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PAPER

CRIMINALISTICS

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Effect of Extraction Procedure and Gas Chromatography Temperature Program on Discrimination of MDMA Exhibits[†,‡,§]

ABSTRACT: Analysis of impurities in seized MDMA tablets can be used to determine the synthesis method used and to identify links among exhibits. However, no standardized method exists to generate impurity profiles, limiting comparisons among different laboratories. This research investigated the effect of extraction procedure and gas chromatography temperature program on the resulting impurity profiles. Five exhibits were extracted using liquid–liquid extraction (LLE) and headspace solid-phase microextraction (HS-SPME), then analyzed using two different temperature programs. Profiles were statistically assessed using principal components analysis. While LLE was more reproducible, more compounds were extracted using HS-SPME, thus providing more informative chemical profiles. The longer temperature program (53 min vs. 36 min) allowed greater discrimination of exhibits, due to improved precision as a result of an extended hold time (12 min). This research further highlights the need for standardized extraction and analysis procedures to allow comparison of chemical profiles generated in different laboratories.

KEYWORDS: forensic science, chemical profiling, 3,4-methylenedioxymethamphetamine, Liquid–liquid extraction, headspace solid-phase microextraction, gas chromatography–mass spectrometry, principal components analysis

Despite regulation as a Schedule I substance in the Controlled Substances Act, 3,4-methylenedioxymethamphetamine (MDMA) continues to be clandestinely synthesized and abused. In a 2011 survey, 12.0% of eighth graders and 37.1% of 12th graders questioned responded that it was "fairly easy" or "very easy" to obtain MDMA (1). Furthermore, 2.6% and 8.0% of eighth graders and 12th graders, respectively, admitted using MDMA at least once in their lifetime (1).

A variety of methods are used for the clandestine synthesis of MDMA, with the method chosen often dependent on the availability of starting materials and the typical yield of final product obtained. The synthesized MDMA contains impurities and by-products, some of which are specific to the synthesis method (2–4). Chemical profiling aims to identify these compounds to determine the synthesis method, which may be further used to link MDMA tablets to a common production source (2–6).

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To generate chemical profiles, illicit MDMA tablets are typically subjected to a liquid–liquid extraction (LLE) and the extract is analyzed by gas chromatography–mass spectrometry (GC-MS) (2–8). However, a variety of different LLE procedures have been used. For example, diethyl ether (2,5,9), heptane (4), and toluene (7,8) have all been used as the extraction solvent. Headspace solid-phase microextraction (HS-SPME) has also been posed as an alternative extraction procedure for both methamphetamine and MDMA (10–14). But again, the actual extraction parameters vary. For example, in the method reported by Kongshaug et al. (12), tablets were dissolved in an acetate buffer and the SPME fiber was exposed to the headspace for 30 min at 90°C. In contrast, in the procedure optimized by Bonadio et al. (10), samples were simply crushed and the SPME fiber exposed to the headspace for 15 min at 80°C. Due to the differences not only in type of extraction but also extraction parameters, comparison of chemical profiles generated in different laboratories is challenging.

To address this, standardized LLE and HS-SPME procedures have been developed for chemical profiling of MDMA and are reported in the literature (10,15). For LLE, standardization of the procedure was one of the aims of the CHAMP (Collaborative Harmonization of Methods for Profiling Amphetamine Type Stimulants) project that was conducted in Europe and involved six partner laboratories (15). In the developed LLE procedure, MDMA samples were dissolved in a neutral 0.33 M phosphate buffer, filtered through regenerated cellulose membranes to remove fatty acids, and finally extracted with toluene (7). The standardized HS-SPME procedure was developed and optimized by Bonadio et al. (10) specifically for compound extraction from MDMA. The fiber type, sample mass, extraction time, and

extraction temperature were investigated, based on the number and abundance of the compounds extracted. The optimized procedure used a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber and 40 mg of sample, which was extracted at 80°C for 15 min.

Despite the availability of standardized extraction procedures, the comparison of chemical profiles generated by different laboratories remains challenging as different GC temperature programs have been used to analyze the extracts. Programs reported in the literature have included one-, two-, and three-step ramps, with differences in the ramp rate, as well as the initial and final temperatures and hold times (2–5,7,9). Such differences affect retention time of the same compound, preventing direct comparison of profiles generated by different laboratories.

Furthermore, the comparison of chemical profiles based on visual assessment remains subjective. To overcome this, various statistical procedures have also been applied to provide more objective comparison of MDMA profiles (5,8,16). Cheng et al. (5) used hierarchical cluster analysis to demonstrate similarities among 89 MDMA samples based on organic impurity profiles. Samples were extracted by LLE using diethyl ether as the extraction solvent and analyzed by GC-MS. Following cluster analysis, four distinct clusters of samples were obtained, and for samples within two of the clusters, it was possible to determine the likely synthesis method based on the impurities present.

Weyermann et al. (8) conducted a study involving four different laboratories located in different countries. Each laboratory used the LLE procedure developed in the CHAMP project (7) to extract impurities from the same 26 MDMA samples. Correlation methods and principal components analysis (PCA) were used to assess association and discrimination of samples based on 32 common compounds, as well as eight compounds that were most variable in abundance among the samples. All samples were discriminated using all 32 compounds, while 99% of the samples were associated and discriminated appropriately using only the eight selected compounds. The same statistical procedures were then applied to 80 exhibits collected in the respective countries. Links were discovered not only between separate exhibits but also, among the countries, based on consistencies in their profiles and correlation values. This large scale, multilaboratory comparison was possible using the standardized extraction and analysis procedures developed as part of the CHAMP project.

Bonadio et al. (16) extracted impurities from 62 MDMA samples using both LLE and HS-SPME to investigate the effect of extraction procedure on the resulting chemical profile. All extracts were analyzed by GC-MS albeit with slight differences in temperature program: for LLE extracts, an one-step ramp from 90°C to 310°C was used while for HS-SPME extracts, a three-step ramp from 60°C to 260°C was used. In total, 46 compounds were extracted using LLE and 31 using HS-SPME. The lower number of compounds extracted using SPME was not unexpected as this procedure is limited by the volatility of the compounds present. Of the total number of compounds extracted by LLE, 32 were extracted reproducibly, with eight of these deemed discriminating compounds. For HS-SPME, 31 compounds were extracted reproducibly with 10 compounds defined as discriminating. The ability to associate and discriminate three different MDMA samples based on the chemical profiles generated using LLE and HS-SPME was then investigated statistically. Using correlation coefficients, similar association of the samples was achieved irrespective of the extraction procedure used.

