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PAPER

CRIMINALISTICS

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Development of Microwave-Assisted Extraction Procedure for Organic Impurity Profiling of Seized 3,4-Methylenedioxymethamphetamine (MDMA)*,†

ABSTRACT: Organic impurity profiling of seized 3,4-methylenedioxymethamphetamine (MDMA) tablets aims to link tablets to common production sources. Conventionally, organic impurities are extracted from tablets using a liquid–liquid extraction (LLE) procedure prior to analysis by gas chromatography–mass spectrometry (GC-MS). In this research, the development of an alternative microwave-assisted extraction/headspace solid-phase microextraction (MAE/HS-SPME) procedure is described. The optimal procedure used phosphate buffer (1 M, pH 8), with an HS-SPME extraction temperature of 70°C for 40 min, using a divinylbenzene/Carboxen™/polydimethylsiloxane (DVB/CAR/PDMS) fiber. Impurities were extracted from seized MDMA exhibits using the MAE/HS-SPME procedure, as well as HS-SPME alone, and a conventional LLE procedure. The HS-SPME procedure was deemed to be the most practical because of the affordability and need for less analyst involvement. Although the LLE was limited in the number of impurities extracted, the procedure is still useful for the extraction of less volatile impurities that are not extracted by HS-SPME.

KEYWORDS: forensic science, 3,4-methylenedioxymethamphetamine, microwave-assisted extraction, headspace solid-phase microextraction, gas chromatography–mass spectrometry, impurity profiling

Owing to their clandestine production, 3,4-methylenedioxyamphetamine (MDMA) tablets often contain impurities, some of which are considered to be characteristic of the method used to synthesize the controlled substance (1–3). Subsequent analysis of the impurities generates an impurity profile of the tablet, which can be used for drug intelligence purposes. Profiles with similar impurities may indicate the same synthesis method, and profiles with similar levels of the same impurities may indicate a common production source. This information can be used by law enforcement agencies to link tablets seized at different times and in different locations, ultimately identifying dealer–user networks and exposing drug-trafficking rings (4,5).

Conventionally, organic impurities are extracted from tablets using liquid–liquid extraction (LLE) procedures, and the extract is analyzed by gas chromatography–mass spectrometry (GC-MS) to generate the impurity profile. The success of LLE in this capacity is well documented in the literature (3,6–8). Weyermann et al. (8) demonstrated the potential of organic impurity profiling, using a standardized method that included a LLE procedure to extract the impurities from MDMA samples. Using multivariate statistical procedures, discrimination among the MDMA samples was possible based on eight organic impurities.

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Despite the noted success of this type of LLE in profiling applications, the procedure does suffer disadvantages. The reported procedures typically use 100–200 mg of tablet, the upper end of which is approximately equivalent to one full tablet. The use of organic solvents during the extraction also requires specialized disposal procedures (3,6,9). Additionally, MDMA, which is present in the tablet at relatively high concentrations with respect to the organic impurities, is also extracted and can dominate the resulting impurity profile, masking the presence of trace-level impurities. As a result, the most discriminatory information may be lost.

Because of these drawbacks, alternative extraction procedures must be pursued, aiming to improve the extraction efficiency of impurities from illicit tablets. Microwave-assisted extraction (MAE) is a relatively recent extraction procedure that has become prominent in a variety of research fields (10–19). In MAE, an extraction solvent is added to the sample in a closed vessel and the mixture is then exposed to microwave irradiation. The solvent is heated above its atmospheric boiling point, not only reducing the solvent viscosity but also increasing the solubility of the target compound in the solvent. The applied microwave field is strictly controlled to ensure homogeneous irradiation. Samples are therefore heated at the same rate, and the extraction temperature for each sample is uniform, ensuring high precision in the extraction.

Although MAE offers highly efficient extractions, all compounds in the sample that are soluble in the extraction solvent are extracted. In the case of illicit MDMA tablets, the controlled substance (i.e., MDMA) will therefore be extracted along with trace-level impurities, as well as other adulterants and diluents. The presence of MDMA in the extract is not desirable as the MDMA peak

tends to dominate the impurity profile to the detriment of trace-level impurities. Thus, solid-phase microextraction is often used as an intermediate step after MAE but before GC-MS analysis of the extract (10–13,15,16,20).

Solid-phase microextraction (SPME) involves exposing a polymeric fiber to the sample, by one of two common sampling modes (21). In direct immersion SPME, the fiber is placed in a liquid sample, while in headspace solid-phase microextraction (HS-SPME), the fiber is exposed to the headspace of a liquid or solid sample. Impurities absorb and/or adsorb to the fiber and are preconcentrated on the fiber during the extraction procedure. The preconcentration ability of the fiber is particularly advantageous in the analysis of trace impurities because of the increased sensitivity afforded. The fiber is then removed from the sample vial and inserted into the heated inlet of the GC, where impurities are desorbed directly from the fiber and analyzed. With the correct choice of polymeric coating to selectively extract impurities from the MAE extract of an illicit MDMA tablet, SPME has the potential to enhance the selectivity of analysis.

The success of SPME for impurity profiling is documented in the literature, primarily for methamphetamine (22–24), with few studies using SPME to extract impurities from MDMA (25–27). Kongshaug et al. (27) were the first to report impurity profiling of MDMA using HS-SPME. Samples of MDMA were also extracted using a LLE procedure with ethyl acetate for comparison. For the HS-SPME procedure, the relative standard deviation (RSD) of impurities identified ranged from 2.2% to 12.6% for replicate analysis ($n = 6$), which was comparable to the RSDs calculated for the LLE procedure (2.5–16.1%).

Bonadio et al. (26) optimized a HS-SPME procedure for the extraction of impurities from a ground MDMA tablet. In a later study by the same authors, the previously developed HS-SPME method was compared with a LLE procedure for profiling 62 different MDMA exhibits (25). Both extraction procedures proved successful in associating samples originating from the same batch while distinguishing samples from different batches. However, the HS-SPME procedure offered the additional advantages of minimal sample preparation and did not involve the use of any solvents.

The combination of MAE with SPME has not yet been reported for impurity profiling purposes although the two procedures have been used in tandem for a multitude of applications (10–13,15–17,19), including the extraction of cocaine from coca leaves (20). Hence, the objective of this research was to investigate the potential of microwave-assisted extraction/headspace solid-phase microextraction (MAE/HS-SPME) for the extraction of organic impurities from seized MDMA. First, the MAE/HS-SPME procedure was developed using a simulated sample of MDMA. The pH and concentration of the microwave extraction buffer as well as the ramp time, extraction time, and extraction temperature were optimized for the MAE procedure. Extraction time and extraction temperature were then optimized for the HS-SPME procedure. Finally, the developed MAE/HS-SPME procedure was compared with a HS-SPME procedure as well as to a conventional LLE procedure for the extraction of organic impurities from seized MDMA tablets.

