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TECHNICAL NOTE

Carolyn J. Koester,[1] *Ph.D; Brian D. Andresen,*[1] *Ph.D.; and Patrick M. Grant,*[1] *Ph.D.*

Optimum Methamphetamine Profiling with Sample Preparation by Solid-Phase Microextraction

ABSTRACT: Solid-phase microextraction (SPME) is a relatively new technique in which a small, polymer-coated fiber is employed to extract volatile and semivolatile organic compounds from the sealed headspace above a questioned sample. SPME, coupled with gas chromatography/mass spectrometry (GC/MS), was used to characterize impurities in illicit methamphetamine samples. Trace impurities present in a specimen were tentatively identified using mass-spectral databases and included 1,2-dimethyl-3-phenyl-aziridine (indicating synthesis via a halogenated ephedrine intermediate), ethyl vanillin (a flavoring compound), and caffeine (a stimulant used as cutting agent). The types and numbers of organic compounds sampled by SPME were compared with those collected by various solvent extraction protocols. In addition to unambiguously confirming the presence of methamphetamine, SPME-GC/MS analyses detected approximately 30 more organic analytes than were found by GC/MS following the ethyl acetate extraction method adopted by the United Nations International Drug Control Programme. SPME-GC/MS is a superior method for generating material "fingerprint" profiles in methamphetamine samples. The detection and characterization of increased points of comparison in drug samples provide more detailed chemical signatures for both intelligence and operational information.

KEYWORDS: forensic science, methamphetamine, chemical profiling, signatures analysis, material "fingerprint," solid-phase microextraction (SPME), GC/MS

The explicit characterization of a questioned sample through empirical measurements of material composition is a familiar tactic in forensic science. These data can provide a chemical "fingerprint" or impurity profile for a vast array of evidentiary specimens. Although a non-routine technique, such signature analysis and profiling can be of value to forensic investigators for both operational and intelligence purposes. For example, product impurities or impurity patterns can allow sample categorization into groups of associated specimens to relate a finished material to starting components and possibly identify an origin. Thus, minor- and trace-element compositions have been used to compare glass (1,2) and sources of bullet lead (3), while gas chromatographic-coupled techniques have been implemented for drug (4–6) and accelerant (7) classifications.

Material profiling can be particularly useful for the evaluation of synthetic drugs produced in clandestine laboratories. In these instances, an initial fingerprint determined by precursor chemical impurities and synthesis procedures is often augmented by the presence of additional compounds associated with cutting and flavoring agents. Forensic analyses of diverse signature species can provide information on the scope of an illicit activity, the estimated period of operation, potential sources of precursor supplies, drug trafficking routes, distribution patterns, and any chemical linkage between specimens of interdicted materials. Such intelligence also allows an analyst to differentiate specific synthetic methods and differing recipes. Monitoring the chemicals and methods employed for illicit drug manufacture can lead to trend analyses, the association of a specimen or group of specimens with an individual chemist or specific lab, distinctions between illicit drugs and those diverted from commercial sources, and an evolution of precursor controls.

Following a resolution adopted by the United Nations General Assembly in 1998, international attention was directed toward the production of methamphetamine from ephedrine precursor in Southeast Asia (8). The Scientific Section of the U. N. International Drug Control Programme began development of a method for the impurity profiling of methamphetamine tablets interdicted specifically in that area of the world. The result was a recommended, standard protocol for the analysis of Southeast Asian samples for implementation at both national and regional forensic laboratories (8). This UN Standard Method was an impressive undertaking, based on methamphetamine samples seized in 17 different countries, and refined through the analyses of $>$500 samples from Thailand, Laos, Myanmar, and Vietnam.

