TECHNICAL NOTE

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Application of Solid-Phase Microextraction to the Profiling of an Illicit Drug: Manufacturing Impurities in Illicit 4-Methoxyamphetamine*

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ABSTRACT: This article describes the application of solid-phase microextraction (SPME) to the recovery of manufacturing by-products and impurities from an illicit drug seizure. The preparation chosen for examination using this technique contained 4-methoxyamphetamine, an hallucinogenic amphetamine that has been encountered frequently in South Australia.

Compounds found in the PMA preparation included 4-methoxyphenol, 4-methoxybenzaldehyde, 4-methoxyphenyl-2-propanone, 4-methoxyphenyl-2-propanol, 4-methoxyphenylpropene, and (tentatively) 4-methyl-5-(4'–methoxyphenyl) pyrimidine. The presence of these compounds suggests that the active drug was prepared from 4-methoxybenzaldehyde via 4-methoxyphenyl-2-propanone using a Leuckardt reductive amination.

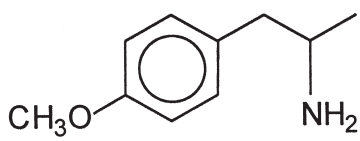
In this instance, SPME was found to be a simple, rapid, and non-destructive recovery technique that gave results complementary to those provided by conventional liquid-liquid extraction. There is an indication that SPME might find application in profiling of illicit drugs.

KEYWORDS: forensic science, 4-methoxyamphetamine, solid-phase microextraction, substance abuse, chemistry, profiling, hallucinogen

4-Methoxyamphetamine [**1**] (PMA) has been encountered in Australia, and in South Australia in particular, since 1995. As reported elsewhere (1–3) many fatal overdoses have been associated with its abuse.

Several seizures of PMA were examined for the presence of manufacturing impurities in order to identify the synthetic route employed in the illicit manufacture of the drug. In relation to one case, a fatal overdose, the only remaining example of the deadly material was a single tablet. In cases such as this, it can be important to preserve the tablet as much as possible. Solid-phase microextraction (SPME) was investigated as a tool for non-destructive recovery of drug manufacturing impurities.

SPME is a relatively new technique applicable to the recovery of organic compounds from either aqueous phase or headspace. The recovery device is thin fiber of fused silica coated with a thin layer of adsorbent polymer. The fiber is attached to the plunger of a "syringe" equipped with a thin stainless steel hypodermic needle. By moving the plunger, the fiber can be either withdrawn into the needle, or caused to protrude from the end of it. In order to initiate an extraction, the fiber is exposed either to headspace or aqueous specimens, thus allowing the polymer coating to adsorb analytes. After extraction is complete, the fiber is withdrawn into the needle and the analytical operation can commence. The needle is placed into the injector of a gas chromatograph (GC), and the fiber is then pushed out to expose it to the hot surroundings. The heat causes any adsorbed compounds to desorb, and they are then swept into the chromatograph for analysis in the usual fashion. SPME can also be used in conjunction with high performance liquid chromatography. Further discussion of the technique can be found in articles by Pawliszyn et al (4–6).



[**1**]

Methods

The gas chromatograph used was a Hewlett Packard 5890 equipped with a 5972 MSD and electronic pressure programming. For SPME a glass liner of 0.75 mm internal diameter was used in the injector, while a standard split/splitless liner was used for liquid injections.

Helium was used as carrier gas at a constant linear flow rate of 62 cm/s for SPME and 55 cm/second for liquid injection; the column was a 15 m × 0.257 mm × 0.25 μm DB-1 fused silica capillary; for SPME the oven program went from 50°C (2 min delay) to 300°C at a rate of 30°C/min; for liquid injection the oven program started at 90°C (3 min delay) and went to 300°C at 45°C/min, the injector was kept at 290°C (for SPME) or 300°C for liquid injec-

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tion; for SPME the injector was run in the splitless mode for 30 s with a pressure pulse of 20 psig for 30 s; for liquid injections the instrument was run in split mode using a 1 μL injection and a split flow of 50 mL/min; the mass spectrometer operated from 40 to 400 Daltons in electron impact mode.

The SPME "syringe" used was a Supelco manual fiber holder equipped with a fiber coated with an 85 μm thick polyacrylate solid phase.

