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Solid-Phase Extraction for Profiling of Ecstasy Tablets*

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ABSTRACT: A solid-phase extraction (SPE) procedure has been developed for impurity profiling of illicit tablets containing 3,4-methylenedioxy-N-methyl-amphetamine (MDMA, ecstasy). Following initial comparison of liquid-liquid extraction and solid-phase extraction, SPE was found to be preferable because it afforded higher extraction efficiencies and shorter extraction times. Procedure blank samples were also analyzed to identify constituents of the extracts which did not originate in the ecstasy tablets. The developed procedure was subsequently applied to 12 samples of seized ecstasy tablets and a comparison was made of these samples to determine similarities and obtain inferences with respect to commonality of origin.

KEYWORDS: forensic science, solid-phase extraction, ecstasy, profiling, impurities

Profiling of clandestinely manufactured drugs is concerned with the detailed chemical examination of impurities derived from their manufacture, if the drugs are synthetic, or co-extracted, if they are derived from natural products. These should be distinguished from diluents and adulterants which have been added deliberately. Many workers have reported on profiling of amphetamine (1–8), methamphetamine (9–12), heroin (13–18), cocaine (19–25), and 3,4-methylenedioxy-N-methyl-amphetamine (MDMA) (26–31). Profiling serves as a tool to relate different street drug seizures to a common source, to determine the origin of drugs manufactured from natural sources (32) or synthetic routes for synthetic drugs, and to identify impurities found in illicit drugs which may cause public health risks because of their inherent chemical or biological hazards (33).

"Ecstasy" is the common name for MDMA, a synthetic hallucinogenic amphetamine, which has become one of the most widely used illicit substances in Europe. It is almost always produced in clandestine laboratories and very often contains various impurities such as reaction by-products, synthetic intermediates and contaminants from reagents, which accumulate during the synthetic sequence because of the lack of quality control in clandestine laboratories. These impurities often represent a very small percentage of the total weight of the finished product and their analysis by instrumental methods usually requires a preliminary extraction process to isolate and concentrate the analytes from the total tablet content. In the process, interfering materials are removed and the target substances are concentrated into a solvent that is suitable for introduction into the analytical instrument selected. Although lengthy and time-consuming, these procedures are of paramount importance, resulting in the removal of the major constituents—the active drug substances, diluents and adulterants—and concentration of the minor impurities.

Liquid-liquid extraction (LLE) of impurities from street drugs has until now been the method of choice for sample preparation. Many problems are associated with LLE, including lengthy handling time and the need to concentrate the sample after extraction. Sample preparation techniques using solid-phase extraction (SPE) have not been widely used for the profiling of street drugs, and there are few reports in the literature (34). In this study, SPE and gas chromatography (GC) were used for the extraction and analysis of impurities in ecstasy tablets obtained from different street drug seizures, and comparisons were made of their chemical signatures (profiles).

Experimental

Materials and Reagents

Sixteen different samples of ecstasy tablets used in the study were provided by the Forensic Science Services, Metropolitan Laboratory, London. All reagents used were of analytical or high-pressure liquid chromatography (HPLC) grade. Solid-phase extraction columns (Bond Elut®) were obtained from Varian Associates, Harbor City, CA.

Gas Chromatography

Gas chromatography was carried out using a Hewlett-Packard 5890 Series II instrument fitted with a flame ionization detector and equipped with a fused silica capillary column (HP5, 30 m × 0.32 mm inside diameter 0.25 μm film thickness). Samples (volume 1 μL) were injected with an injection port temperature of 270°C at a linear velocity of 30 cm/s. The column oven temperature was programmed from an initial temperature of 80°C (1 min) at 35°C/min to 180°C (held for 18 min) then at 50°C/min to 300°C (held for 2 min).

Solid-Phase Extraction Versus Liquid-Liquid Extraction

A comparison of SPE and LLE was carried out to evaluate the use of SPE as an alternative extraction procedure to LLE. Ten portions of a ground and homogenized ecstasy sample, 50 mg each, were separated into two groups of five portions. One group was subjected to LLE, the other to SPE. Both groups were analyzed by gas chromatography/flame ionization detection (GC/FID) using the conditions mentioned above.