The UN Method entails dissolution of a methamphetamine specimen in high-pH buffer, solvent extraction with ethyl acetate, and instrumental analysis by means of gas chromatography with flame-ionization detection (GC/FID). The procedure was optimized for Southeast Asian methamphetamine samples "with regard to the extracting solvent, the pH for extraction, the amount of sample required, and the analytical parameters (9)." However, in our experience, sample preparation by modern solid-phase microextraction (SPME) can be more advantageous prior to GC analyses than conventional solvent extraction, and we surmised that it would be particularly valuable for material signature profiling. SPME has begun to find diverse application in many areas of forensic science (10–13), including drug analyses (14–20).

[1] Forensic Science Center, Livermore National Laboratory, Livermore, CA.
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Materials and Methods

Materials

All reagents used were of analytical-grade quality or higher. Dichloromethane, UltimAR grade for HPLC and GC, was obtained from Mallinckrodt (Phillipsburg, NJ). Acetone, 99.9+% (HPLC grade), ethyl acetate, 99.8% (HPLC grade), and isopropanol, 99.5% (ACS spectrophotometric grade) were obtained from Sigma-Aldrich (Milwaukee, WI). Water was either HPLC grade from Sigma-Aldrich or 18.2-MΩ quality from a Milli-Q UF Plus system (Millipore, Bedford, MA). Phosphate buffer (pH = 7) and sodium carbonate were obtained from J. T. Baker (Phillipsburg, NJ). SPME fibers of 65-μm polydimethylsiloxane/divinylbenzene and manual fiber holders were purchased from Supelco (Bellefonte, PA). Samples extracted either with solvent or by SPME fiber were contained in 4-ml, screw-cap vials with PFTE-lined silicone septa (Supelco).

Sample Preparation

Specimens of illicit, Southeast-Asian methamphetamine were interdicted by local law enforcement and transferred into glass containers. They were maintained and transported under ambient conditions, with temperatures ranging from approximately 20–40°C. Methamphetamine tablets from a common batch were ground with mortar and pestle. Small portions of the powdered material were weighed and prepared for analysis via direct dissolution, solvent extraction, or solid-phase microextraction (SPME). The specific protocols used to prepare experimental extracts for analysis by gas chromatography-mass spectrometry (GC/MS) are summarized in Table 1.

Blank control samples were designed so that they were as close as possible to the real specimens, except that they contained no methamphetamine. Treating blanks identically to questioned samples is standard laboratory practice and allows the correction of analytic results for any contamination contributed by such vectors as the glass vials, SPME fibers, or solvents used for extraction.

TABLE 1—*Procedures used for sample preparation prior to GC/MS analyses.*

Method	Procedure
Acetone dissolution	30 mg powder dissolved in 10.0 mL acetone.
Isopropanol dissolution	30 mg powder dissolved in 10.0 mL isopropanol.
Water dissolution and extraction into CH_2Cl_2	30 mg powder dissolved in 10.0 mL water;1.00 mL of the resultant solution was then extracted by shaking for 2 min with 1.00 mL CH_2Cl_2.
Acid dissolution (pH~2) and extraction into CH_2Cl_2	30 mg powder dissolved in 10.0 mL water acidified to pH 2 with dilute H_2SO_4;1.00 mL of the resultant solution was then extracted with CH_2Cl_2.
Base dissolution (pH~10) and extraction into CH_2Cl_2	30 mg powder dissolved in 10.0 mL water adjusted to pH 10 with NaOH;1.00 mL of the resultant solution was then extracted with CH_2Cl_2.
UN Method (8)	30 mg powder dissolved in 1.00 mL of pH 10.5 phosphate buffer;this solution was extracted, by shaking for 5 min, with 500 μl ethyl acetate.
SPME, 80–110°C	30 mg powder placed in a sealed, 4-mL vial and positioned on a hot plate set between 80–110°C;SPME fiber was equilibrated with the vial headspace for 25 min.