Materials and Methods

Simulated MDMA Sample

A simulated MDMA sample of known composition was prepared and used for the optimization of the MAE procedure. The simulated sample consisted of benzylamine, phenethylamine, methamphetamine, ephedrine, and caffeine.

Benzylamine and phenethylamine were included because of their structural similarity to methamphetamine, MDMA, and impurities commonly observed in MDMA tablets. Methamphetamine was included as MDMA tablets can contain methamphetamine as a second controlled substance. Ephedrine was also included as this is a common starting material for methamphetamine synthesis (28). Finally, caffeine was included in the simulated tablet as this is often used as an adulterant in MDMA tablets.

Benzylamine hydrochloride (0.5–2% of sample), 2-phenethylamine hydrochloride (0.5–2% of sample), methamphetamine hydrochloride (0.5–2% of sample), and ephedrine (0.5–2% of sample) were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Caffeine (92–98% of sample) was purchased from Eastman (Rochester, NY) and used as received. All components were combined in the appropriate proportions and homogenized using a mortar and pestle. No MDMA was included in the simulated sample as the aim was to extract impurities, not MDMA.

MDMA Exhibits

Three exhibits of seized MDMA tablets were received from the Michigan State Police Forensic Science Division. In this research, an exhibit is defined as a set of tablets with similar physical properties, which was seized at the same time and from the same location. Table 1 summarizes the physical properties of the MDMA exhibits used in this work. Several tablets from each exhibit were homogenized using a mortar and pestle prior to extraction.

Optimization of MAE Buffer

Preliminary work conducted in our laboratory investigated the effect of different buffers (acetate, phosphate, and tris) on the microwave extraction procedure. From these studies, phosphate buffer was determined to be the most promising in terms of the number of impurities extracted and was the focus of further optimization in this research.

Phosphate buffers at concentrations of 1, 0.5, and 0.1 M were prepared using potassium phosphate-monobasic (KH_2PO_4; Mallinckrodt, Paris, KY) and sodium phosphate-dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$; Jade Scientific, Canton, MI). For each concentration, buffers were prepared at three different pH values (pH 6, 7,

TABLE 1—*Physical characteristics of MDMA exhibits.*

Exhibit	Total Number of Tablets	Tablet Color	Tablet Logo	Tablet Shape	Average Diameter/mm ($n = 10$)	Average Height/mm ($n = 10$)	Average Mass/g ($n = 10$)
A	100	Pink/green/purple	Alligator	Circular, beveled edge	8.0	5.0	0.2705
B	20	Blue	Omega	Circular, beveled edge	8.0	4.0	0.2423
C	20	Pink	Heart	Circular, beveled edge	8.0	4.8	0.2693

MDMA, 3,4-methylenedioxymethamphetamine.

and 8), using sodium hydroxide (2 M NaOH; Spectrum, New Brunswick, NJ) to adjust the pH.

For every combination of buffer concentration and pH, three extractions were performed. For the extraction, 75 mg of the homogenized simulated sample was placed in a 100-mL Teflon™ microwave vessel (Milestone Inc., Shelton, CT) and 10 mL of the appropriate buffer was added. The vessel was assembled (Fig. 1*A*) according to the manufacturer's instructions and placed in the microwave system (Ethos Labstation; Milestone Inc.). For the buffer optimization study, the following MAE program was used: heat from room temperature to an extraction temperature of 100°C with a ramp time of 15 min and hold at 100°C for an extraction time of 15 min. Following extraction, the vessel was allowed to cool to at least 50°C, before being opened. A 5-mL aliquot of the resulting extract was then transferred to an amber vial (Supelco, Bellefonte, PA) that contained a stir bar.

The extract was then subjected to HS-SPME, using a procedure previously developed in our laboratory. The vial was suspended in a water bath and preheated to 70°C for 5 min, with stirring, before a divinylbenzene/Carboxen™/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Supelco) was exposed to the headspace for 20 min, with stirring. The fiber was retracted and subsequently analyzed by GC-MS.

Following each microwave extraction, procedural blanks (containing buffer only with no sample) were prepared and analyzed as described to assess the cleanliness of the vessels. In initial studies, procedural blanks indicated methamphetamine carryover in the vessels, potentially because of methamphetamine adsorbing to the Teflon™ walls of the vessel. The vessels were subjected to further cleaning as necessary prior to subsequent extractions.

Optimization of MAE Parameters

The microwave ramp time, extraction time, and extraction temperature were optimized using a circumscribed central composite (CCC) experimental design. The CCC design consists of a factorial (or fractional factorial) design with star points and center points. Factorial points account for all interactions of the factors being considered, star points allow an estimate of curvature, and the center points allow an estimation of the overall error. In this study, the CCC design was generated using commercially available software (Statgraphics Centurion, version XV; Statpoint Inc., Herndon, VA), and the maximum and minimum values for each extraction parameter were chosen based on practical and instrumental limitations. The calculated values for the factorial, star, and center points in the CCC design are summarized in Table 2.

Extractions for the microwave optimization study were conducted in 30-mL quartz vessels (Milestone Inc.) that were placed inside the Teflon™ microwave vessel (Fig. 1*B*). In this way, adsorption of the sample to the Teflon™ walls was minimized, resulting in minimal carryover. For the extraction, 50 mg of the homogenized simulated sample was placed in 5.5 mL of the optimal buffer solution in the quartz insert. The insert was capped and placed in the Teflon™ vessel that contained 10 mL of the optimal buffer. The Teflon™ vessel was assembled as described previously and placed in the microwave. Extractions were performed using the combinations of ramp time, extraction time, and extraction temperature specified in by the experimental design.

Following microwave extraction, 5 mL of the extract was transferred to a separate vial and subjected to HS-SPME using the procedure described previously, except with a 40-min extraction time. Extracts were subsequently analyzed by GC-MS. Peak areas for the simulated sample components were integrated, and linear regression procedures were used to generate a mathematical model that optimized the response for each component in the simulated sample. A desirability function was generated for each component (Statgraphics Centurion, version XV; Statpoint Inc.), and the individual functions were combined to generate a global desirability function. The resulting set of parameters theoretically gives the optimal settings for each parameter that allow extraction of all components at acceptable levels, based on the range of extraction parameters studied in the design.