The aforementioned studies have highlighted the utility of both LLE and HS-SPME for chemical profiling purposes, as well as the use of statistical procedures (e.g., cluster analysis, correlation coefficients, and PCA) for the comparison of profiles. Although LLE procedures and HS-SPME procedures have been optimized specifically for MDMA (10,15), these procedures are not necessarily applied routinely. Additionally, while GC-MS is the analytical method of choice in these studies, the profiles generated are not readily comparable as different GC parameters and oven temperature programs were used. Such differences can affect the compounds detected, which, in turn, may affect the association and discrimination of exhibits when the resulting profiles are subjected to statistical procedures.

The research reported herein investigates the effect of both extraction procedure and GC temperature program on chemical profiles obtained from seized MDMA exhibits. Five different exhibits were extracted using previously optimized LLE and HS-SPME procedures then analyzed in replicate using two different temperature programs, ranging from 36 min to 53 min in length (2,10). The generated chemical profiles were subjected to PCA to assess the effect of each extraction/temperature program combination on the association of replicates and the discrimination among exhibits.

Materials and Methods

MDMA Exhibits

Five MDMA exhibits were received from the Michigan State Police Forensic Science Division and the physical characteristics of each exhibit are given in Table 1. Seven tablets from each exhibit were homogenized with a mortar and pestle, and samples were taken from these homogenized batches for subsequent extraction and analysis.

Liquid–liquid Extraction and GC-MS Analysis of Extracts

The LLE procedure was adapted from the procedure previously optimized by van Deursen et al. (7). Briefly, a pH 7.00 phosphate buffer was prepared by combining 6.83 g KH_2PO_4 (Mallinckrodt, Paris, KY) and 27.45 g Na_2HPO_4 $7H_2O$ (Jade Scientific, Canton, MI) in a 500-mL volumetric flask and filling to the mark with high purity water (Burdick & Jackson Laboratories, Inc., Muskegon, MI). For each MDMA exhibit, 200 mg homogenized sample were dissolved in 4.0 mL buffer, vortexed for 10 sec, sonicated for 10 min, and centrifuged for 8 min. Then, 400 μL toluene (Fluka, St. Louis, MO) were added to the buffer. The sample was vortexed for 2 min, inverted ten times, and centrifuged for 10 min. Finally, the toluene layer was removed and transferred into a GC vial for subsequent analysis.

The LLE extracts were analyzed by GC-MS, using an Agilent 6890N gas chromatograph coupled to an Agilent 5973 mass spec-

TABLE 1—*Physical characteristics of methylenedioxymethamphetamine exhibits.*

Exhibit	Shape	Edge	Color	Motif	Mean Mass* (mg)
A	Round	Beveled	Blue	Omega	242.3
B	Round	Beveled	Pink	Heart	267.9
C	Round	Flat	Yellow	Waving Man	261.6
D	Round	Beveled	Green	"ME"	262.7
E	Round	Beveled	Green/Purple	Alligator	267.4

*Mean mass calculated based on seven tablets that were homogenized for analysis.

trometer (Agilent Technologies, Inc., Santa Clara, CA). The carrier gas was ultra-high purity helium with a nominal flow rate of 1 mL/min. A 4-mm i.d. tapered inlet liner with glass wool (Restek, Bellefonte, PA), an 11 mm septum (Agilent Technologies, Inc.), and a cross-bond 5% phenyl 95% dimethylpolysiloxane column (Rxi-5 ms, 30 m × 0.25 mm × 0.25 μm) (Restek) were used. The injection port was maintained at 250°C and, as no autosampler was available, a 1-μL aliquot of each extract was manually injected in splitless mode, using a 1-μL syringe (Hamilton, Reno, NV).

The two GC oven temperature programs investigated were previously published in the literature for the analysis of MDMA exhibits and are detailed in Table 2 (2,10). These procedures were primarily chosen due to differences in the number of ramp steps, as well as the total analysis time. The transfer line to the mass spectrometer varied according to the temperature program and was equivalent to the final oven temperature. For both temperature programs, the ion source temperature was 230°C and the mass spectrometer was operated in electron ionization mode (70 eV) at 3.58 scans/sec, with a mass scan range of 50–500 m/z. Each extract was analyzed in replicate (n = 5) by each temperature program.

Headspace Solid-Phase Microextraction Procedure and GC-MS Analysis of Extracts

The HS-SPME procedure used in this research was previously optimized by Bonadio et al. (10). Briefly, a 65-μm PDMS/DVB fiber (23 gauge, Supelco, St. Louis, MO) was conditioned in the GC inlet for 30 min at 250°C, following the recommended guidelines. A 40 mg portion of homogenized exhibit was transferred into a 4-mL vial with a screw cap and silicone septum (Supelco), and the vial was heated in a water bath at 80°C for 15 min. The SPME fiber was then exposed to the headspace within the vial and, after 15 min, the fiber was retracted, and removed from the vial. As a SPME autosampler was not available, the fiber was manually inserted into the GC inlet for subsequent analysis. For each exhibit, three separate extracts, using three separate 40-mg aliquots of homogenized sample, were prepared for analysis and treated as replicates.

Due to instrument availability, the HS-SPME extracts were analyzed using an Agilent 6890N with 5975B inert XL MS (Agilent Technologies, Inc.). The column previously used for the analysis of the LLE extracts was transferred to this instrument so that all extracts were analyzed using the same column. The instrument parameters were the same as before except that a narrow bore liner (0.75 mm i.d., Sigma-Aldrich, CO) and a Merlin Microseal (Merlin Instrument Company, Half Moon Bay, CA) were used in place of the traditional liner and septum.

After analyzing an extract, a fiber blank was analyzed to ensure the cleanliness of the fiber prior to the next extraction. The blanks were analyzed using the same GC-MS instrument parameters as described previously, except using a 50:1 split rather than the splitless mode. The oven temperature program for the blanks was similar to the program being used to analyze the extracts but with no initial hold, a ramp of 40°C/min, and a final hold of 3 min.

Because a newer model mass spectrometer was used for analysis of the HS-SPME extracts compared with the LLE extracts, the sensitivity of the two detectors was investigated to ensure that all compounds previously observed would be detected. To do this, one exhibit (Exhibit B) was again extracted using LLE and analyzed in triplicate by Temperature Program 1 on the newer model mass spectrometer (5975 MSD). The total ion chromatograms were then compared based on a qualitative assessment of the compounds present, as quantitative assessment of the resulting data was not necessary in this research. All compounds were detected in the chromatograms generated by each detector.