Instrumental

All samples were analyzed with an Agilent Technologies (Palo Alto, CA) 5973 GC/MS system. Experimental liquid aliquots were introduced into the GC/MS in 1-μl injection volumes, while SPME fibers were introduced directly into the injection port of the GC/MS to thermally desorb analytes for 1.25 min. The injection port of the GC/MS was maintained at 250°C. Gas-chromatographic separations of organic analytes were performed on a 30 m, DB-1 column having 0.25-mm inner diameter and 0.25-μm film thickness (J&W Scientific, Folsom, California). The GC temperature programming was: isothermal at 50°C for 10 min, increased at 15°C/min to 150°C, held at 150°C for 5 min, increased at 15°C/min to 300°C, and held at 300°C for 5 min. The GC column pressure was held constant at 12 psig.

The MS was operated in scan mode, with a source temperature of 230°C, at a rate of 1.5 scans/sec over the range of 45–450 amu. The *NIST/EPA/NIH Mass Spectral Library* (NIST 1998, National Institute of Standards and Technology, Gaithersburg, MD) and the *Wiley Registry of Mass Spectral Data* (6th Edition, Wiley, New York, NY) were used to tentatively identify detected species in the samples.

Results and Discussion

A multiplicity of organic compounds was found in the exemplar of Southeast Asian methamphetamine. The chemical species tentatively identified, in order of their elution from the GC column under the present experimental conditions, and listed as a function of different sample-preparation methodologies, are given in Table 2. Only compounds that produced distinct chromatographic peaks, and were not present in blank control samples, were included in Table 2. Other identified analytes, such as phthalates (which are ubiquitous environmental contaminants) and organo-silane compounds (which bleed from the GC/MS septa and column and from the SPME fibers), were not included in the Table. Moreover, a large number of hydrocarbons measured in the SPME experiments, and not present in either the solvent-extracted samples or in the SPME blank runs, were not tabulated either. These species will be discussed further below.

The measured compounds were identified through comparisons of their mass spectra with those included in the standard mass-spectral databases. For the vast majority of chemical species in Table 2, the library quality matches were > 70 (a quality fit of 100 is a perfect spectral match). Upon subsequently evaluating the credibility of a library match, an analyst additionally considered the scan range of the collected data, as well as all discernable mass-spectral peaks, for a final interpretation. However, because the identities of the majority of the compounds in this study were not further confirmed by analyses of known standards, most compound identifications were considered to be tentative only. The presences of methamphetamine and caffeine in the questioned samples were established through standards analyses, thus allowing unambiguous identifications of these two compounds.

Multiple SPME samplings were conducted over a temperature range of 80–110°C to optimize the collection of fingerprint compounds. Good agreement was achieved between these approximately replicate analyses. The SPME data reported in Table 2 are those generated from a 110° exposure. For material profiling, the larger the number of valid points of comparison the better, and in-

TABLE 2—*Analytes measured in Southeast Asian methamphetamine samples by GC/MS, following different sample preparation methods.*

Tentative Compound Identification	Acetone	IPA	CH_2Cl_2	pH=2/ CH_2Cl_2	pH=10/ CH_2Cl_2	UN Method: pH=10/ EtOAc	SPME PDMS/ DVB	MS Data for Unidentified Peaks: Ion m/z (Relative Abundance)
Benzaldehyde							X	
Dimethyloxamide							X	
Benzylmethylketone					X	X	X	
1,2-dimethyl-3-phenyl-aziridine		X	X		X	X	X	
D-amphetamine						X		
Ethoxyphenol							X	
Unknown aromatic compound							X	105 (100), 77 (70), 51 (20), 126 (5), 134 (5)
Methamphetamine	X	X	X		X	X	X	
Dimethylbenzaldehyde							X	
Ethoxy-methyl-phenol							X	
Ethyl amphetamine	X	X					X	
Unknown compound						X	X	56 (100), 158 (85), 144 (40), 103 (25)
Methylbenzaldehyde							X	
Unknown aromatic compound							X	132 (100), 91 (20), 117 (5), 77 (5), 148 (5)
Dihydro-5-pentyl-2(3H)-furanone							X	
Unknown compound							X	170 (100), 68 (100), 144 (50)
3,4-dimethyl-5-phenyloxazolidine	X	X			X	X	X	
Tetradecane							X	
Ethyl vanillin	X		X	X			X	
Unknown aromatic compound							X	150 (100), 179 (90)
Di-tert-butyl-phenol							X	
Butylated hydroxytoluene							X	
Formyl methamphetamine or n-methyl phenylethylamine acetate					X		X	
Acetylated meth						X	X	
Benzophenone							X	
Hexadecane							X	
Unknown aromatic compound	X							128 (100), 72 (60), 91 (45)
Similar to amino indane or tetrahydroquinoline							X	132 (100), 133 (40), 91 (20), 117 (20), 118 (15)
Unknown aromatic compound	X							128 (100), 70 (60), 91 (25)
Unknown compound							X	57 (100), 120 (70), 71 (45), 85 (25), 138 (25), 222 (10)
Unknown aromatic compound							X	146 (100), 118 (80), 132 (25), 147 (25)
Unknown aromatic compound		X						116 (100), 158 (80), 91 (5), 162 (<5)
Heptadecane							X	
Caffeine	X	X	X	X	X	X	X	
Total	7	6	4	2	6	8	30	

