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## USE OF BONDED-PHASE SILICA SORBENTS FOR RAPID SAMPLING OF IMPURITIES IN ILLICIT AMPHETAMINE FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSES

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### SUMMARY

A simple and rapid method has been developed for the extraction of impurities from illicit amphetamine samples using bonded-phase silica sorbents. The drug is dissolved in phosphate buffer (pH 7) and added to a C<sub>8</sub> Bond Elut™ extraction column. The column is washed with water, and the impurities are then eluted with acetonitrile. The eluate is directly injected into the liquid chromatograph. This sample preparation technique has been compared with the traditional liquid–liquid extraction method.

High-performance liquid chromatographic analysis of the impurities is carried out on a reversed-phase C<sub>18</sub> column with an acetonitrile–water gradient as mobile phase. Peaks are monitored by UV detection at 220 and 254 nm.

A series of seized amphetamine samples has been analysed, and the procedure gives detailed impurity patterns suitable for the comparison of samples. Compounds are identified by absorbance ratios ( $A_{220}/A_{254}$ ).

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### INTRODUCTION

Recently there has been an increasing interest in the application of advanced chromatographic methods for the comparison of seized drugs<sup>1</sup>. This is because sources and traffickers of drugs are easier to counteract if intelligence information is available on which seizures are related. One means of obtaining such information is through the comparison of trace impurity profiles<sup>2</sup>. Profiling work has been performed on different kinds of drug<sup>3–9</sup>, and capillary gas chromatography (GC) is usually employed for these analyses.

Capillary GC has also been used for the profiling of impurities in illicit amphetamines<sup>10,11</sup>. However, a high-performance liquid chromatographic (HPLC) method was recently shown to be suitable for the analysis of these impurities<sup>12</sup>.

The sample preparation methods currently available involve liquid–liquid extraction of the impurities from the bulk drug. Problems related to these methods include lengthy handling time and the need to concentrate the sample after extraction.

In recent years, numerous sample preparation techniques using the liquid–solid approach have been introduced. Adsorbent cartridge methods have been developed for a number of drugs in biological fluids<sup>13</sup>. The work described in this report is based on this principle; bonded-phase silica cartridges for the extraction of impurities.

Attention has been focused on impurity profiles obtained from amphetamine synthesized by the popular Leuckart route. Several of the impurities found in amphetamine synthesized by this method have been isolated and characterized, and most of them are neutral or weakly basic<sup>14</sup>. These compounds can be retained on a reversed-phase Bond Elut matrix when an aqueous solution of amphetamine salt is applied to the column. The amphetamine and the diluents are then washed out with water, and the impurities are eluted with an organic solvent. This procedure has the advantage of minimizing handling time and eliminating the need to concentrate the extract.

The extracts are analysed by HPLC and the impurities are monitored by UV detection at 220 and 254 nm. The response ratios at these wavelengths can be used for identification purposes.

## EXPERIMENTAL

### *Chemicals*

Analytical grade dichloromethane and methanol were obtained from Riedel de Haën (Hannover, F.R.G.). HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, U.K.). Phosphate Buffer-Titrisol (pH 7) was provided by E. Merck (Darmstadt, F.R.G.). Samples of amphetamine were obtained from the Forensic Laboratory Department, National Bureau of Crime Investigation (Oslo, Norway).

### *Apparatus*

*Bond Elut extraction.* A Vac Elut vacuum manifold and Bond Elut<sup>TM</sup> columns from Analytichem International (Harbour City, CA, U.S.A.) were used to process the samples. The extraction columns were packed with 100 mg of C<sub>18</sub>, C<sub>8</sub> or C<sub>2</sub> bonded-phase silica adsorbents (Part No. 607101, 606101, 603101).

*High-performance liquid chromatography.* The liquid chromatograph was an SP 8700 (Spectra-Physics, San Jose, CA, U.S.A.) equipped with a SpectroMonitor III variable-wavelength UV detector (LDC, Riviera Beach, CA, U.S.A.) and an SP 4270 integrator (Spectra-Physics). The eluent was monitored at 254 and 220 nm. The UV spectra were recorded with an HP 1040 A diode array UV detector (Hewlett-Packard, Palo Alto, CA, U.S.A.) connected to the liquid chromatograph. The injector was a Rheodyne Model 7125 (Berkeley, CA, U.S.A.) with a 20- $\mu$ l sample loop.