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Solid-Phase Extraction

The tablets were crushed and 50 mg of the homogeneous powder were added to 1 mL phosphate buffer at pH 9. The suspension was then mixed on a rolling extractor for 30 min, centrifuged for 5 min and the supernatant was taken off. A period of 30 min was required for complete equilibrium to be achieved. SPE procedures were carried out using Varian Bond Elut C18 (200 mg) columns. The SPE column was conditioned with 10 mL of deionized water (to remove water-soluble impurities due to manufacture of the cartridge), 10 mL methanol (to solvate the column and remove other impurities), and 10 mL deionized water (to remove excess solvation solvent). The sample was then applied to the column at 1 to 2 mL/min. The column was then washed with 10 mL deionized water (to remove sugars and other unwanted water-soluble compounds) and dried under maximum vacuum (15 psi) for 5 min. The cartridge was subsequently eluted with 0.7 mL isopropanol (to remove any remaining water and recover neutral analytes retained only by lipophilic interaction with the sorbent surface) followed by 1 mL ethyl acetate with 2% ammonia (to elute basic analytes retained by ion-exchange interactions). These two fractions were combined and evaporated in a nitrogen stream to about 100 μL. Care was taken not to evaporate to dryness to avoid evaporating the amphetamine impurities present in ecstasy samples.

Liquid-Liquid Extraction

Phosphate buffer (1 mL, pH 9) was added to 50 mg of the same homogeneous powder used for the SPE procedure. The solution was then mixed on a rolling extractor for 30 min. Ethyl acetate (1 mL) was added to the solution and the tube was again mixed on a rolling extractor for 30 min. A period of 30 min was required for complete equilibrium to be achieved. The sample was then centrifuged for 5 min at 3000 rpm and the organic layer was taken off and evaporated in a nitrogen stream to about 100 μL, with care being taken not to evaporate to dryness. At this stage 100 μL of the internal standard, triethylamine, was added. Only 1 μL of the sample was injected in the GC-FID.

Blank Extraction by SPE and LLE

Two blank aliquots of phosphate buffer were extracted by SPE and LLE using the same solvent and cartridges used in the rest of the work to ensure that analytical artifacts were not introduced.

Application to Real Case Samples

Twelve different samples of seized ecstasy tablets were analyzed using solid-phase extraction and GC-FID. The extraction procedure and the GC-FID conditions were as described above.

Comparison of Tablets from Same Seizure

Two tablets from the same seizure were chosen at random and were analyzed for the purpose of establishing common origin. Each tablet was ground, extracted, and analyzed separately to avoid cross contamination.

Results and Discussion

Solid Phase Extraction Versus Liquid-Liquid Extraction

Five portions of the same ecstasy sample were extracted by SPE and analyzed by GC-FID. The principal peaks of chromatograms representing impurities of each portion were numbered. The ratios of their peak areas to that of the internal standard and the means of the relative peak areas were calculated. The same procedure was performed for the other five portions extracted by LLE.

To evaluate the data collected from the various chromatograms of ecstasy samples extracted by SPE and LLE, statistical analysis was performed to compare the means of the relative peak areas of seven major impurity peaks present in the chromatograms. The two-sided *t*-test was used to assess if means of the selected peaks were significantly different for each extraction method. In addition, a comparison was made of the means of relative peak areas of peaks number 1, 2, and 7 for each extraction method and the *p* value was calculated using a computer software package (Minitab). The *p* value of each *t*-test as shown in Table 1 indicated that there are significant differences between the evaluated means. Since peak areas for samples extracted by SPE were larger than those extracted by LLE, and the *p* values showed significant differences between the mean values, it is safe to conclude that SPE provided a better extraction yield of impurities in ecstasy samples.

Another illustration of the differences in the mean of relative peak area of peaks 1, 2, and 7 is shown in Fig. 1 where SPE yielded better peak recovery than LLE.

Blank Extraction by LLE and SPE

Blank extracts showed no major peaks especially at the retention time as the ecstasy impurities peaks where present. Figure 2 demonstrates chromatograms of two blank samples extracted by LLE and SPE.

Applications to Seized Samples

The magnitude of inter- and intra-batch variation of impurity profiles governs the establishment of a "common source." Intra-batch variation should be less than inter-batch variation since samples belonging to the same batch expected to show more similarities than samples belonging to different batches.