creased signature species usually result in more credible categorization and forensic inference. In these experiments, the SPME method resulted in 30 entries in Table 2, while the next-best protocol, the UN Method, gave 8. A graphic display of the increased analytic information possible with SPME sample-preparation is given in Fig. 1, which shows representative total-ion chromatograms (TIC) from the GC/MS analyses. The disparity between methods would have been even greater had the number of unknown hydrocarbons measured by SPME also been included in the Table (e.g., 41 SPME analytes vs. 8 for the UN Method). However, they were not incorporated in the data results because the diverse hydrocarbon species were presumed to have less applicability to the methamphetamine synthetic process than the compounds listed in Table 2. However, they could still provide valuable intelligence information for considerations such as purity of the synthesis solvents, cleanliness of the tablet press, or local environments of packaging and distribution operations. Figure 2 depicts a similar GC/MS comparison between the UN Method and SPME for the m/z = 57 fragment ion, which is characteristic of hydrocarbon species.

SPME is the superior tactic for methamphetamine profile analysis, and Fig. 1 is a good example. In all extraction and direct-dissolution methods, the GC/MS TIC was dominated by the bulk quantities of the drug and caffeine cutting agent. However, with SPME, although both compounds were also collected (allowing courtroom testimony on the presence of a controlled substance), trace and ultra trace species present in the sample were concentrated by the fiber relative to the major components. For example, ethyl vanillin (tent.) became the dominant peak in the SPME GC/MS TIC (as compared to the methamphetamine peak by the UN Method). Such selective preconcentration of diverse methamphetamine signatures by the SPME fiber prior to analysis is the feature of this protocol that makes it superior for these studies.

The disparity in GC/MS profiles produced by the UN and SPME methods can be explained by the major difference between the two