Samples were chromatographed on C<sub>18</sub> Spheri-5 Brownlee Labs. MPLC<sup>TM</sup> cartridges (Santa Clara, CA, U.S.A.), and the guard column (30  $\times$  4.6 mm I.D.) was directly connected to the analytical column (100  $\times$  4.6 mm I.D.). The mobile phase was an acetonitrile–water gradient. The gradient was programmed linearly from 35 to 100% acetonitrile in 15 min, then isocratic 100% acetonitrile for 3 min. The flow-rate was 1 ml/min, and the analyses were carried out at ambient temperature.

*Gas chromatography–mass spectrometry (GC–MS).* A Micromass 7070 F mass spectrometer (VG-Micromass, Altrincham, U.K.) combined with a Fractovap 4200 gas chromatograph (Carlo Erba, Milan, Italy) was used for the identification of im-

purities eluted from the HPLC column. The eluate from ten injections was collected and evaporated to dryness under a stream of nitrogen at 35°C. The residue was dissolved in 50  $\mu\text{l}$  of dichloromethane, and 1  $\mu\text{l}$  was injected into the gas chromatograph. The experimental conditions for the GC-MS analysis have been described earlier<sup>12</sup>.

#### *Extraction of impurities*

*Sample solutions.* Seized amphetamine sulphate was ground to a fine powder and dissolved in phosphate buffer (pH 7). Solutions were prepared for liquid-solid extraction (50 mg/ml) and for liquid-liquid extraction (60 mg/ml).

*Liquid-solid extraction.* One Bond Elut C<sub>8</sub> column for each sample was inserted into the Vac Elut, which was attached to a vacuum source. A 500-ml vacuum flask was placed between the vacuum pump and the Vac Elut to collect the washes and waste materials. With the vacuum on, each column was conditioned with two column volumes of methanol, followed by two column volumes of distilled water. At this point, the vacuum was discontinued to keep the columns from dehydrating. With the vacuum off, 1 ml of the sample solution was added to each column. The vacuum was then reapplied to draw the samples through the columns at *ca.* 3 ml/min. The matrix was washed with two column volumes of distilled water, and the vacuum was left on for 3 min to remove any traces of water.

A 1.5-ml polypropylene sampling tube (Greiner, Nürtingen, F.R.G.) in the Vac Elut rack was placed under each column. With the vacuum off, 100  $\mu\text{l}$  of acetonitrile was added to each Bond Elut column. After 2 min the vacuum was applied to draw the acetonitrile into the collection tubes, then turned off. Altogether this process was carried out four times. After the last application, the acetonitrile was immediately drawn off.

Each collection tube was thoroughly vortexed before injection into the liquid chromatograph.

*Extraction efficiency — Bond Elut procedure.* The waste and wash water after the application of samples to the Bond Elut columns were collected and analysed for impurities. The amount of impurities left on the columns after the last acetonitrile elution was then checked by passing two additional column volumes of acetonitrile and three column volumes of chloroform through the Bond Elut cartridges. The eluate was collected, evaporated to dryness under a stream of nitrogen at 35°C and dissolved in 0.4 ml of acetonitrile.

The extraction efficiency study was performed on ten different extraction columns after the addition of samples with various contents of impurities and diluents.

*Liquid-liquid extraction.* A 5-ml sample solution was extracted with 2 ml of dichloromethane by being vigorously shaken for 10 min. The dichloromethane extract was then transferred to a glass tube and evaporated to dryness under a stream of nitrogen at 35°C. The residue was dissolved in 50  $\mu\text{l}$ –1 ml of acetonitrile, depending on the purity of the sample.