Data Analysis

Major compounds in the resulting total ion chromatograms were provisionally identified by comparing mass spectra with spectra in the National Institute of Standards and Technology (NIST) Mass Spectral Search Program (version 2.0, NIST, Gaithersburg, MD). Compounds present in each of the five exhibits and above the baseline were selected for subsequent data analysis. Peak areas of the selected compounds were integrated using ChemStation software (version E.01.01.335, Agilent Technologies), using a peak threshold of 14.

Data sets were generated comprising peak areas of the selected compounds for each extraction procedure and temperature program combination, giving a total of four data sets. Replicates of each exhibit in each data set were subjected to total area normalization prior to data analysis to account for slight differences in abundance due to slight differences in injection volume. To do this, the total area of the selected compounds in each replicate was calculated, and then the average total area across all replicates of that exhibit was calculated. The peak area of each compound in a replicate was divided by the total area of that replicate and then multiplied by the average area of all replicates. All calculations were performed in Microsoft Excel (Microsoft Corp., Redmond, WA).

Following normalization, each data set was subjected to PCA, which was performed in Matlab (version R2010b, The Mathworks, Natick, MA) and scores and loadings plots were generated in Microsoft Excel (Microsoft Corp.). Association of replicates and discrimination of exhibits was assessed using the scores plots while the loadings plots were used to identify those compounds contributing most to the variance described by the principal components.

TABLE 2—*Gas chromatography temperature programs investigated.*

Temperature Program	Initial Temp. (°C)	Solvent Delay (min)	Initial Hold (min)	Ramp Rate (°C/min)	Final Temp. (°C)	Final Hold (min)	Total Time (min)
1*	60	5	3	10	150	2	36
				2	170	0	
				10	260	3	
2†	50	5	1	5	150	12	53
				15	300	10	

*Bonadio et al. (10).
†Gimeno et al. (2).

Results

Liquid–Liquid Extracts

An exemplar total ion chromatogram of each exhibit extracted using LLE and analyzed using Temperature Program 1 is shown in Fig. 1. Common compounds were observed in each of the MDMA exhibits, including methamphetamine, 3,4-methylenedioxyphenyl-2-propanol (MDP2P-OH), and MDMA, although the abundances of these compounds varied according to exhibit. Some compounds identified were unique to certain exhibits. For example, ketamine and diphenhydramine were only identified in Exhibit B, whereas 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide and 2-(diethylamino)ethyl-4-aminobenzoate, which are also known as lidocaine and procaine, respectively, were only identified in Exhibit D. Exhibit C did not contain caffeine, but this compound was a prominent cutting agent in the remaining four exhibits. In fact, for each of these exhibits, chromatographic peaks for caffeine displayed extensive fronting, indicating that the column was overloaded with this particular compound.

All five exhibits also contained a compound that eluted with a retention time of 18.6 min using Temperature Program 1. When the mass spectrum was searched against the NIST database, this compound was interchangeably identified as either 3,4-methylenedioxy-N-ethylamphetamine (MDEA) or methylenedioxydimethylamphetamine (MDDMA). Both MDEA and MDDMA have a molecular mass of 207 amu and only differ in the substitution of the nitrogen atom: for MDEA, nitrogen is substituted with an ethyl group, whereas in MDDMA, the nitrogen is substituted with two methyl groups. As appropriate reference standards were not available and the selected mass scan range did not incorporate masses less than m/z 50, definitive identification of this compound using both retention time and mass spectral data was not possible. Hence, this compound is referred to as "MDEA/MDDMA" though the remainder of the discussion.

The exhibits also contained impurities that served as indicators of the probable method used to synthesize the MDMA present in the tablets. Piperonal, which was present at low levels in all five exhibits, can be used to synthesize the precursor 3,4-methylenedioxyphenyl-2-propanone (MDP2P). Furthermore, for Exhibits A and C, the presence of 3,4-methylenedioxy-N,N-dimethylbenzylamine (MDDMB) indicated a reductive amination route due to the amination of residual piperonal using methylamine (2). However, despite a similar synthesis method, Exhibits A and C were differentiated based on the lack of caffeine in the latter exhibit.

Each of the compounds identified in the five exhibits was also observed in all LLE extracts when analyzed using Temperature Program 2. An exemplar chromatogram of Exhibit A analyzed using Temperature Program 2 is shown in Fig. 2. In this exhibit, there was a difference in the ratio of MDEA/MDDMA and caffeine between the two temperature programs; however, this was attributed to differences in extraction efficiency rather than temperature program.

Association and Discrimination of Liquid-Liquid Extracts Using PCA

Principal components analysis was performed on selected compounds for each data set. Compounds were selected as those that were present in all replicates of all exhibits and that were present above the baseline. The selected compounds for each temperature program are shown in Table 3. It should be noted that benzaldehyde was not included in the analysis of Temperature Program 2 because this compound eluted during the solvent delay and, hence, was not detected in any exhibits analyzed using this program.

The PCA scores plot based on selected compounds for the five exhibits analyzed using Temperature Program 1 is shown in Fig. 3*A*, in which the first two PCs accounted for 90.9% of the variance. Replicates of Exhibits B and C were closely clustered, while more spread was apparent, especially on PC2, among replicates of the other three exhibits. In particular, clear distinction of Exhibits A and E based on visual assessment of the scores plot was not possible.

The compounds contributing to the variance described by the principal components are shown in the corresponding loadings plot (Fig. 3*B*). MDMA and MDP2P-OH contributed most to the variance described: MDMA had the greatest positive weighting on PC1, while MDP2P-OH had the greatest positive weighting on PC2. The remaining compounds were positioned close to zero on both PCs and therefore had less contribution to the positioning of the exhibits in the scores plot. This was particularly true for benzaldehyde and isothiocyanic acid, which were positioned at zero on both PCs in the loadings plot.

The positioning of exhibits in the scores plot was explained with reference to the corresponding loadings plot (Fig. 3*B*). For example, Exhibit B contained the highest abundance of MDMA of all five exhibits and, because MDMA was weighted positively on PC1 in the loadings plot, Exhibit B was positioned most positively on PC1 in the scores plot. This exhibit also contained the lowest abundance of MDP2P-OH among the five exhibits. As a result, when the data were mean-centered prior to PCA, there was a negative contribution from MDP2P-OH in Exhibit B. The mean-centered data were then multiplied by the PC to generate the score for the exhibit on PC2. In this case, MDP2P-OH had a positive contribution to PC2 such that, when multiplied by the negative contribution in the mean-centered data, there was a negative contribution at this retention time. This negative contribution, along with the negative contribution of MDMA on PC2, resulted in Exhibit B being positioned negatively on PC2 in the scores plot. The position of all other exhibits in the scores plot could be explained in a similar manner, either directly from the loadings plot or with reference to the mean-centered data.