Solid-Phase Microextraction of Illicit Preparations

The illicit PMA preparation (crushed if tablet form, approximately 100 mg containing between 30 and 60% PMA) was placed into a 2 mL glass GC autosampler phial and capped with a teflon backed septum cap. After placing the phial on its side, the septum was carefully pierced with the SPME needle; it was allowed to protrude only about 5 mm into the phial. The fiber was then exposed to the headspace while taking care not to touch powder. Upon completion of headspace extraction, the fiber was withdrawn into the needle, and then placed into the injector of the gas chromatograph. The fiber was then exposed immediately to the hot interior of the chromatographic injector and data acquisition commenced; the fiber was left in place, exposed to the hot environment, until required for the next extraction.

Liquid Extraction of Illicit Preparations

The illicit PMA preparation (130 mg) was treated with an aqueous solution of sodium bicarbonate (5 mL, 10%) and extracted with dichloromethane (5 mL). The extract was passed through a column of anhydrous sodium sulfate (200 mg) and then blown to dryness using a stream of dry nitrogen. The concentrate was taken up in isooctane (0.5 mL) and an aliquot (1.0 μL) analyzed using GC-MS.

Results and Discussion

Samples of illicit PMA preparations were placed into a standard glass GC autosampler phial (2 mL capacity) equipped with a teflon-backed septum. It was a simple matter to pierce the septum with the needle of the SPME "syringe" and expose the adsorbent fiber to the headspace above the specimen.

Previous work in this laboratory (7) and by others (4,5,8) has established that important variables affecting the outcome of headspace solid-phase microextraction are the temperature at which adsorption takes place, the time span over which adsorption takes place, and the polarity of the SPME adsorbent.

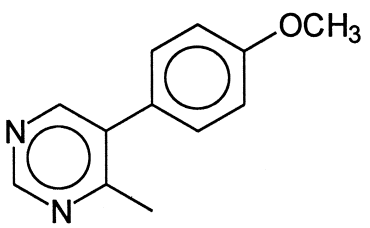
For this study, an adsorbent of medium polarity (polyacrylate resin) was chosen because analytes covering a range of polarity were expected.

Adsorption was performed at room temperature (25°C) over 5 min, 10 min, and 30 min. Desorption at 290°C into the injector of a gas chromatograph-mass spectrometer gave the data shown in Fig. 1. As is to be expected, adsorption over a longer period of time enables recovery of greater amounts of analyte. However, even after only 5 min adsorption, the technique yields valuable information regarding manufacturing impurities in the illicit preparation.

In order to assess the affects of temperature, adsorption was also performed for 5 min at 45°C and at 65°C; Figure 2 shows a small window of the signals obtained at these temperatures compared with that obtained from 5 min adsorption at room temperature. Elevation of the adsorption temperature from 25°C to 65°C resulted in approximately an order of magnitude increase in detector response. The response at 65°C for 5 min even exceeded that achieved over 30 min at 25°C. Recovery gains are to be expected when an increase in temperature results in a favorable change in the equilibrium between specimen, headspace, and adsorbent. Although it is generally true that an increase in adsorption temperature results in an increase in response, some compounds, particularly those with a high volatility for example, exhibit the opposite behavior (7). This arises because a higher temperature leads to desorption of analyte from the fiber, even though the headspace might be richer in the analyte. It is, therefore, not wise to select arbitrarily a very high adsorption temperature without first assessing its influence upon adsorption thermodynamics.

In order to "clean" the fiber between analyses, it was merely left in the injection port of the GC until required. The chromatogram displayed in Fig. 3 is a system blank produced by cleaning the fiber in the injection port after the adsorption at 65°C described above. Bearing in mind that the maximum peak abundance in Fig. 3 is about 8000 (many times smaller than the smallest peaks detected in the other chromatograms displayed) it can be seen that carry over of analytes between injections is minimal.

The compounds detected in the PMA preparation were 4-methoxyphenol [**2**], 4-methoxybenzaldehyde [**3**], 4-methoxyphenyl-2-propanone [**4**], 4-methoxyphenyl-2-propanol [**5**], and 4-methoxyphenylpropene [**6**]. A tentative identification of 4-methyl-5-(4'-methoxyphenyl)pyrimidine [**7**] was also made; work towards the unambiguous synthesis of this compound is underway.