## RESULTS AND DISCUSSION

### *Liquid-solid extraction using Bond Elut sorbents*

The retention of the impurities was studied as a function of several sampling

and elution parameters. The ability of various solvents to elute adsorbed impurities from C<sub>2</sub>, C<sub>8</sub> and C<sub>18</sub> Bond Elut materials was first examined. Hexane, diisopropyl ether, diethyl ether and ethyl acetate gave low-intensity chromatograms. Almost no peaks were detected when hexane was used as eluent. Dichloromethane, chloroform,

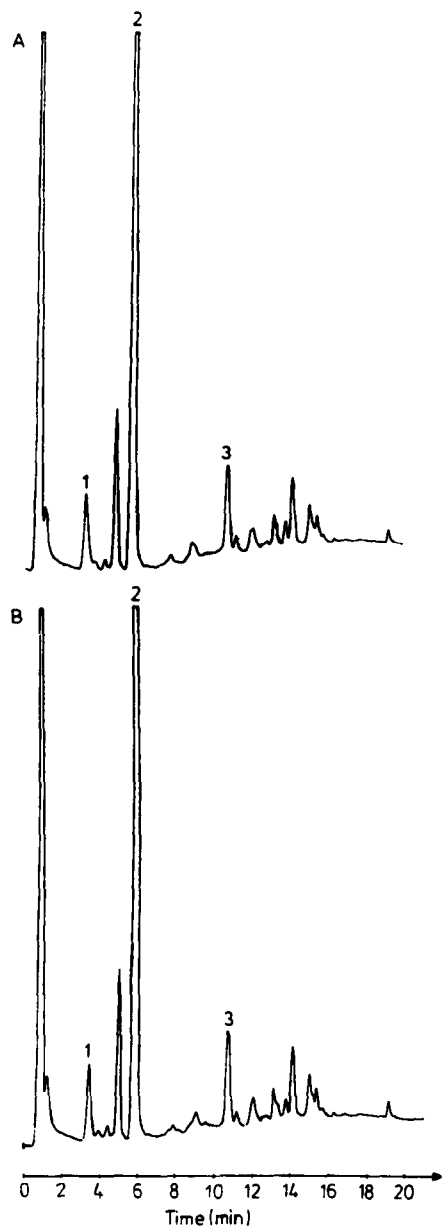


Fig. 1. HPLC impurity profiles of two separately analysed samples of Seizure I prepared by the Bond Elut procedure; detection by UV absorption at 254 nm. Peaks: 1 = N-formylamphetamine; 2 = 4-methyl-5-phenylpyrimidine; 3 = N,N-di( $\beta$ -phenylisopropyl)formamide.

acetonitrile and methanol showed similar extraction properties, and they all gave detailed impurity profiles. Acetonitrile was chosen as the extraction solvent, because the eluate can then be directly injected into the liquid chromatograph. Furthermore, sample blanks with acetonitrile as eluent gave no extra peaks.

The  $C_2$ ,  $C_8$  and  $C_{18}$  reversed phases performed similarly. However, the  $C_{18}$  sorbent did not demonstrate as good a reproducibility for the late eluting compounds as the others. In this work  $C_8$  columns were used, but  $C_2$  cartridges can also be employed.

Washing with one, two or three column volumes of water had no influence on the impurity profiles, but two column volumes were required to wash out most of the amphetamine and the diluents.

Various elution volumes of acetonitrile were tested. Two repeated applications of 0.1 ml gave the highest intensity chromatograms, but the best reproducibility of the extraction process was obtained after four applications of acetonitrile. To obtain a good reproducibility, it was important to wait for 2 min after the first three applications before drawing off the eluate. The extracts can be stored in capped polypropylene tubes at 4°C for two weeks without any change of the profile.

Fig. 1 shows the impurity profiles of a seizure synthesized by the method of Leuckart (Seizure I). The sample, which consisted of 80% amphetamine and was diluted with glucose, was prepared by the Bond Elut extraction procedure. The impurities N-formylamphetamine (N-f-A) and 4-methyl-5-phenylpyrimidine (4-me), peaks 1 and 2 respectively, are associated specifically with the Leuckart route<sup>14</sup>. N,N-Di( $\beta$ -phenylisopropyl)formamide (di-iso), peak 3, also provides strong evidence for this manufacturing method<sup>15</sup>. Identification of these compounds is consequently important for the verification of the Leuckart procedure. The extraction-efficiency study showed that less than 2% of these impurities and, on average, less than 5% of other trace compounds, were retained on the Bond Elut matrixes. Only amphetamine and diluents were detected in the waste water. The solubility properties of the impurities in the phosphate buffer are therefore the limiting factor concerning the intensities of profiles prepared by the Bond Elut procedure.