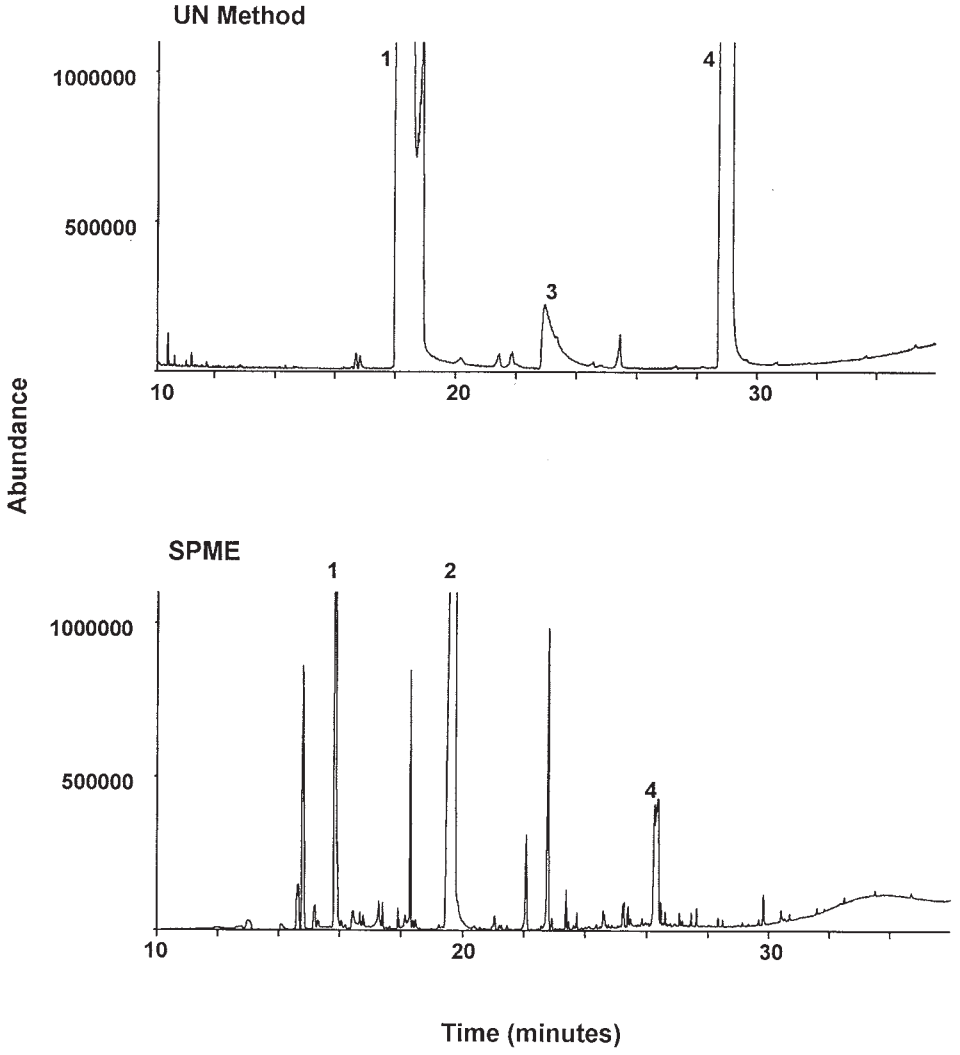

FIG. 1—*GC/MS total-ion chromatograms of replicate methamphetamine samples obtained by solvent extraction with the UN Method and by SPME. Peak 1 = methamphetamine. Peak 2 = ethyl vanillin (tent; not detected by the UN Method), and peak 4 = caffeine. [For both this figure and Fig. 2, the data for the UN Method were obtained with a 60-m GC column, while those for SPME were from the 30-m column. Consequently, the corresponding retention times for analytes by the UN Method were greater than those by the SPME method.] Peak 3 = methylparaben (tent.), which was observed only in the UN-Method experiments, and is used as a preservative in foods, beverages, and cosmetics (26). It was not present in the methamphetamine samples themselves, but was a component of the pH buffer used for the aqueous phase of the EtOAc extraction. It was likely added intentionally to the buffer by the manufacturer as an antimicrobial preservative.*