Optimization of HS-SPME Procedure

All HS-SPME extractions were conducted using a DVB/CAR/PDMS fiber (Supelco) that was conditioned daily prior to use following the manufacturer's instructions. After conditioning,

TABLE 2—*Summary of factorial, star, and center points used in circumscribed central composite design.*

Microwave Parameter	Factorial Point (min/max)	Star Point (min/max)	Center Point
Ramp time (min)	10/20	7/23	15
Extraction time (min)	10/20	7/23	15
Extraction temperature (°C)	80/120	67/134	100

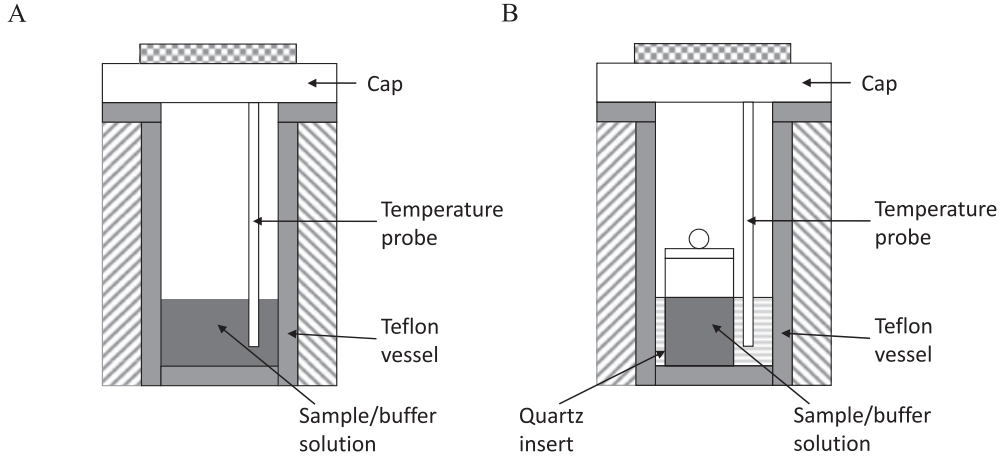

FIG. 1—*Schematic representation of microwave vessel assembly (A) without quartz insert and (B) with quartz insert.*

the fiber was analyzed to ensure cleanliness before conducting any extractions.

A homogenized sample from one MDMA exhibit (exhibit A) was used for the HS-SPME optimization studies. A 50-mg aliquot of the homogenized sample was transferred to a 10-mL amber glass vial (Supelco), and 5 mL of the optimal buffer was added, along with a stir bar. The vial was capped, suspended in a water bath, and preheated for 5 min at the extraction temperature under investigation. The SPME fiber was then exposed to the headspace of the extract for the specified extraction time. Extraction times ranging from 10 to 60 min, in 10 min increments, were investigated, holding the extraction temperature constant at 70°C. Extraction temperatures ranging from 40 to 80°C, in 10°C increments, were investigated, holding extraction time constant at 40 min. Following extraction, the fiber was retracted and subsequently analyzed by GC-MS.

LLE Procedure

The LLE procedure used for comparison was based on a procedure available in the literature (29). Briefly, phosphate buffer (0.33 M, pH 7) was prepared using potassium phosphate-monobasic (Mallinckrodt) and sodium phosphate-dibasic (Jade Scientific), and the pH was adjusted using 2 M sodium hydroxide (Spectrum). A 200-mg aliquot of sample (either homogenized simulated sample or homogenized MDMA exhibit) was transferred to a centrifuge tube, and 4 mL of buffer was added. The sample was vortexed for 10 sec, sonicated for 10 min, and centrifuged for 8 min. A 400-μL aliquot of toluene (Mallinckrodt) with eicosane (Sigma-Aldrich) as an internal standard (0.020 mg eicosane/mL toluene) was added, and the sample was agitated, then sonicated for 10 min and centrifuged for 5 min. The toluene layer was transferred into a GC vial insert (Restek Corp., West Chester, PA), and 1 μL of the toluene extract was analyzed by GC-MS.

GC-MS Analysis

All analyses were conducted using a Focus gas chromatograph coupled to a Polaris Q ion trap mass analyzer (Thermo Fisher Scientific Inc., Waltham, MA). The GC was equipped with a Rxi™-5ms column (30 m × 0.25 mm id, 0.25 μm df; Restek Corp.). For MAE/HS-SPME and HS-SPME extracts, a Merlin Microseal™ septum replacement (Merlin Instrument Company, Half Moon Bay, CA) and a narrow splitless inlet liner (0.8 mm i.d.) were used. The injection port temperature was 260°C, splitless for 1 min, then 100:1 split. Ultrahigh purity helium was used as the carrier gas, at a nominal flow rate of 1 mL/min. The oven was initially held at 60°C for 2 min, then ramped at 8°C/min to a final temperature of 300°C, and held for 15 min. The transfer line was maintained at 275°C, and the mass spectrometer was operated in electron ionization mode (70 eV). Full mass scans were performed, with a mass scan range of m/z 50–500 to avoid detection of low-mass contaminants such as nitrogen, water, and carbon dioxide.

For LLE extracts, the same instrumentation was used except using a low-bleed, high-temperature septum with a conventional split/splitless liner. A 1 μL of extract was injected in split mode (50:1). The injection port temperature, carrier gas flow rate, and oven temperature program were taken directly from the literature, with no modification (29). The injection port was set to 250°C, the carrier gas flow rate was 0.5 mL/min, and the oven temperature program was as follows: 90°C for 1 min, then ramped at 8°C/min to a final temperature of 300°C and held for 10 min. The transfer line was maintained at 275°C, and the mass spectrometer was operated in electron ionization mode (70 eV). Full mass scans were performed, with a mass scan range of m/z 50–500.

Impurities in all extracts were provisionally identified through comparison of the mass spectral data with a library database (NIST Mass Spectral Search Program, version 2.0; National Institute of Standards and Technology, Gaithersburg, MD), as well as comparison with mass spectral data in literature sources (3,30).

Results

Optimization of MAE Buffer

Triplicate MAE/HS-SPME extractions were conducted using the simulated sample and each of the phosphate buffers under consideration. Representative chromatograms of extracts obtained using 1 M phosphate buffer at pH 6 and pH 8 are shown in Fig. 2. At pH 6, only methamphetamine and caffeine were extracted. At pH 7, all components in the simulated sample except phenethylamine were extracted, while at pH 8, all five components were extracted.