[7]

Based on the work of Van Der Ark et al. (9), the presence of [**7**] indicates that PMA was likely to have been prepared using the Leuckardt reductive amination of 4-methoxyphenyl-2-propanone, while the presence of 4-methoxybenzaldehyde suggests that this aldehyde might have been the precursor for the ketone. Although a direct intermediate between the aldehyde and the ketone was not detected in the preparations, the presence of 4-methoxyphenyl-2-propanol suggests the involvement of reductive chemistry. It is, therefore, possible that 4-methoxybenzaldehyde was converted into 4-methoxyphenyl-2-nitropropene [**8**] which was then reduced to the ketone.[3] Such a reduction is likely to produce 4-methoxyphenyl-2-propanol as a by-product. If 4-methoxyphenyl-2-propanone contaminated with 4-methoxyphenyl-2-propanol is subjected to the Leuckardt reaction, then 4-methoxyphenylpropene could easily arise from simple dehydration of the alcohol. 4-Methoxyphenol is likely to originate from 4-methoxybenzaldehyde by the action of adventitious oxygen, either during storage of the aldehyde or during its elaboration into 4-methoxyphenyl-2-propanone. Although this oxidation is obscure, it might proceed by a mechanism similar to the Dakin Reaction (10). The chemistry de-

[3] Analysis of a preparation using inductively-coupled plasma-mass spectrometry showed a high concentration of tin to be present. This could be a residue of a tin-based reducing agent.

A contribution from Forensic Science and the University of South Australia.

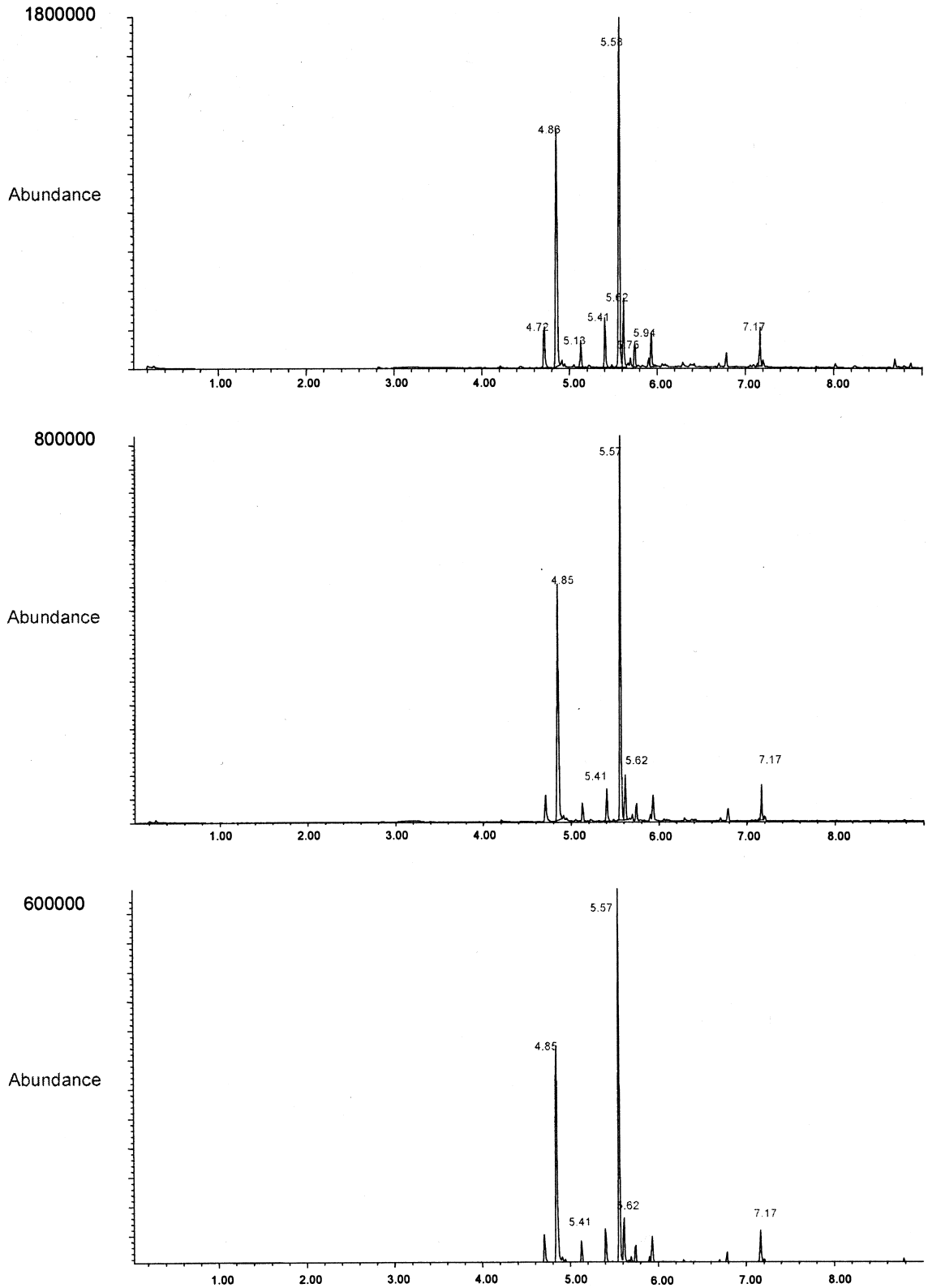
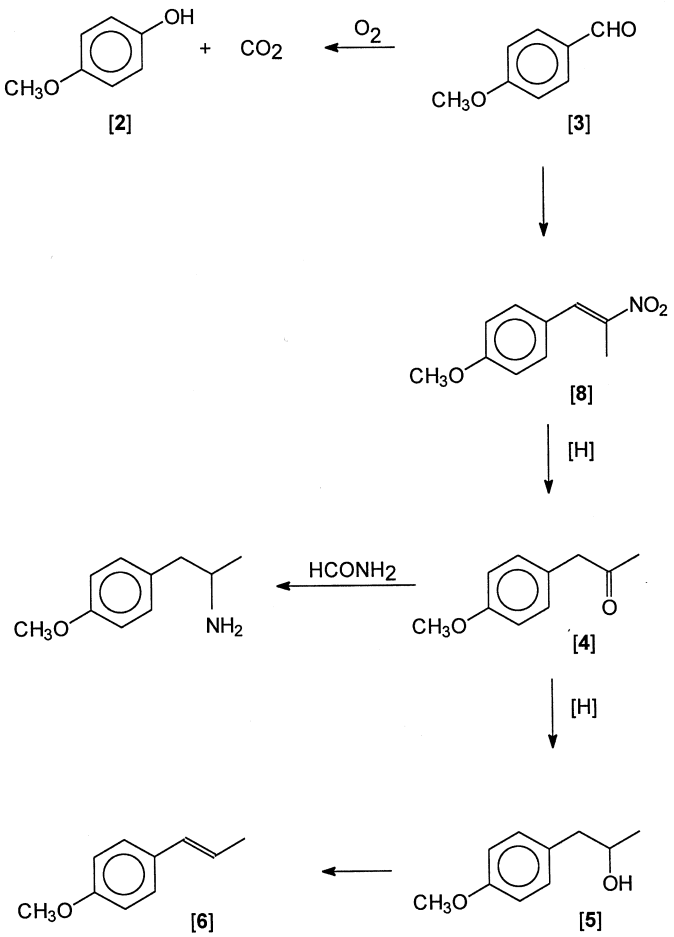