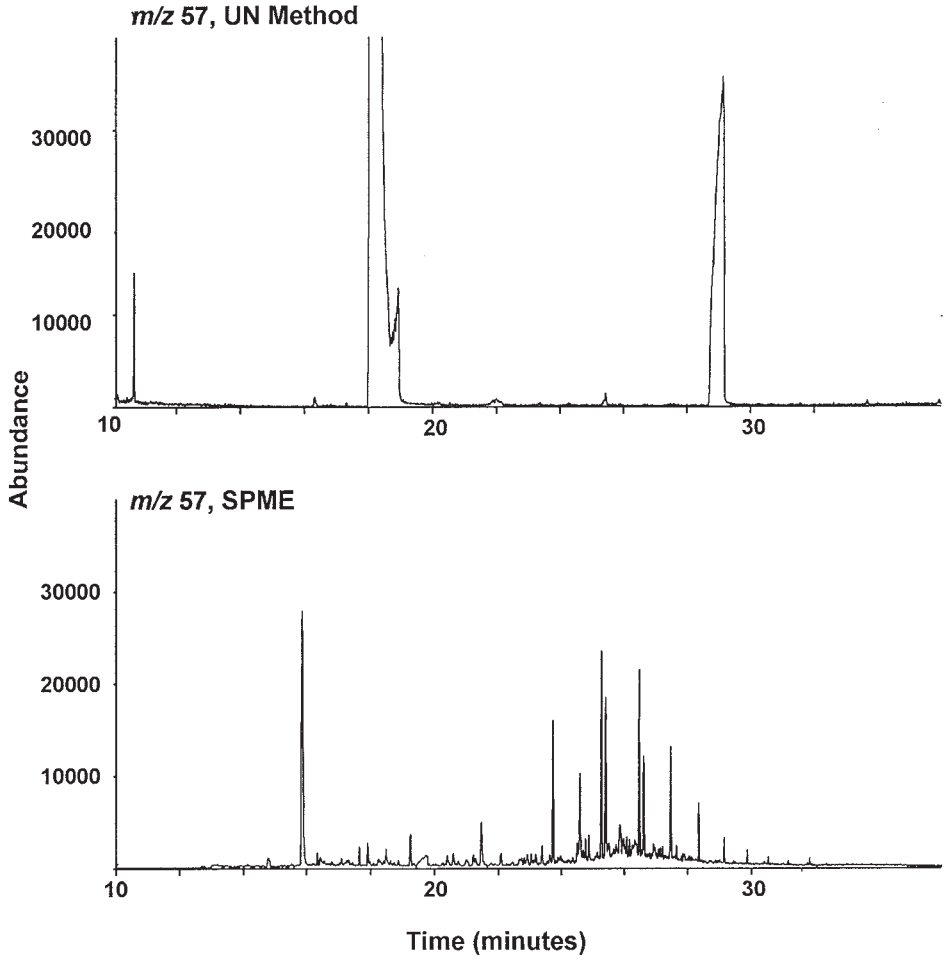

FIG. 2—*Mass chromatograms for the m/z = 57 fragment ion (a characteristic hydrocarbon signature) produced by replicate methamphetamine samples analyzed by the UN Method and by the SPME protocol.*

extraction techniques. The UN Method is based on solvent extraction, which ideally can be considered an exhaustive (i.e., quantitative) removal of analytes from a sample matrix. By contrast, SPME is not an exhaustive extraction method. The efficiency of SPME as a collection/concentration modality depends on a number of factors, and such discussion is beyond the scope of this paper. However, recent comprehensive literature sources are readily found (21–23).

Some of the chemical species in Table 2 could be interpreted within the context of the synthetic drug procedures. For example, 1,2-dimethyl-3-phenyl-aziridine was indicative of the Emde synthesis route with a chloroephedrine intermediate (24,25), as opposed to the P2P or Nazi methods. Similarly, benzyl methyl ketone is a methamphetamine decomposition product through chemical oxidation. Other compounds could be directly assessed as flavor components (e.g., ethyl vanillin and benzaldehyde) or as antioxidants and fixatives (butylated hydroxytoluene, benzophenone). However, the majority of the Table 2 entries were, at face value, empirical signatures in a product "fingerprint" for potential comparison with other questioned specimens.

Thus, the SPME sample-preparation method has been shown to be the most favorable protocol of those studied for empirical measurements of a multiplicity of signature species for methamphetamine profiling. At the same time, the SPME method also retains the four guiding principles of the UN-recommended method (8): simplicity; optimal peak resolution; robustness and reproducibility over long periods of time; and reliable and rapid searching/comparisons of the resultant data.

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Additional information and reprint requests:
Pat Grant
Forensic Science Center, L-231
Livermore National Laboratory
Livermore, CA 94550
Email: patgrant@llnl.gov