Triplicate extractions of the simulated sample were then conducted using phosphate buffer pH 8 at three different concentrations: 1.0, 0.5, and 0.1 M. RSDs of the peak areas for each

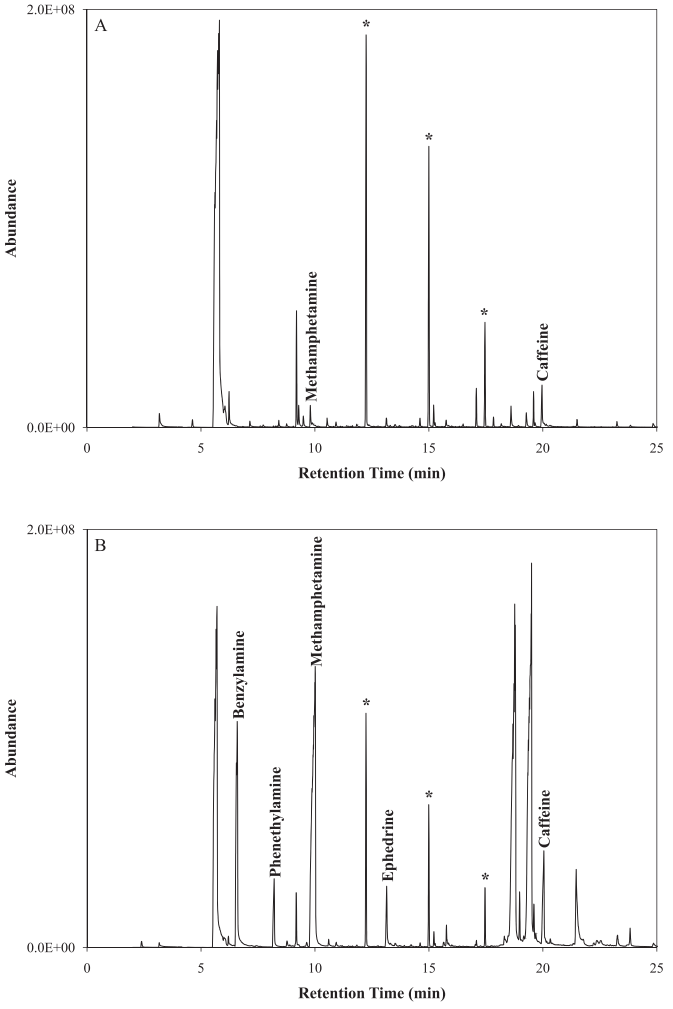

FIG. 2—*Representative chromatograms for simulated 3,4-methylenedioxymethamphetamine sample extracted using 1 M phosphate buffer (A) pH 6 and (B) pH 8. Note that additional peaks observed in each chromatogram are due to siloxanes from the fiber (denoted *) as well as impurities in the buffer solutions.*

TABLE 3—*Relative standard deviation for each buffer concentration based on peak area of simulated sample components.*

Simulated Sample Component	Relative Standard Deviation (%) Based on Peak Area for Extractions Using Phosphate Buffer pH 8		
	1 M	0.5 M	0.1 M
Benzylamine	1.9	20.2	15.6
Phenethylamine	5.6	11.8	27.1
Methamphetamine	1.1	1.4	5.5
Ephedrine	7.9	*	*
Caffeine	5.4	23.7	6.9
Carry-over	No	Yes	Yes

*Peak area not determined because of co-elution with siloxane peak from fiber.

component at each buffer concentration were calculated and are given in Table 3.

At lower buffer concentrations (0.5 and 0.1 M), ephedrine was extracted but co-eluted with siloxane from the fiber, preventing accurate peak area determination, and hence, no RSDs are shown for this compound. For the remaining components, extractions using buffer concentrations of 0.5 and 0.1 M resulted in relatively high RSDs, particularly for the early eluting components. In addition, methamphetamine carryover proved particularly problematic, requiring multiple cleanings of the microwave vessels prior to subsequent extractions.

At a buffer concentration of 1 M, RSDs were <8% for all components, which is considered acceptable for SPME extractions (21). In addition, no carryover was observed between extractions using the higher buffer concentration. As a result, the optimal buffer for the MAE procedure was determined to be a 1 M phosphate buffer, pH 8.

Optimization of MAE Parameters

Each extraction defined in the CCC design (nine in total) was conducted at least in triplicate, with six replicates at the center point conditions (15-min ramp time, 15-min extraction time, 100°C extraction temperature). Although extracted, ephedrine was not included in the data analysis owing to co-elution with siloxane from the SPME fiber.

Following completion of all extractions, component peaks were integrated and linear regression analysis was used to fit a second-order mathematical model to describe each component. Next, the responses (peak areas) of each impurity were optimized individually. Responses for the impurities benzylamine and phenethylamine were maximized as the goal of the design was to maximize the extraction of impurities. Meanwhile, responses for methamphetamine and caffeine were minimized as these compounds are adulterants in MDMA tablets rather than impurities.

Individual desirability functions were generated for each component, and the functions were then combined to generate a global desirability function. In this way, the extraction parameters that maximized extraction of the impurities (benzylamine and phenethylamine) and minimized extraction of the adulterants (methamphetamine and caffeine) were determined. The resulting optimal parameters for the MAE procedure were as follows: ramp time 23 min, extraction time 23 min, and extraction temperature 100°C.

All subsequent MAE/HS-SPME extractions were conducted using the optimal buffer and MAE parameters. That is, 50 mg of sample (simulated or MDMA tablet) was dissolved in 5.5 mL of 1 M pH 8 phosphate buffer and microwave extracted with a ramp time of 23 min, an extraction time of 23 min, and an extraction temperature of 100°C. In order to minimize potential carryover between extractions, all subsequent extractions were conducted in quartz vessels that were placed inside the Teflon™ microwave vessel.

Optimization of HS-SPME Extraction Time and Extraction Temperature Following MAE

For optimization of the HS-SPME procedure, a seized MDMA exhibit (exhibit A) was used, rather than the simulated sample, and no MAE was performed.

Initially, extraction temperature was held constant at 70°C and extraction times from 10 to 60 min, in 10-min increments, were investigated. Representative chromatograms are shown in Fig. 3.

With a 10-min extraction time (Fig. 3*A*), piperonal was not extracted and detected in the exhibit. Piperonal is a starting material in the synthesis of 3,4-methylenedioxyphenyl-2-propanone (MDP2P). Hence, the presence of piperonal gives information concerning the synthesis of MDP2P, which is a common precursor for MDMA. Although the same impurities were extracted with extraction times of 40 and 60 min (Figs. 3*B*,*C*), at the longer extraction time, broadening of the more abundant peaks was observed. This is potentially because of overloading the SPME fiber at the longer extraction times, which is undesirable as broad peaks may mask impurities present at lower concentrations. As a result, the 40-min extraction time was deemed to be optimal, offering a compromise between the number of impurities extracted and chromatographic efficiency.

The HS-SPME extraction time was held constant at 40 min, and extraction temperatures in the range 40–80°C were investigated, in 10°C increments. With an extraction temperature of 40°C, piperonal and diethyl phthalate were not extracted from exhibit A. Diethyl phthalate is a plasticizer that can be added to tablets as a binder during the tabletting process, hence providing information related to the production source.

At the higher extraction temperatures investigated, the same impurities were extracted and detected. However, as before, broadened peaks were observed at the higher extraction temperature, potentially because of overloading the SPME fiber. As broad peaks are undesirable, an extraction temperature of 70°C was deemed to offer the best compromise between the number of impurities extracted and the efficiency of the chromatography.

Thus, for HS-SPME, the optimal extraction parameter was an extraction time of 40 min, with an extraction temperature of 70°C.