TABLE I  
REPRODUCIBILITY OF PEAK HEIGHT RATIOS OF IMPURITIES FROM SEIZURE I

$h_{N-f-A}$  = Peak height of N-formylamphetamine (peak 1);  $h_{4-me}$  = peak height of 4-methyl-5-phenylpyrimidine (peak 2);  $h_{di-iso}$  = peak height of N,N-di( $\beta$ -phenylisopropyl)formamide (peak 3). R.S.D. = relative standard deviation.

	Peak height ratio (mean)	S.D.	R.S.D. (%)
Liquid-solid extraction ( $n = 8$ )			
$h_{N-f-A}/h_{4-me}$	0.060	0.002	3.3
$h_{di-iso}/h_{4-me}$	0.067	0.002	3.0
Liquid-liquid extraction ( $n = 8$ )			
$h_{N-f-A}/h_{4-me}$	0.079	0.002	2.5
$h_{di-iso}/h_{4-me}$	0.085	0.003	3.5

As described earlier<sup>10</sup>, the amount of impurities is strongly influenced by the experimental conditions of the Leuckart synthesis. In order to remove ambiguities in the comparison of samples, ratios between peak heights of selected peaks from each profile can be measured. Coincidental peak height ratios and visual similarities in the profiles are therefore important indications of a common origin of the seizures. The reproducibility of the peak height ratios of  $h_{N-f-A}/h_{4-me}$  and  $h_{di-iso}/h_{4-me}$  after eight runs of samples from Seizure I is reported in Table I. The relative standard deviations (R.S.D.) which were less than 4%, are satisfactory for the comparison of samples. Visual inspection of the impurity profiles also showed that they were similar (Fig. 1A and B). Equivalent results were obtained for other seizures containing various amounts of diluents and impurities.

#### *Comparison with the liquid-liquid extraction procedure*

The liquid-liquid extraction procedure was optimized before being compared with the Bond Elut procedure, and several organic solvents were evaluated for the extraction of impurities. Dichloromethane was chosen as extraction solvent because of its low boiling point and high purity. Fig. 2 shows the impurity profile of Seizure I prepared by liquid-liquid extraction. Figs. 1 and 2 demonstrate the similarity between profiles obtained from the two sample preparation procedures when starting with the same amount of sample and dissolving in the same amount of acetonitrile. The intensity of most of the impurities from the traditional procedure was lower than the intensity of the Bond Elut profile. The reproducibility of peak height ratios when eight replicated samples from Seizure I were extracted by the liquid-liquid procedure is given in Table I. The R.S.D. is of the same magnitude as was obtained with the

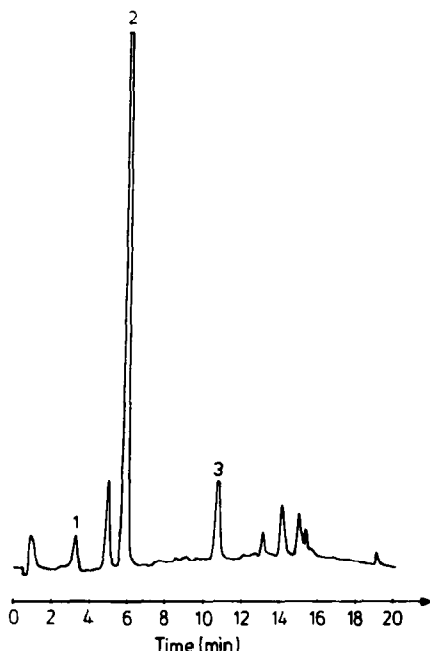


Fig. 2. HPLC impurity profile of Seizure I prepared by liquid-liquid extraction; detection by UV absorption at 254 nm. Peaks as in Fig. 1.