FIG. 1—*Solid-phase microextraction; time dependency. Total ion chromatograms obtained after SPME of headspace sampled for 5 min (bottom trace), 10 min (middle trace), and 30 min (top trace). Each trace is normalized to the highest peak in the chromatogram, the abundance figures given on the vertical scale indicate the relative sizes of the signals.*

scribed above is outlined in Scheme 1, mass spectral data for these compounds are given in Table 1.



SCHEME 1

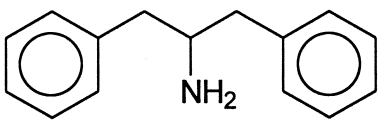
In order to compare SPME with liquid-liquid (LL) extraction a PMA preparation that had been subjected to SPME was dissolved in buffer (pH 9.6), extracted with dichloromethane, and analyzed using GC-MS after concentration at room temperature. The results of this analysis are shown in Fig. 4.

The most obvious difference between SPME and LL extraction is that the former did not indicate the presence of the major component of the preparation, PMA. Presumably, this is because the drug is present as its sulfate salt, which would have a very low vapor pressure. This aspect of SPME might be of benefit to drug profiling in general; attention can be focused upon minor components of the mixture, chromatographic artefacts arising from injection of large quantities of drug might not be a problem, and poor GC-MS performance as a result of the drug overloading the column and ion source might be avoided. On the other hand, impurities and by-products that happen to be in their salt form might not be detectable using SPME; detection of such compounds might be vital for profiling purposes.