The PCA scores plot based on selected compounds for the five exhibits analyzed using Temperature Program 2 is shown in Fig. 4*A*, in which the first two PCs accounted for 85.6% of the variance. Replicates of each exhibit were more closely associated using this temperature program resulting in less spread in the scores plot. As a result, greater distinction of the five exhibits was possible. As before, the positioning of all exhibits in the scores plot was explained with reference to the loadings plot (Fig. 4*B*) and, in this case, positioning of the exhibits was primarily based on the presence, and differences in abundance, of MDMA and N-formyl MDMA.

In the scores plot for each temperature program (Figs 3*A* and 4*A*), Exhibits A and E were closely associated, mainly due to their similar chemical composition; that is, the dominant compounds in both exhibits, MDP2P-OH and MDMA, were present at similar abundance. Furthermore, abundances of the other selected compounds were similar between the two exhibits such that greater discrimination between A and E was not possible.

While the general positioning of exhibits in the scores plot was similar irrespective of temperature program, replicates of each exhibit (particularly for Exhibits A, D, and E on PC2) were

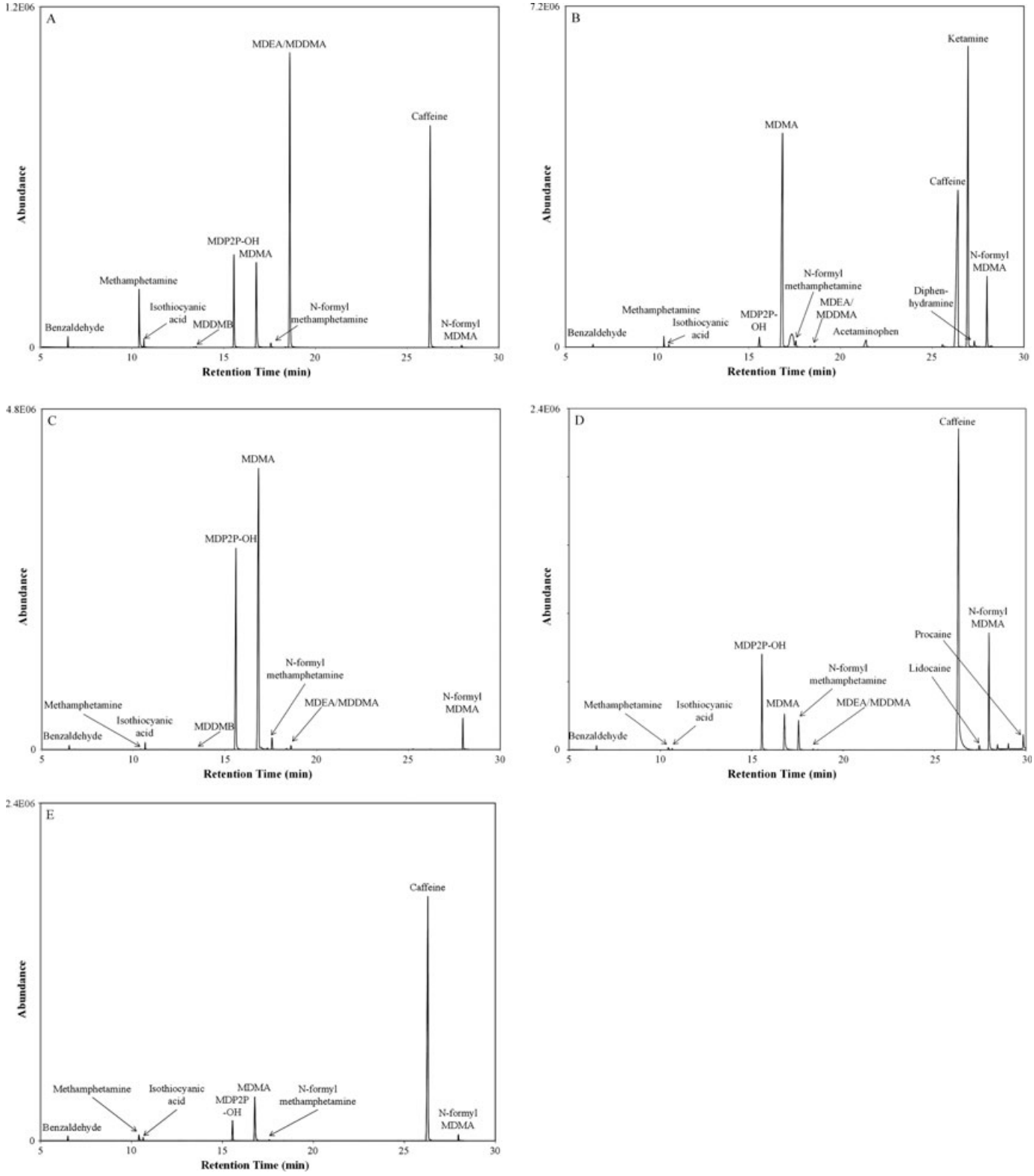

FIG. 1—*Total ion chromatograms for five methylenedioxymethamphetamine exhibits extracted using liquid–liquid extraction and analyzed using Temperature Program 1 (A) Exhibit A, (B) Exhibit B, (C) Exhibit C, (D) Exhibit D, and (E) Exhibit E. Peaks are labeled with provisional identities based on a mass spectral database search. Although present in all exhibits, piperonal was at levels too low to be observed on this scale and is not labeled.*

more spread, indicating poorer precision, when analyzed using Temperature Program 1. To assess precision in the analysis, relative standard deviations (RSDs) were calculated based on peak area abundance of the selected compounds (Table 4). For Temperature Program 1, the RSDs based on the average of all selected compounds ranged from 1.9% to 38.2%, while for Temperature Program 2, RSDs ranged from 2.5% to 12.3%.