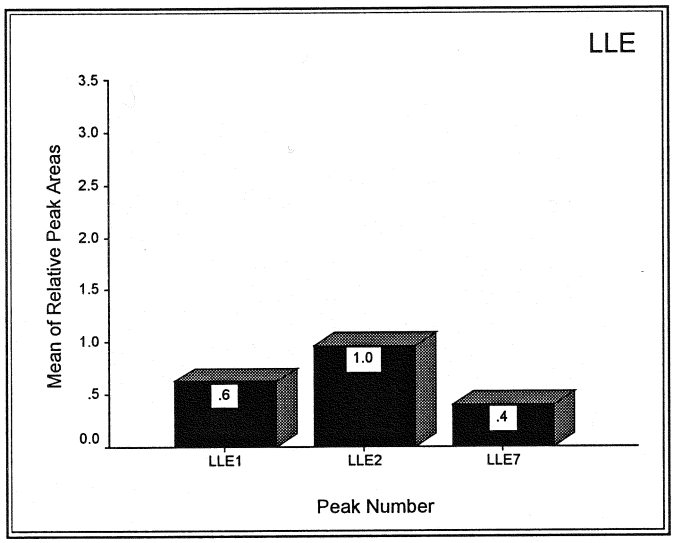
Comparison of Different Seizures (Inter-Sample Variation)

Ecstasy samples from 14 different seizures (packages) were extracted by solid-phase extraction and analyzed by GC-FID for the purpose of establishing commonality. Figure 3 shows a comparison of impurity profiles of three ecstasy samples from three different seizures.

The above impurity profiles allowed for visual comparison of three different ecstasy samples. It is clear that they contain great variations and consequently do not belong to a common batch (source).

TABLE 1—*Two sample* t*-test results of peaks 1, 2, and 7 for both SPE and LLE extraction methods.*

Two Sample *t*-test for Peak 1
95% C.I. for LLE 1 − SPE 1: (−1.859, −0.12)
t-Test LLE 1 = SPE 1 (not=): $t = -2.57$
$p = 0.030$ DF = 9
Two Sample *t*-test for Peak 2
95% C.I. for LLE 2 − SPE 2: (−2.564, −2.053)
t-Test LLE2 = SPE 2 (not=): $t = -20.82$
$p = 0.001$ DF = 9
Two Sample *t*-test for Peak 7
95% C.I. for LLE 7 − SPE 7: (−0.176, −0.057)
t-Test LLE 7 = SPE 7 (not=): $t = -4.63$
$p = 0.0025$ DF = 9



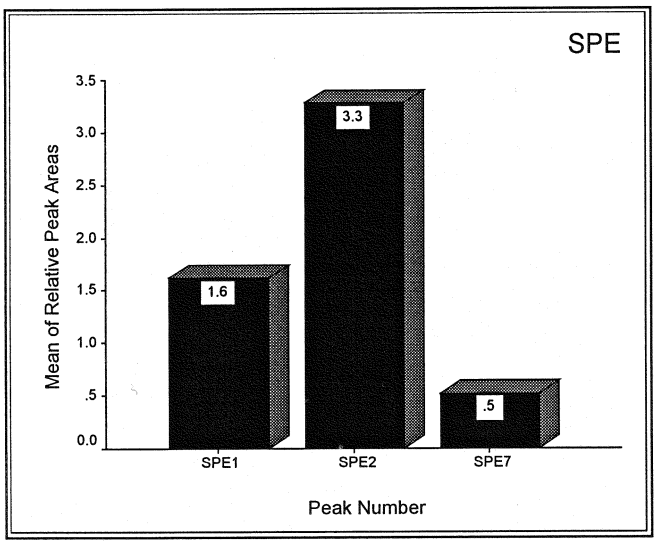


FIG. 1—*Comparison of mean relative peak areas for three different peaks from chromatograms of an ecstasy sample extracted by LLE and SPE.*

Comparison of Tablets from Same Seizure (Intra-Sample Variation)

Impurity profiles of two randomly chosen ecstasy tablets from the same seizure (package) were obtained using solid-phase extraction and GC-FID. The generated impurity profiles showed similarities indicating that both samples belong to the same batch. Figure 4 demonstrates an overlay comparison of their impurity profiles showing little variation.

Future Work

Among the numerous advantages of using solid-phase extraction for profiling of illicit drugs are efficiency, no cross contamination of phases, and no emulsion problems, as with LLE, due to the presence of fatty acids in ecstasy tablets. Further profiling can be very difficult with a sample of high purity since the impurity profile is dependent on impurity abundance in the sample. An excellent extraction of impurities in high-purity drug exhibits by LLE requires repeated extractions and the use of a large volume of solvents, which can cause loss of analytes and consequently poor impurity profiles. SPE can provide excellent impurity profiles with superior selectivity, high recovery, and reduced organic solvents consumption. Finally, there is the possibility of automating the extraction procedure, which is more easily automated with SPE than with LLE. The automated SPE procedure will provide an attended extraction, saving precious analyst time and manpower, establishing more consistency and repeatability in the analysis, and allowing for a total automated procedure including extraction, chromatography, and data and statistical analysis.