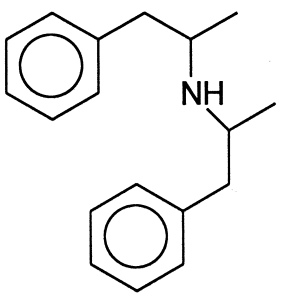
All the major peaks at high retention time in Fig. 4 appear to be due to compounding agents present in the table (e.g., derivatives of stearic acid and palmitic acid). Such compounds were not recovered in our SPME experiments. This also can be a positive feature in that the performance of the chromatographic injection liner does not deteriorate over the course of many injections of such substances.

In conclusion, SPME offered a very rapid, solvent-free, and nondestructive means of recovering volatile compounds from the PMA preparations. The technique did not overload chromatographic components or the mass spectrometer source, nor did it lead to confusing artefacts arising from reactions between amphetamines and extraction solvents. SPME appeared to be a poor technique for the recovery of PMA in its sulfate salt form. Although this might not be a limitation with respect to the major drug present in seizures examined by us, sensitivity for complex amines such as 1,3-diarylaminopropanes (analogues of [**9**]) or di-(arylisopropyl)amine (analogues of [**10**]) therefore might also be low. Given the robust nature of the sampling device and its small size, it is conceivable that SPME could be used for covert analysis of an illicit seizure without the need to significantly disrupt packaging.

For amphetamine profiling in general, SPME might represent a very effective and efficient screening technique that complements standard LL extraction.



[**9**]



[**10**]

Acknowledgments

Thanks are due to Dr. J. Skopec, Senior Research Chemist, Research and Development, Australian Government Analytical Laboratory, Sydney, Australia for performing the inductively-coupled plasma-mass spectrometric analysis.

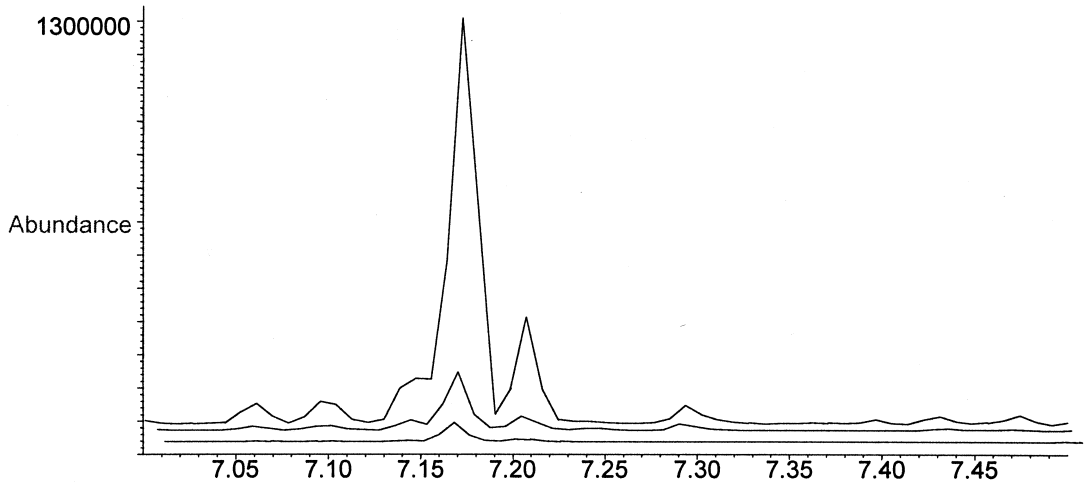


FIG. 2—*Solid-phase microextraction; temperature dependency. Total ion chromatograms obtained after SPME of headspace sampled for 5 min at 25°C (bottom trace), 45°C (middle trace), and 65°C (top trace). For clarity, each trace is an enlargement of the small peak at 7.17 min (tentatively identified as the pyrimidine [7]). Each peak in this figure is plotted against a single vertical axis. From Fig. 1 it can be seen that adsorption over 30 min gives a total ion abundance of 200,000 for [7]. From the above figure it is evident that adsorption for only 5 min at 65°C results in abundance approximately 5 times greater for this compound.*