In both cases, the sample and extraction procedure contributed to the variation observed; however, there was also some contribution from the temperature programming, particularly

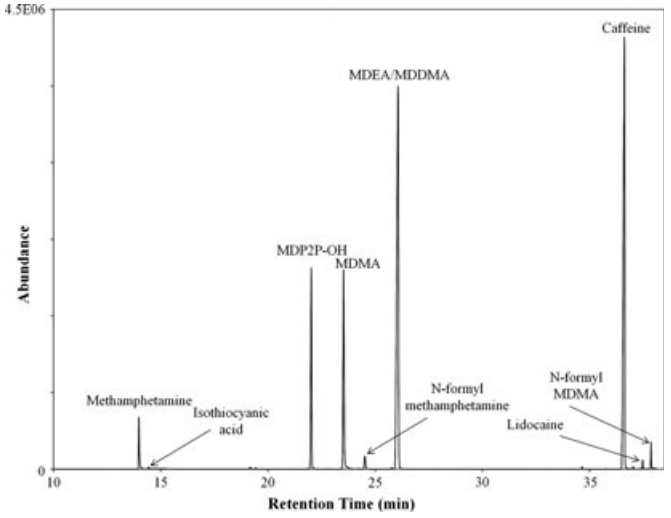

FIG. 2—*Total ion chromatogram of Exhibit A extracted using liquid–liquid extraction and analyzed using Temperature Program 2.*

differences in the ramp rates and intermediate hold times between the two programs. Temperature Program 1 included a slow ramp of 2°C/min from 150°C to 170°C; however, the tolerance associated with the oven temperature is ± 1°C. Hence, there is the potential for more fluctuation in temperature during such a slow ramp rate, resulting in greater variation (poorer precision) in the peak area of compounds eluting during the temperature ramp. Thus, in PCA, variance described by the first two PCs includes instrumental contribution rather than solely chemical contribution from variation in the sample and extraction procedure. In comparison, Temperature Program 2 includes a 12 min hold at 150°C. The greater stability in the instrument minimizes variation in the peak area of compounds eluting during the hold, resulting in greater precision and, hence, lower RSDs. As such, in subsequent PCA, the first two PCs account for more chemical, rather than instrumental, variance, allowing greater discrimination of exhibits using this temperature program.

Headspace Solid-Phase Microextraction Extracts

An exemplar total ion chromatogram of each exhibit extracted using the headspace solid-phase microextraction (HS-SPME) procedure and analyzed using Temperature Program 1 is shown in Fig. 5. The major compounds observed in the HS-SPME extracts were also observed in the LLE extracts, with the exception of benzaldehyde, isothiocyanic acid, and diethyl phthalate. Benzaldehyde was only present in Exhibit A following HS-SPME but was present in all five exhibits after LLE. While this compound has been identified as an impurity in the manufacture of MDMA (2, 10), benzaldehyde is also an impurity in toluene, which was used as the extraction solvent in LLE. Hence, when both LLE and HS-SPME are considered, it is likely that benzaldehyde is an impurity in Exhibit A, but was present in the LLE extracts of the other exhibits as a solvent impurity. Diethyl phthalate was only observed in HS-SPME extracts because the compound is not soluble in toluene and was therefore not extracted by LLE. Diethyl phthalate is used as a binder in the tabletting process and could be useful in linking tablets to a common production source (10).

Several compounds, such as piperonal, MDDMB, 2-(diethylamino)-N-(2,6-dimethylphenyl)acetamide (lidocaine), and 2-(diethylamino)ethyl-4-aminobenzoate (procaine), which were present at levels close to the background in the LLE extracts, were observed at significant levels in the HS–SPME extracts, primarily due to the pre-concentration ability of the fiber. Additionally, phenyl-2-propanone (P2P) and MDP2P, which were both present in all five exhibits, were only observed in the HS-SPME extracts. P2P is a common precursor in the manufacture of methamphetamine (10), while MDP2P is an intermediate in the production of MDMA, when either isosafrole or piperonal is used as the starting material (2). Furthermore, (3,4-methylenedioxyphenyl)-2-propanone-2-oxime was provisionally identified in the HS-SPME extracts of Exhibits D and E. This compound is indicative of the nitropropene synthesis method in which piperonal is used as the precursor for MDP2P (2).

While pre-concentration of trace level impurities is an obvious advantage of the HS-SPME extraction procedure, pre-concentration can also result in peak broadening in the chromatograms (compare Fig. 1 with Fig. 5). This can result in fronting peaks and poor baseline resolution, both of which were observed in chromatograms of the HS-SPME extracts.

Association and Discrimination of HS-SPME Extracts Using PCA

As previously, compounds selected for PCA of the HS-SPME extracts (Table 3) were those that were present in all replicates

TABLE 3—*Selected compounds used for principal components analysis.*

	Liquid–Liquid Extracts		Headspace Solid-Phase Microextraction Extracts	
Compound	Temperature Program 1	Temperature Program 2	Temperature Program 1	Temperature Program 2
Benzaldehyde	✓			
Phenol			✓	✓
P2P				✓
Methamphetamine	✓	✓	✓	✓
Piperonal			✓	✓
Isothiocyanic acid	✓	✓		
MDP2P			✓	✓
MDP2P-OH	✓	✓	✓	✓
MDMA	✓	✓	✓	✓
N-formyl MA	✓	✓	✓	✓
Diethyl phthalate			✓	
MDEA/MDDMA			✓	
N-formyl MDMA	✓	✓	✓	✓

MDEA, methylenedioxy-N-ethylamphetamine; MDDMA, methylenedioxydimethylamphetamine; MDMA, methylenedioxymethamphetamine
Shading indicates compounds not used for data analysis for that extraction procedure.