Comparison of MAE/HS-SPME, HS-SPME, and LLE Procedures for Organic Impurity Extraction from MDMA

The developed MAE/HS-SPME was then compared with a HS-SPME procedure and a conventional LLE procedure. The HS-SPME involved an extraction time of 40 min, with an extraction temperature of 70°C, while the LLE procedure was taken from the literature (29). Triplicate extractions of the simulated sample were conducted using each of the three extraction procedures, and all extracts were analyzed by GC-MS. Peak areas of components were integrated, and RSDs were calculated to assess the precision of each extraction procedure. A summary of the RSD values is given in Table 4.

Using the LLE procedure, only two of the five simulated sample components were extracted and detected (methamphetamine and caffeine), while using MAE/HS-SPME and HS-SPME, all five components were extracted and detected. The precision was similar

for the two components extracted by all three procedures, in the range 5–10%, which is acceptable for SPME extractions (21).

For MAE/HS-SPME, ephedrine co-eluted with a siloxane peak from the SPME fiber, preventing accurate peak area determination. As a result, no RSD is reported for ephedrine in Table 4. However, this is a limitation of the SPME fiber rather than the extraction procedure, as no co-elution of siloxane and ephedrine was observed in HS-SPME, which used a new fiber of the same type. The precision of the extraction was otherwise similar for MAE/HS-SPME and HS-SPME, with the exception of phenethylamine. In MAE/HS-SPME, the RSD for phenethylamine was *c*. nine times greater than the corresponding RSD for the HS-SPME extraction. The variability in the phenethylamine peak area in the microwave extract may be due to thermal degradation of the molecule at the higher temperatures used during the extraction. For HS-SPME, all RSD values were <7%, indicating acceptable precision in the extraction and analysis procedures.

Each of the three MDMA exhibits (exhibits A, B, and C) was extracted in triplicate using each of the three extraction procedures. Extracts were analyzed using GC-MS to generate impurity profiles for each exhibit, which were subsequently compared. It should be noted that since the SPME extracts and the LLE extracts were analyzed using different GC temperature programs, there are differences in retention time for common impurities.

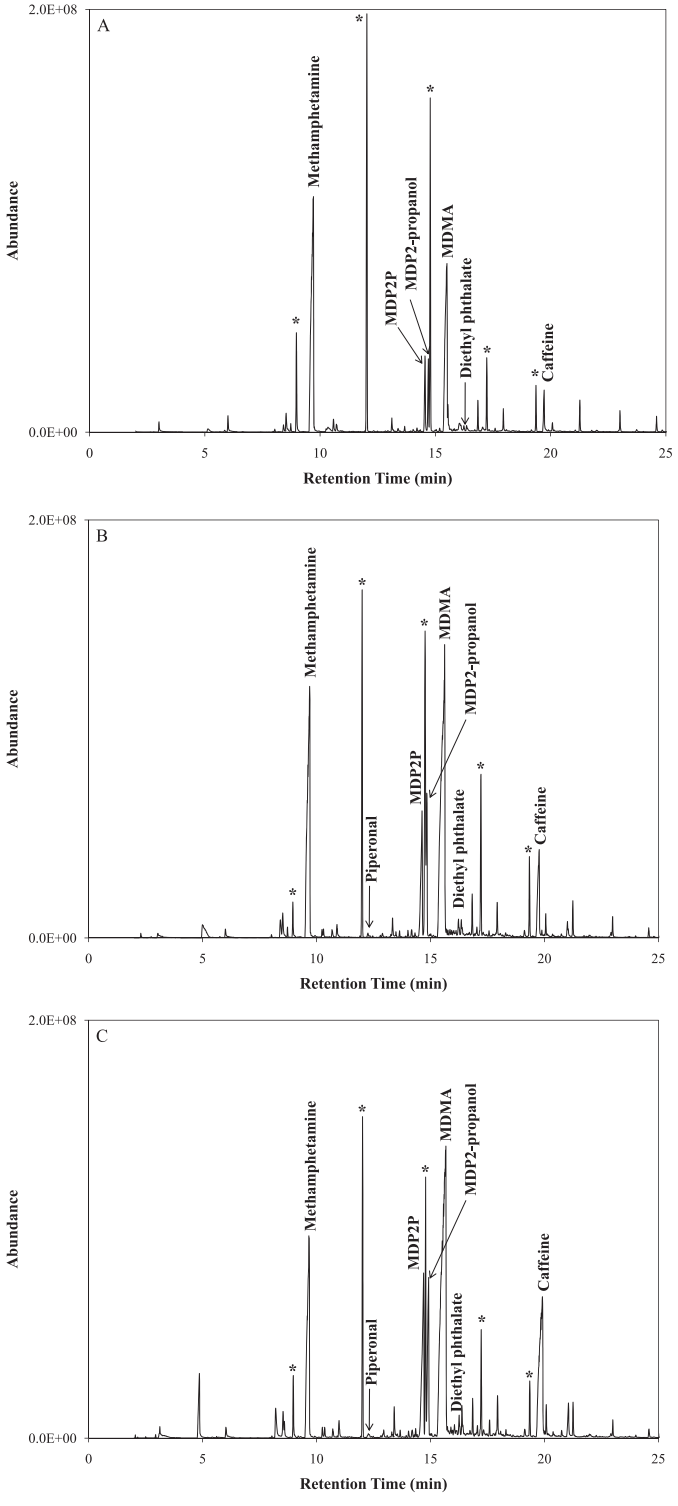

FIG. 3—*Representative chromatograms for headspace solid-phase microextraction (HS-SPME) of exhibit A at an extraction temperature of 70°C and extraction times of (A) 10 min, (B) 40 min, and (C) 60 min. Peaks marked * are siloxane peaks from the SPME fiber.*

Exhibit A

Representative impurity profiles for exhibit A are shown in Fig. 4, while Table 5 lists the number of impurities extracted from this exhibit using each extraction procedure. The impurities MDP2P and MDP2-propanol were extracted and detected using all three procedures. However, the abundance of each impurity was greater using the MAE/HS-SPME and HS-SPME procedures because of the preconcentration ability of the fiber. MDP2P is a precursor for MDMA synthesis, and MDP2-propanol is a by-product of MDMA synthesis *via* reductive amination of MDP2P. Additionally, N-methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl)benzene

TABLE 4—*Comparison of extraction procedure precision based on relative standard deviations of simulated sample component peak areas.*

	Relative Standard Deviation (%) of Integrated Peak Area		
Simulated Sample Component	MAE/HS-SPME (n = 4)	HS-SPME (n = 5)	LLE (n = 3)
Benzylamine	4.61	5.74	Not detected
Phenethylamine	28.32	3.46	Not detected
Methamphetamine	9.81	5.50	9.37
Ephedrine	*	4.88	Not detected
Caffeine	8.73	6.35	7.61

MAE/HS-SPME, microwave-assisted extraction/headspace solid-phase microextraction; LLE, liquid–liquid extraction.