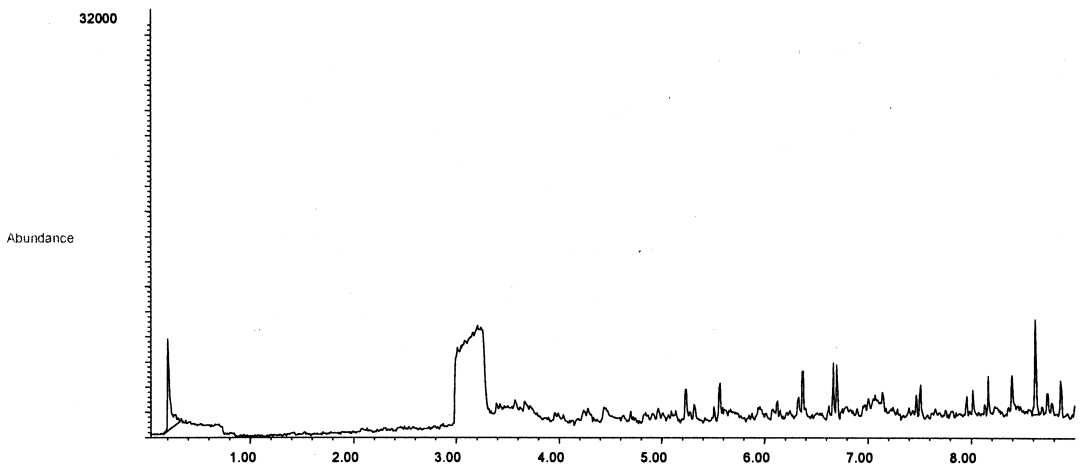


FIG. 3—*Solid-phase microextraction: carry-over assessment. Total ion chromatogram conducted using a fiber that had been exposed to headspace for 5 min at 65°C (i.e., as shown in Fig. 2) then left in the injection port of the gas chromatograph at 290°C for 10 min in order to clean it. The total ion abundance of the largest peak in the chromatogram is only about 8000, this is many times smaller than the smallest peaks depicted in Figs. 1 and 2.*

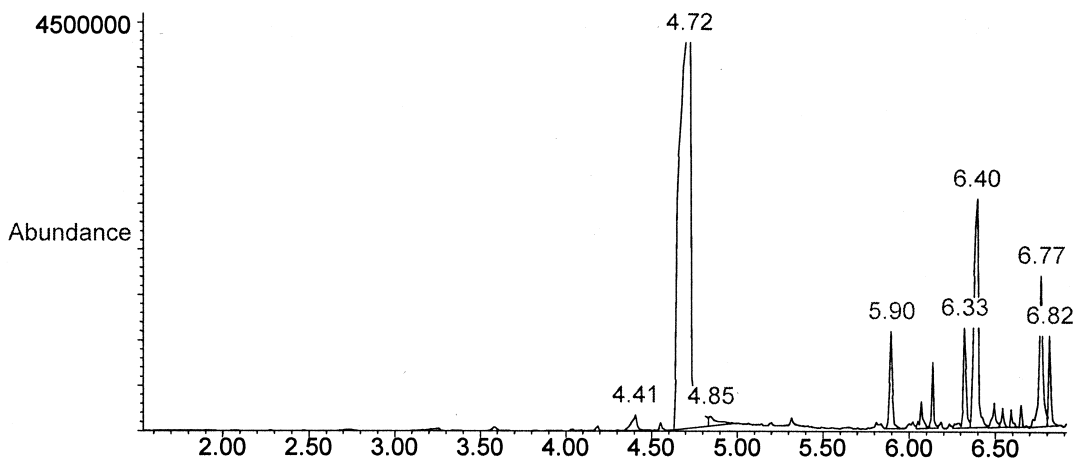


FIG. 4—*Liquid-liquid extraction. Total ion chromatogram resulting from liquid extraction of a powder sample identical to that used for SPME.*

TABLE 1—*Mass spectral data.*

Compound	Mass Spectral Fragmentation Pattern (Daltons/% Abundance)
2	109(100); 124(81); 81(69), 53(36), 63(10)
3	135(100), 136(70), 77(46), 92(25), 63(24), 50(16), 107(14)
4	121(100), 164(12), 43(12), 77(11), 78(10), 91(10)
5	121(100), 122(55), 107(15), 166(12), 77(12), 78(10), 91(10)
6	148(100), 147(54), 117(31), 77(29), 133(25), 105(22), 51(15), 91(15)
7	200(100), 117(36), 89(21), 132(17), 185(15), 199(15), 201(15), 63(12)

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