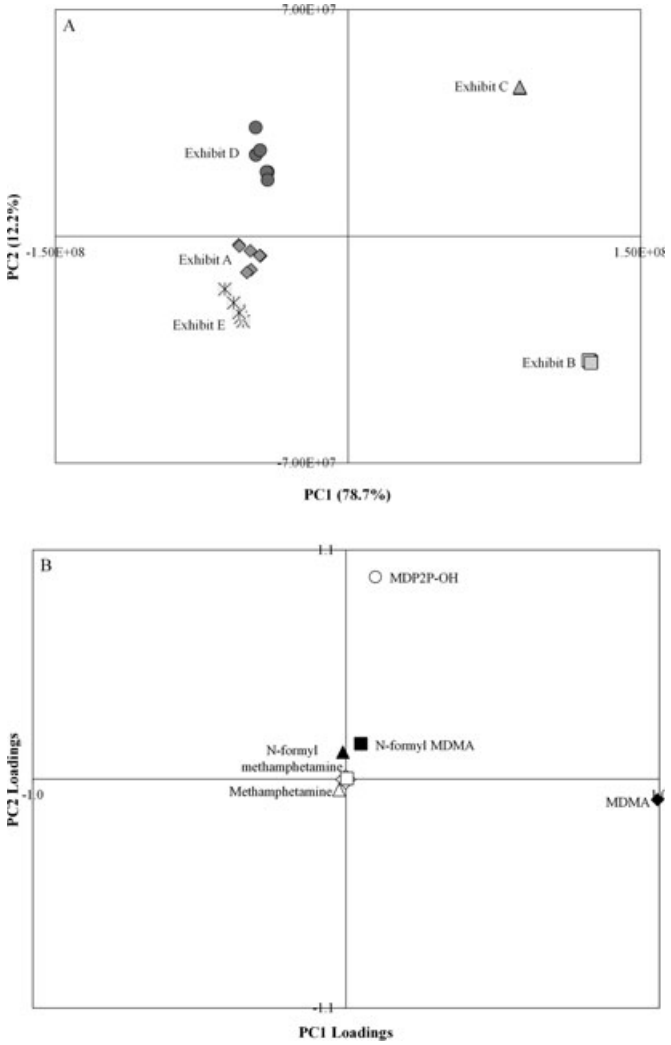

FIG. 3—*Principal components analysis (A) scores plot and (B) loadings plot for five methylenedioxymethamphetamine exhibits extracted using liquid–liquid extraction and analyzed using Temperature Program 1. Each exhibit is labeled in the scores plot and selected compounds are labeled in the loadings plot.*

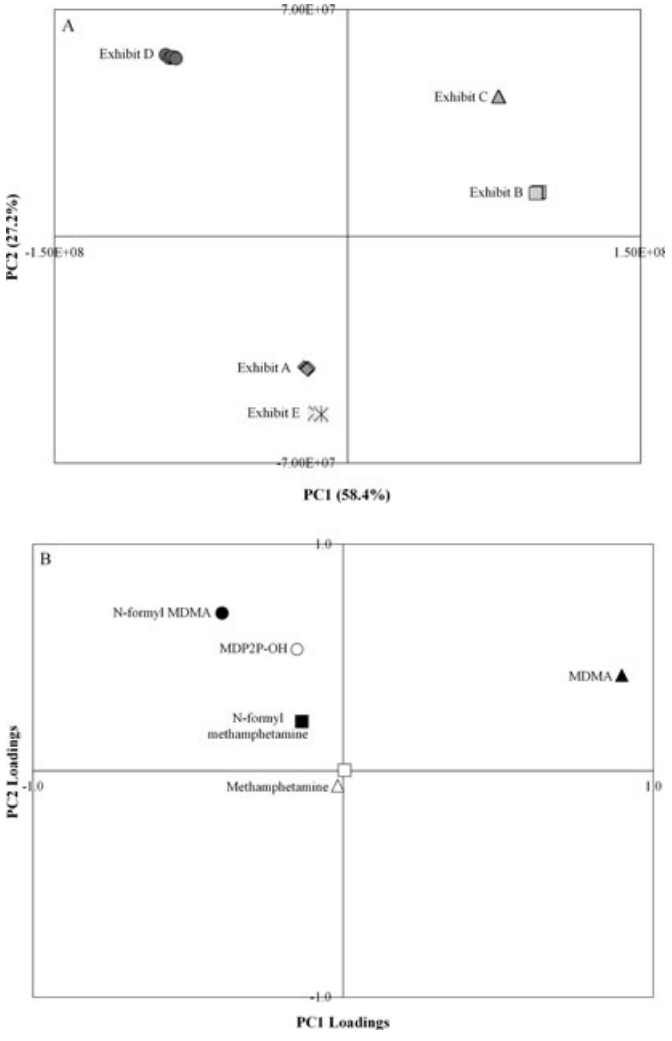

FIG. 4—*Principal components analysis (A) scores plot and (B) loadings plot for five methylenedioxymethamphetamine exhibits extracted using liquid–liquid extraction and analyzed using Temperature Program 2. Each exhibit is labeled in the scores plot and selected compounds are labeled in the loadings plot.*

of all exhibits and that were present above background. The pre-concentration of compounds on the fiber resulted in higher abundance of compounds detected, with the result that more compounds were available for PCA after HS-SPME extraction of the exhibits.

Ten compounds met the selection criteria when the exhibits were analyzed using Temperature Program 1 and nine compounds were selected for Temperature Program 2. Of these, eight were common to both programs. Phenyl-2-propanone was not consistently present at levels above the background in all exhibits when analyzed using Temperature Program 1 and, hence, was not included in the data analysis for this program. Although diethyl phthalate and MDEA/MDDMA were present in all exhibits, the two compounds were not baseline resolved in one extract of Exhibit A when analyzed using Temperature Program 2 and, hence, neither compound was included in the data analysis for this program.

The PCA scores plot for the five exhibits extracted using HS-SPME and analyzed using Temperature Program 1 is shown in Fig. 6*A*, in which the first two PCs accounted for 97.9% of the total variance among the exhibits. This was higher than the variance accounted for by the first two PCs when the LLE extracts were analyzed using the same temperature program (90.9%). The five exhibits were distinguished in the scores plot, although minor spread was observed among extracts of each exhibit, mostly on PC2.

From the corresponding loadings plot (Fig. 6*B*), MDMA had the greatest positive contribution to positioning of exhibits on

TABLE 4—*Relative standard deviation (RSD) of peak area abundance for liquid–liquid extracts (LLE) and headspace solid-phase microextraction extracts (HS-SPME) analyzed using Temperature Programs 1 and 2.*

	RSD (%) of Peak Area Abundance Average of all Selected Compounds				
Temperature Program	A	B	C	D	E
LLE					
1	38.2	4.2	1.9	15.5	34.3
2	12.3	4.3	2.5	4.8	10.0
HS-SPME					
1	26.9	21.8	38.8	12.4	14.0
2	9.3	9.6	15.6	14.9	13.3