*Peak area not determined because of co-elution with siloxane peak from fiber.

TABLE 5—*Number of impurities extracted from seized MDMA exhibits using each extraction procedure.*

	Number of Impurities Extracted		
Extraction Procedure	Exhibit A	Exhibit B	Exhibit C
MAE/HS-SPME	42	40	50
HS-SPME	46	35	46
LLE	8	23	14

MAE/HS-SPME, microwave-assisted extraction/headspace solid-phase microextraction; LLE, liquid–liquid extraction; MDMA, 3,4-methylenedioxymethamphetamine.

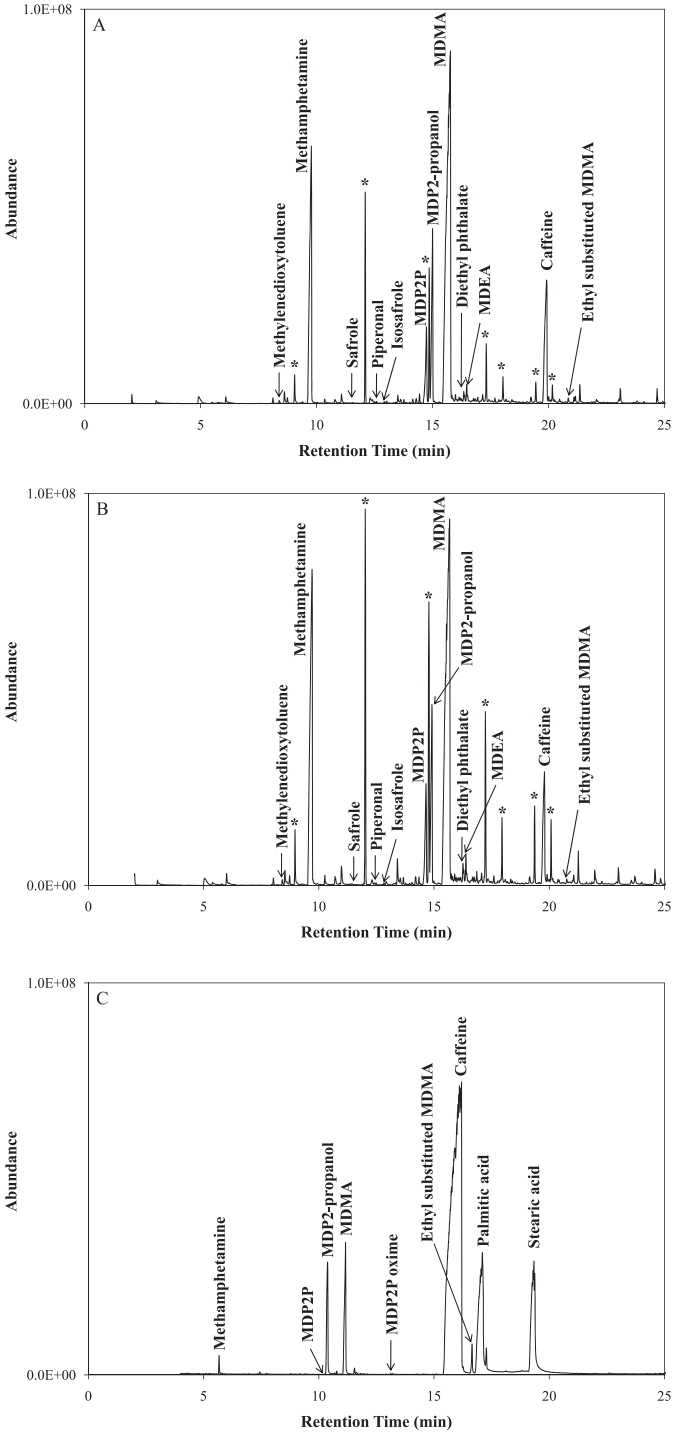

FIG. 4—*Representative impurity profiles obtained for exhibit A extracted using the (A) microwave-assisted extraction/headspace solid-phase microextraction (MAE/HS-SPME) procedure, (B) HS-SPME procedure, and (C) liquid–liquid extraction procedure. Peaks marked * are siloxane peaks from the SPME fiber.*

(ethyl substituted MDMA) was also extracted by all three procedures. Although the origin is unknown, this impurity is similar in structure to N-ethyl-N-methyl(1,2-methylenedioxy)-4-(2-aminopropyl)benzene, which is a by-product of the reductive amination of MDP2P by ethylamine (30).

An impurity, provisionally identified as 3,4-methylenedioxytoluene, was extracted using both MAE/HS-SPME and HS-SPME but was not extracted using LLE. 3,4-methylenedioxytoluene is formed during the synthesis of MDP2P from safrole and hence is potentially useful in determining synthetic route for precursors.

The impurities safrole, piperonal, and isosafrole were only extracted and detected using the MAE/HS-SPME and HS-SPME procedures. These three impurities are important indicators of the method used to synthesize MDP2P. Additionally, 3,4-methylenedioxyethylamphetamine (MDEA) was extracted using MAE/HS-SPME and HS-SPME but not by LLE. The lack of extraction and detection in the LLE extract may be due to the low levels of MDEA in exhibit A and the lack of preconcentration using this type of extraction.

One impurity, 1-(3,4-methylenedioxyphenyl)-2-propanone oxime (MDP2P oxime), was extracted and detected using the LLE procedure but not by the MAE/HS-SPME and HS-SPME procedures. This oxime impurity originates from the synthesis of MDP2P from safrole through the β-nitroisosafrole route (1). The LLE procedure is not limited by volatility as both MAE/HS-SPME and HS-SPME are, and hence, less volatile impurities may be extracted using LLE. As a result, while the impurity profile obtained using the LLE procedure may seem less informative than those obtained using MAE/HS-SPME and HS-SPME, the LLE procedure still has utility in providing additional information regarding the less volatile impurities in the sample.

Exhibit B

Representative impurity profiles obtained using each extraction procedure are shown in Fig. 5, while Table 5 lists the number of impurities extracted from this exhibit using each extraction procedure. As observed for exhibit A, MDP2P and MDP2-propanol were extracted by all three procedures. In addition, in exhibit B, ephedrine and MDEA were also extracted by all three procedures. Ephedrine is a common starting material in the synthesis of methamphetamine (31). The impurities 3,4-methylenedioxytoluene, safrole, piperonal, and N-methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl) benzene (ethyl substituted MDMA) were extracted by both MAE/HS-SPME and HS-SPME. As observed in exhibit A, MDP2P oxime was only extracted using the LLE procedure potentially because of the low volatility of the impurity.