Conclusion

Solid-phase extraction of impurities in ecstasy tablets proved to be more efficient than the traditional liquid-liquid extraction. SPE provided impurity peaks with higher intensities than did LLE and a shorter extraction time. Street samples of ecstasy were analyzed for profiling purposes using the SPE procedure. Samples from different seizures (packages) showed little variations and great similarities, indicating common batch origin, while those from different packages showed great variations, indicating different batch origin.

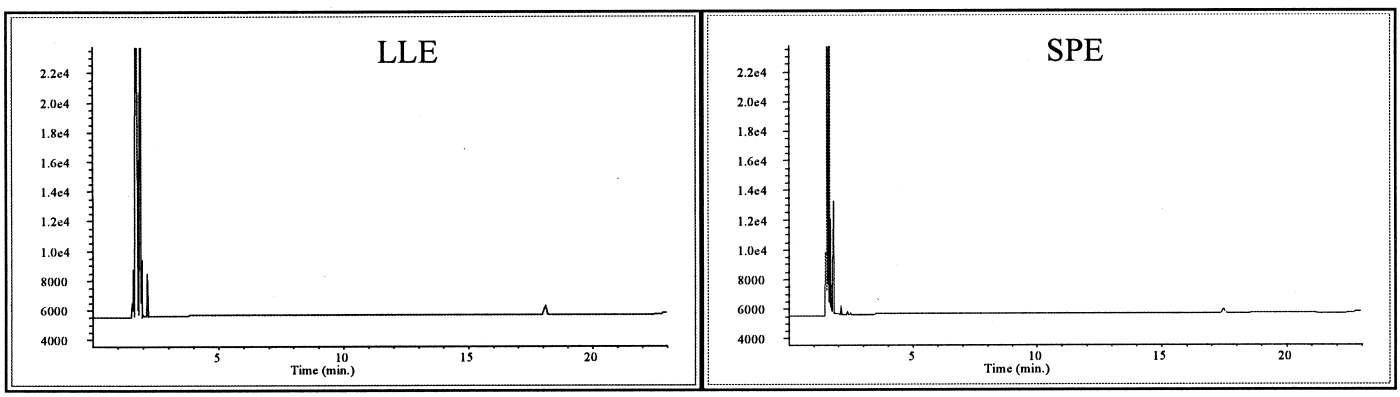


FIG. 2—*Chromatograms of blank sample extracted by LLE and SPE.*

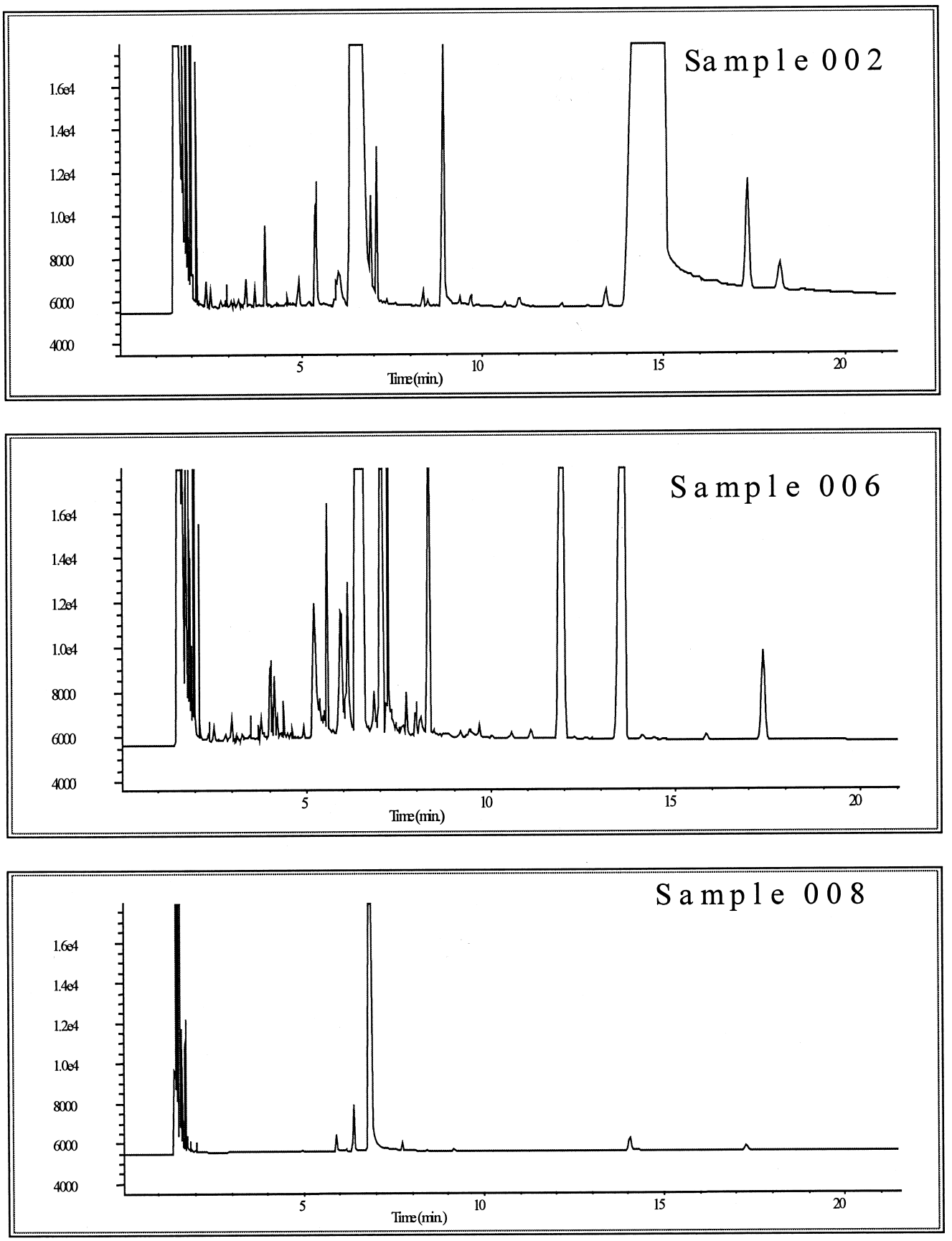


FIG. 3—*Impurity profiles of three different samples 002, 006, and 008 from three different seizures.*

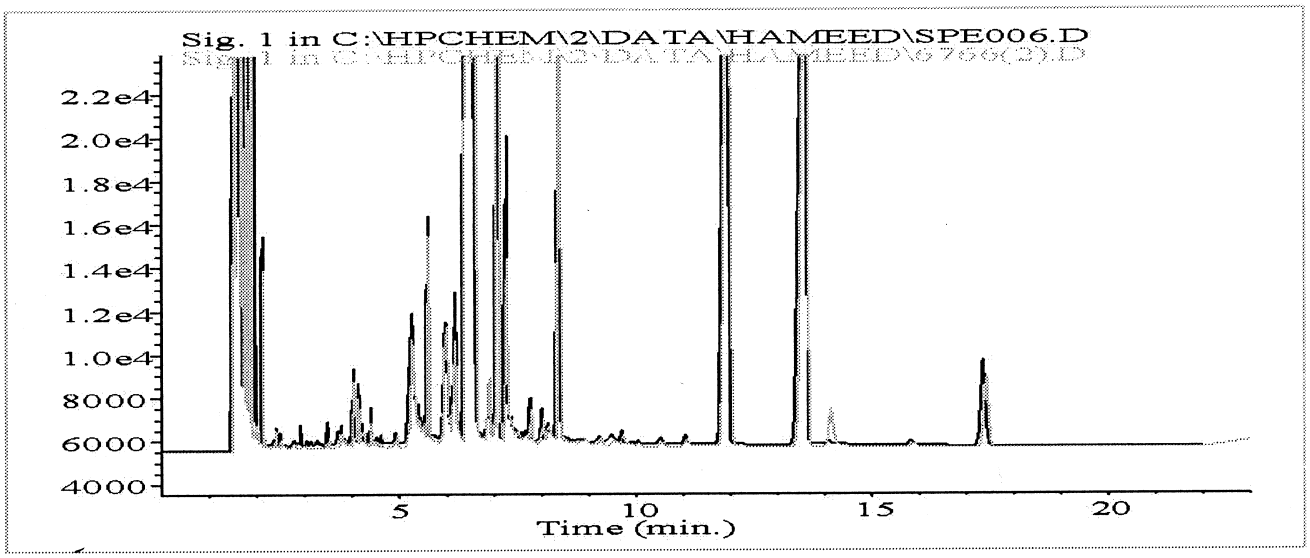


FIG. 4—*Overlay of impurity profiles of two samples from the same seizures.*

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