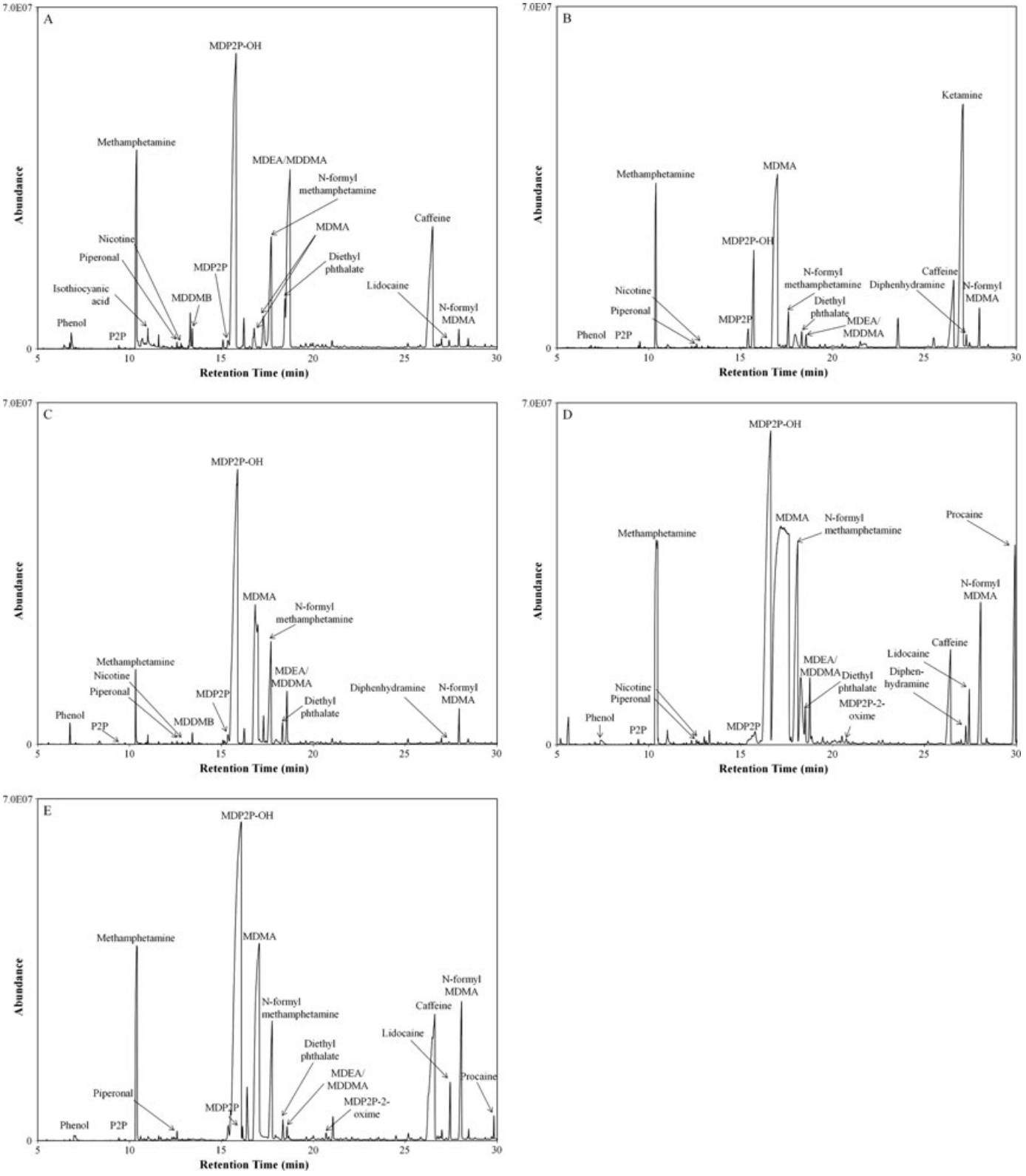

FIG. 5—*Total ion chromatograms for five methylenedioxymethamphetamine exhibits extracted using headspace solid-phase microextraction and analyzed using Temperature Program 1 (A) Exhibit A, (B) Exhibit B, (C) Exhibit C, (D) Exhibit D, and (E) Exhibit E. Peaks are labeled with provisional identities based on a mass spectral database search.*

PC1 in the scores plot, while MDP2P-OH had the greatest positive contribution to positioning on PC2. Methamphetamine, N-formyl methamphetamine, and N-formyl MDMA were each weighted positively on both PCs, while MDEA/MDDMA was weighted negatively on PC1 and slightly positively on PC2. The remaining four compounds were positioned close to zero on both PCs and, therefore, had little effect on the positioning of exhibits in the scores plot. As before, the positioning of each exhibit in

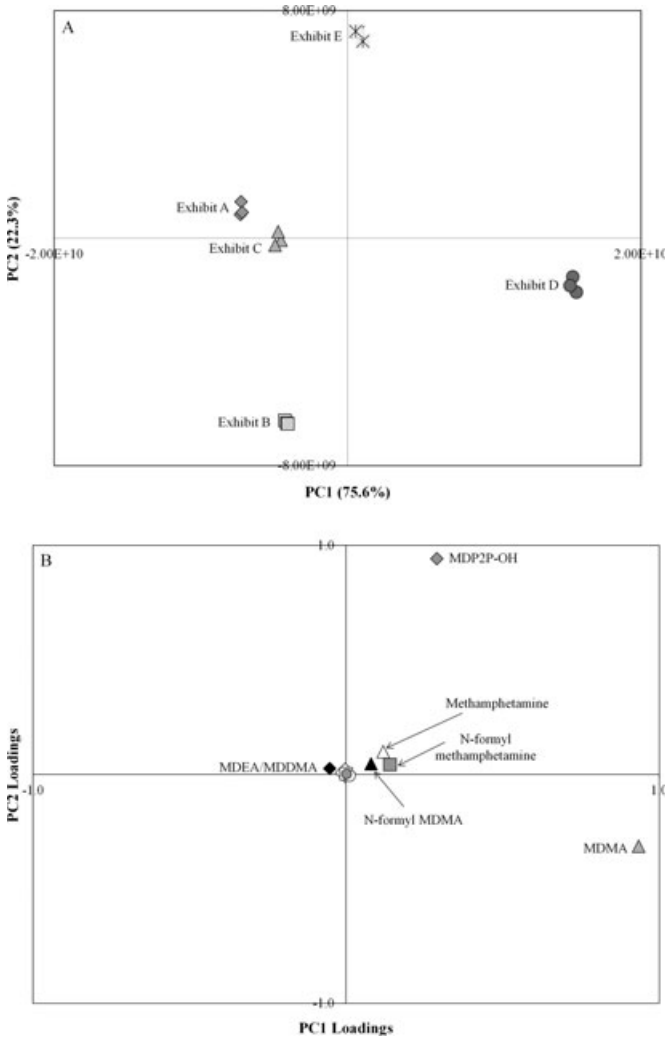

FIG. 6—*Principal components analysis (A) scores plot and (B) loadings plot for five methylenedioxymethamphetamine exhibits extracted using headspace solid-phase microextraction and analyzed using Temperature Program 1. Each exhibit is labeled in the scores plot and selected compounds are labeled in the loadings plot.*

