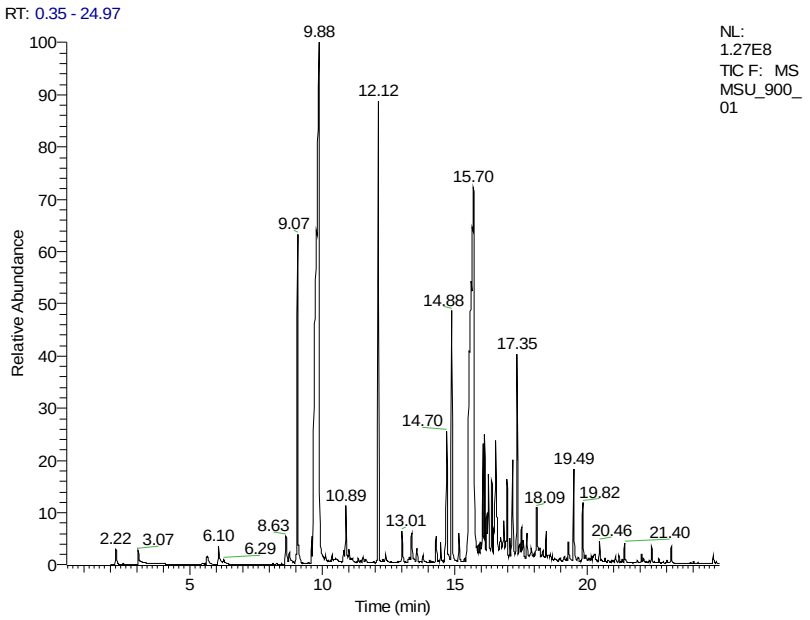


Figure 11 Exemplar impurity profile from exhibit MSU_900_01

Caffeine and methamphetamine were observed in all exhibits with the exception of CJ_FS_01. Due to the relatively high levels present, methamphetamine is more likely added to the MDMA rather than a by-product of a side reaction during MDMA synthesis. Caffeine is also commonly observed as a cutting agent in amphetamine-type drugs, enhancing the stimulant effects.

The absence of caffeine and methamphetamine in CJ_FS_01 served to differentiate this exhibit from the remaining four. N-formyl amphetamine was only observed in exhibit MSU_900_01, while 3,4-methylenedioxy-N,N-dimethylbenzylamine was only observed in exhibit CJ_FS_05. Exhibits CJ_FS_02 and CJ_FS_03 both contained 1,2-dimethyl-3-phenylaziridine, which was not observed in the other three exhibits. However, CJ_FS_03 could be distinguished from CJ_FS_02 based on the presence of N-methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene. Hence, all five exhibits were differentiated based on the impurity profiles obtained using an HS-SPME procedure to extract impurities from an aqueous buffer solution of MDMA.

Dissemination Discussion

This work was presented as a poster at the Fall Meeting of the Midwestern Association of Forensic Scientists in Traverse City, MI (September 2007). An abstract has also been accepted for a poster presentation at the Annual Meeting of the American Academy of Forensic Sciences in Washington DC (February 2008).

Discussion of Problems that have Arisen

Michigan State University Contracts and Grants Administration was delayed in signing the sub-contract agreement with Iowa State University due to conflicts in the language of the agreement. These conflicts were resolved but the sub-contract was only signed on July 12th, 2007. The project was able to start in early May 2007 using supplies and consumables currently available in our laboratory. However, without having a signed agreement, no account number was available and purchasing further materials, supplies, or equipment was not possible, which delayed the progress of the project.

In proposing this research, the intention was to optimize a HS-SPME procedure for organic impurity profiling and then apply the optimized procedure for the extraction of impurities from MDMA tablets from different exhibits. A request was submitted to the Michigan State Police Forensic Science Division for more tablets on August 10th, 2007. On August 20th, 2007, we were notified of the availability of one MDMA exhibit. However, it was not possible to transfer this exhibit immediately and we did not take receipt of the exhibit until September 21st, 2007.

Due to the grant ending September 30th, 2007, we were only able to analyze one tablet in triplicate from this exhibit, along with one tablet from each of our other exhibits. In order to apply chemometric procedures for association and discrimination among tablets from different exhibits, a larger sample population is necessary. Such procedures could not be attempted based on the sample population available in this study. However, this research is an on-going project in our laboratory and work will continue, analyzing larger sample populations to allow more investigation into the statistical and chemometric procedures.

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