Exhibit C

Representative impurity profiles obtained for exhibit C using each extraction procedure are given in Fig. 6, while Table 5 lists the number of impurities extracted from this exhibit using each extraction procedure. The MAE/HS-SPME procedure extracted six impurities that eluted during the first 10 min of the GC analysis, but these impurities were not present in the HS-SPME extract. The higher temperatures used during the microwave extraction may cause thermal degradation of components that would be of lower molecular weight with lower boiling points and hence more likely to elute earlier in the chromatogram. However, as these impurities have not yet been identified, this hypothesis was not confirmed.

The impurities MDP2P and MDP2-propanol were extracted by all three procedures, albeit at lower abundances in the LLE extract than in the other two extracts, which is potentially because of the lack of preconcentration in LLE. The impurity N-methyl-(1,2-methylenedioxy)-4-(1-ethyl-2-aminopropyl) benzene (ethyl substituted MDMA) was extracted by all three procedures, although at very low abundance.

Safrole, piperonal, isosafrole, and MDEA were extracted by both MAE/HS-SPME and HS-SPME but were not observed in the LLE

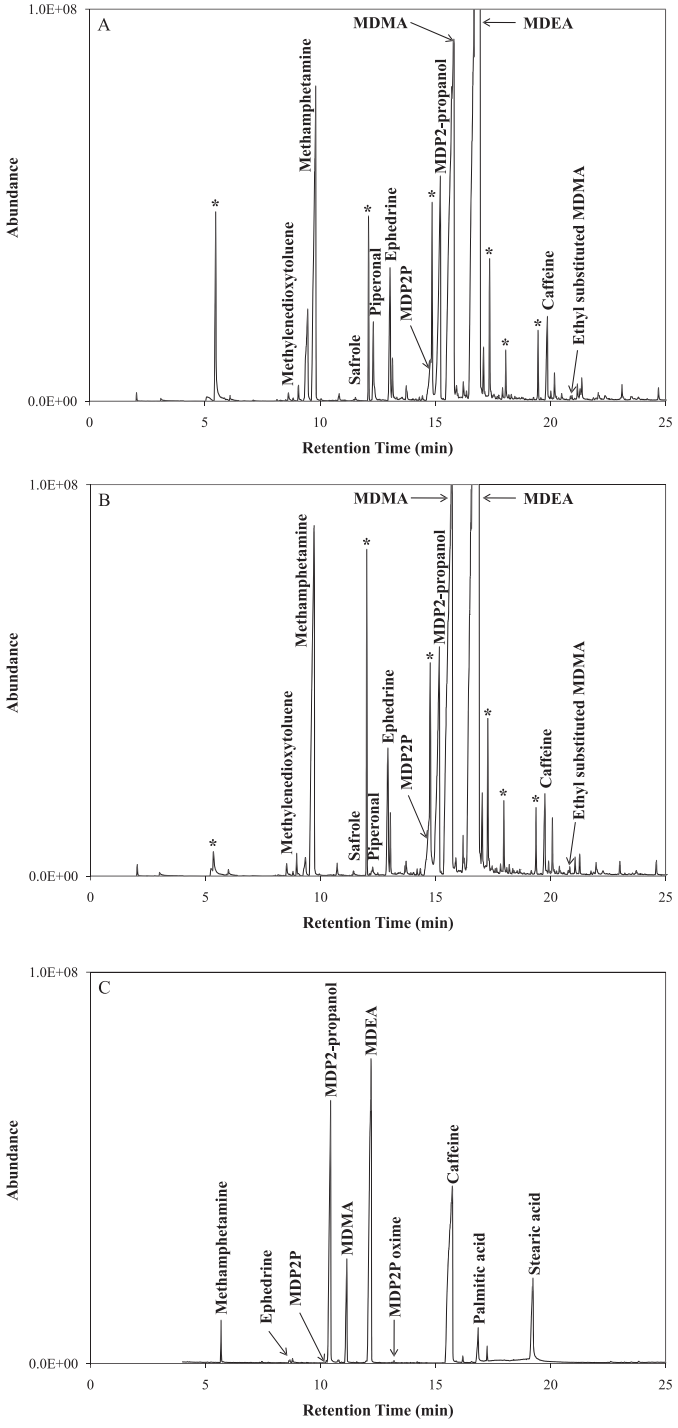

FIG. 5—*Representative impurity profiles obtained for exhibit B extracted using the (A) microwave-assisted extraction/headspace solid-phase microextraction (MAE/HS-SPME) procedure, (B) HS-SPME procedure, and (C) liquid–liquid extraction procedure. Peaks marked * are siloxane peaks from the SPME fiber.*