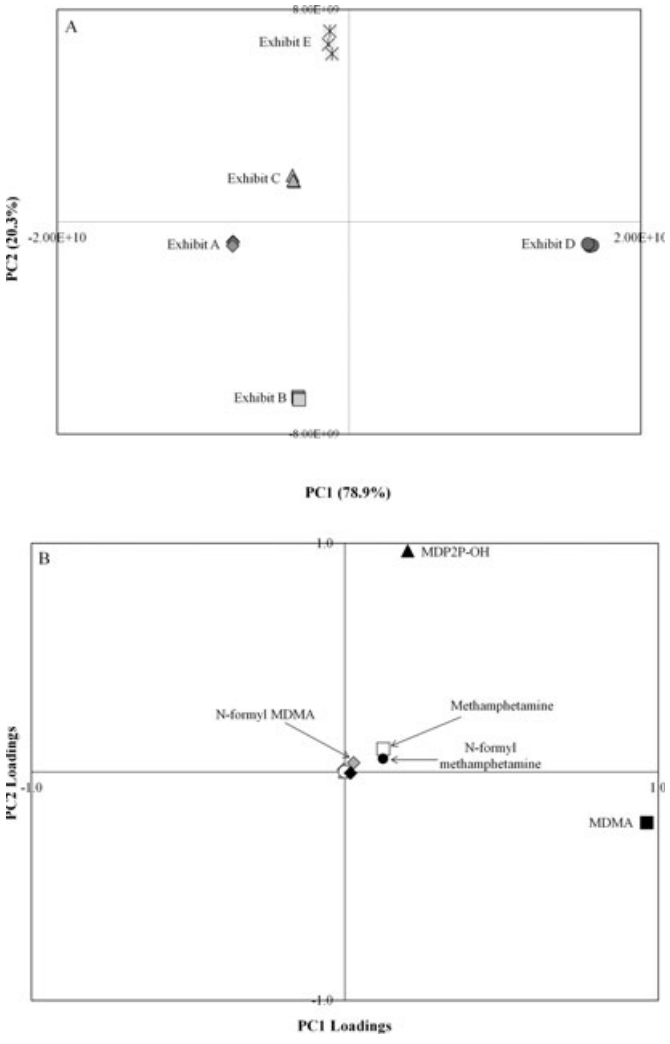

FIG. 7—*Principal components analysis (A) scores plot and (B) loadings plot for five methylenedioxymethamphetamine exhibits extracted using headspace solid-phase microextraction and analyzed using Temperature Program 2. Each exhibit is labeled in the scores plot and selected compounds are labeled in the loadings plot.*

the scores plot could be explained with reference to the loadings plot, mainly based on differences in abundance of MDMA and MDP2P-OH among the exhibits.

The scores and loadings plots for the exhibits extracted using HS-SPME and analyzed using Temperature Program 2 are shown in Fig. 7. The first two PCs accounted for 99.2% of the total variance and extracts of each exhibit were more closely clustered compared to Temperature Program 1, as a result of improved thermal stability as discussed previously. Using Temperature Program 2, discrimination of the exhibits in the scores plot was also mainly based on differences in abundance of MDMA and MDP2P-OH, as evidenced from the loadings plot (Fig. 7*B*).

Slight differences in positioning of the exhibits in the scores plot were observed according to temperature program (Fig. 6*A* compared with Fig. 7*A*). These differences were mainly due to the inclusion of MDEA/MDDMA as a selected compound in the analysis of Temperature Program 1. For example, Exhibit A was positioned positively on PC2 when analyzed using Temperature Program 1 (Fig. 6*A*), but negatively on PC2 when analyzed using Temperature Program 2 (Fig. 7*A*). This exhibit contained the greatest abundance of MDEA/MDDMA of all exhibits. When included as a selected compound for PCA (Temperature Program 1 only), MDEA/MDDMA was weighted positively on PC2 (Fig. 6*B*), resulting in the positive position of Exhibit A on PC2.

Overall, for the HS-SPME extracts, greater association among extracts of each exhibit was observed using Temperature Program 2. In turn, this allowed greater discrimination of the five exhibits, compared to Temperature Program 1.

Comparison of LLE and HS-SPME Procedures Using Temperature Program 2

Greatest association of replicates and discrimination of exhibits was observed when the extracts were analyzed using Temperature Program 2, irrespective of extraction procedure. However, scales on the scores plots generated for the HS-SPME extracts were approximately two orders of magnitude greater than those for the plots based on the LLE extracts (Figs 4*A* and 7*A*). Thus, although discrimination of exhibits appears to be the similar or only slightly improved using HS-SPME, the discrimination is actually greater. The improvement in discrimination is likely due to the additional compounds used for PCA: six compounds were

used when PCA was performed on chromatograms of the LLE extracts, while nine compounds were used in data analysis of the HS-SPME extracts.

Despite the greater discrimination, more spread was observed among replicates of the HS-SPME extracts of each exhibit, compared with the LLE extracts. As mentioned previously, the HS-SPME procedure is less reproducible than LLE, as evidenced in the higher RSDs calculated based on peak area of the selected compounds. For HS-SPME, the RSDs calculated for Temperature Program 2 ranged from 9.3% to 15.6%, while for LLE, the RSDs ranged from 2.5% to 12.3% (Table 4). The poorer precision observed for HS-SPME is primarily due to the pre-concentration ability of the fiber that can lead to overloading the column and subsequently, peak fronting in the chromatogram (Fig. 5).

Discussion

Common compounds and impurities were extracted from each exhibit using both LLE and HS-SPME. For LLE, there was greater precision in the analysis of the extracts, as evidenced by the lower RSDs. For HS-SPME, the pre-concentration ability of the fiber resulted in poorer peak shapes, and poorer precision, of the more dominant compounds in the exhibits. However, using this extraction procedure, additional compounds were extracted, albeit at low levels. Several of these compounds were indicative of synthesis route and hence, to obtain the most informative chemical profiles of exhibits, both LLE and HS-SPME should be performed.

For both LLE and HS-SPME extracts, the greatest association of replicates and discrimination of exhibits was observed using Temperature Program 2, primarily due to the greater thermal stability of the program. However, this program was also the longer of the two programs, with a total analysis time of 53 min compared with 36 min for Temperature Program 1. With a relatively long analysis time, this program may not be practical for implementation in forensic laboratories and minor adaptations (e.g., reducing the duration of the intermediate hold) may be necessary to reduce the total time.

Overall, this research has demonstrated the utility of both LLE and HS-SPME for chemical profiling applications, as well as the potential of PCA for the association of replicates and discrimination of exhibits. Further research in this area is warranted to develop fully standardized analysis protocols, including data analysis procedures, that would enable direct comparison of chemical profiles generated in different laboratories.

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