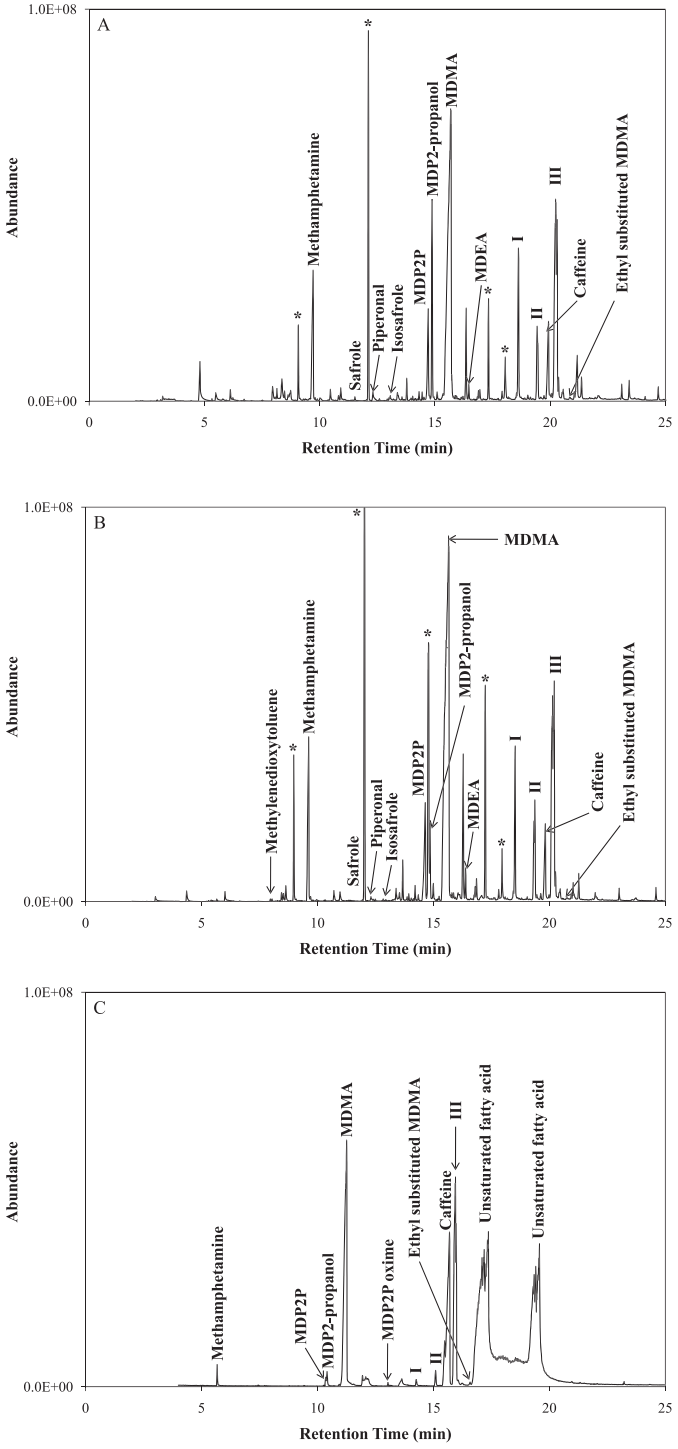

FIG. 6—*Representative impurity profiles obtained for exhibit C extracted using the (A) microwave-assisted extraction/headspace solid-phase microextraction (MAE/HS-SPME) procedure, (B) HS-SPME procedure, and (C) liquid–liquid extraction procedure. Peaks marked I, II, and III are unidentified. Peaks marked * are siloxane peaks from the SPME fiber.*

impurity profiles. Again, this may be due to the low concentration of each impurity in exhibit C as well as the lack of preconcentration in the LLE procedure. As previously observed for exhibits A and B, MDP2P oxime was only extracted by LLE, potentially because of the low volatility of the impurity making it unsuitable for headspace sampling. In addition, as yet unidentified, unsaturated fatty acids were only extracted using the LLE procedure. The poor chromatography observed (Fig. 5*C*) is most likely due to the use of a nonpolar stationary phase in this research (32).

In exhibit C, 3,4-methylenedioxytoluene was only observed in the HS-SPME chromatogram. The impurity may have been present in the MAE/HS-SPME extract, but other unidentified peaks were present in the retention time range, potentially masking this impurity.

Comparison of MDMA Exhibits Extracted Using HS-SPME

Based on the limitations of MAE/HS-SPME and LLE highlighted in the comparison of the MDMA exhibits, the HS-SPME procedure shows the greatest potential for future use in organic impurity profiling applications. For comparison, representative impurity profiles obtained for each exhibit following HS-SPME extraction of organic impurities and subsequent GC-MS analysis are given in Figs 4*B*, 5*B*, and 6*B*, for exhibits A, B, and C, respectively.

The three exhibits can be differentiated based on the organic impurities present. Although MDEA is present in all three exhibits, the abundance of MDEA in exhibit B suggests that MDEA is present intentionally, while the lower levels in exhibits A and C suggest MDEA is present as an impurity. Isosafrole is only present in exhibits A and C, while ephedrine is only present in exhibit B. Exhibits A and C can also be differentiated based on the presence of three (as yet unidentified) impurities that are only present in exhibit C.

Furthermore, identification of the impurities present can indicate the likely method used to synthesize not only the active ingredient in the tablets, MDMA, but also the precursors for MDMA synthesis. All three exhibits contain MDP2P that is the likely precursor for MDMA synthesis. There are two common methods used to synthesize MDP2P using safrole as the precursor, as shown in Fig. 7 (1). The presence of safrole, isosafrole, and piperonal in each exhibit suggests that the route shown in Fig. 7*B* is the more likely method of synthesizing MDP2P. Similarly, while there are numerous methods to synthesize MDMA from MDP2P, reductive amination is more likely due to the presence of MDP2-propanol and ethyl-substituted MDMA. However, such hypotheses require the analysis of a larger number of tablets from each exhibit and thus cannot be confirmed yet.

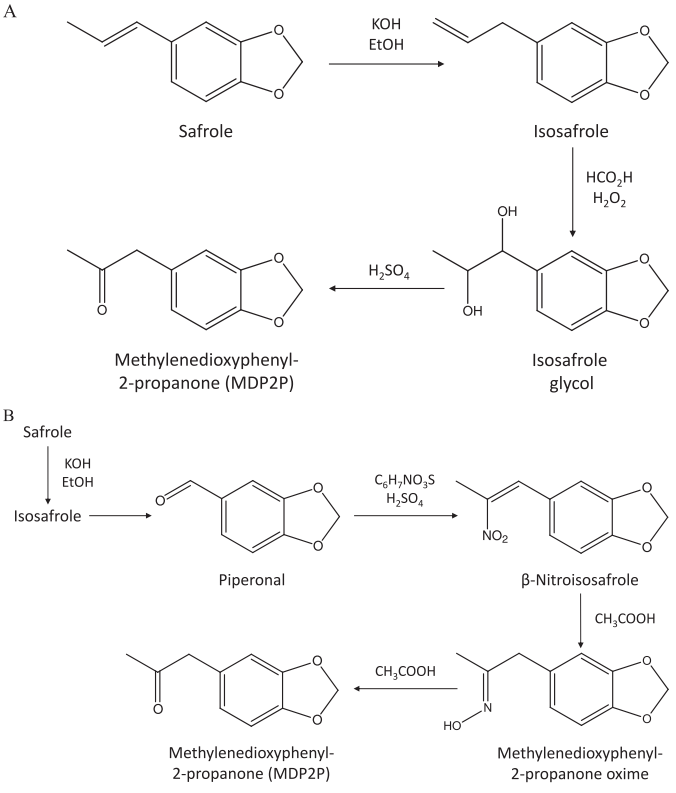

FIG. 7—*3,4-methylenedioxyphenyl-2-propanone synthesis from safrole (A) via the intermediate isosafrole glycol and (B) via the intermediate β-nitroisosafrole.*

Discussion

A MAE/HS-SPME procedure was developed for the extraction of organic impurities from seized MDMA tablets. The developed procedure was then compared with a HS-SPME procedure, as well as with a conventional LLE procedure. While the MAE/HS-SPME procedure generally extracted the greatest number of impurities, the procedure is limited by expensive instrumentation, increased analysis time, and the potential for thermal degradation of the sample. In addition, MAE is limited by the potential for sample carryover in the microwave vessels between extractions. This limitation can be overcome with the use of quartz vessels, although this practice further increases the costs associated with the microwave procedure. While the HS-SPME procedure extracted slightly fewer impurities than the MAE/HS-SPME procedure, HS-SPME was deemed to be the most practical because of the affordability and need for less analyst involvement. Although the LLE was limited in the number of impurities extracted, the procedure is still useful for the extraction of less volatile impurities that are not extracted by HS-SPME.

Using three MDMA exhibits, the potential of HS-SPME in impurity profiling applications was demonstrated. The three exhibits were differentiated based on differences in the impurities present. Furthermore, it was possible to hypothesize the likely methods used to synthesize the precursor, MDP2P, as well as the active ingredient, MDMA, in the tablets.

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