TABLE II  
REPRODUCIBILITY OF RETENTION TIMES

<i>Compound</i>	<i>Retention time (min) (mean, n = 10)</i>	<i>S.D.</i>	<i>R.S.D. (%)</i>
N-Formylamphetamine (peak 1)	3.57	0.06	1.7
4-Methyl-5-phenyl- pyrimidine (peak 2)	5.92	0.08	1.4
N,N-Di-( $\beta$ -phenyl- isopropyl)formamide (peak 3)	10.92	0.08	0.8

Bond Elut procedure. For more diluted samples, the R.S.D. varied from 8 to 13% ( $n = 8$ ).

#### HPLC analysis

The profiling of impurities was carried out by HPLC. With reversed-phase chromatography on a  $C_{18}$  column and an acetonitrile–water gradient as mobile phase, acceptable resolution of the major impurities in the Leuckart-synthesized amphetamine was obtained. A methanol–water gradient has been preferred previously<sup>12</sup>, but in this work the acetonitrile–water gradient was chosen because of less baseline drift, especially when the eluate was monitored at 220 nm. As shown in Table II, reproducible retention times were obtained. The chromatographic system was stable with minimal loss of efficiency after being used continuously for one year.

Several of the impurities in amphetamine synthesized by the Leuckart method were found to have a high response at 220 nm. By monitoring the eluent at 220 and 254 nm and measuring absorbance ratios, the characterization of compounds is strongly enhanced. The absorbance ratios ( $A_{220}/A_{254}$ ) of N-f-A, 4-me and di-iso were reproducible and are reported in Table III.

TABLE III  
ABSORBANCE RATIOS ( $A_{220}/A_{254}$ ) BASED ON PEAK HEIGHT MEASUREMENTS

<i>Compound</i>	$A_{220}/A_{254}$ (mean, n = 8)	<i>S.D.</i>	<i>R.S.D. (%)</i>
N-Formylamphetamine (peak 1)	14.9	0.5	3.4
4-Methyl-5-phenyl- pyrimidine (peak 2)	1.21	0.04	3.3
N,N-Di( $\beta$ -phenyl- isopropyl)formamide (peak 3)	23.0	1.5	6.5

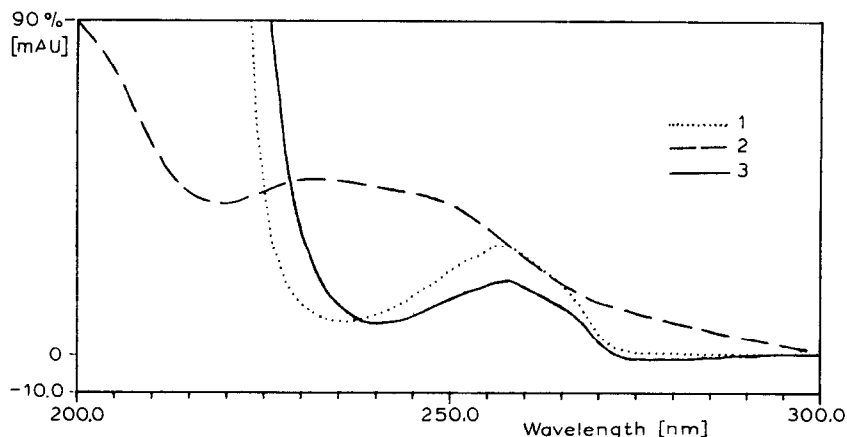


Fig. 3. UV spectra of peaks 1, 2 and 3 from Fig. 1: 1 = N-formylamphetamine; 2 = 4-methyl-5-phenylpyrimidine; 3 = N,N-di( $\beta$ -phenylisopropyl)formamide.

To achieve further characterization of impurities, the UV spectra were recorded with a diode-array UV spectrophotometer connected to the liquid chromatograph. Fig. 3 shows the UV spectra of peak 1 (N-f-A), peak 2 (4-me) and peak 3 (di-iso) from the chromatogram shown in Fig. 1. Identification of these compounds was verified by GC-MS analyses of collected eluates. In addition to N-f-A and 4-me, which have been identified earlier<sup>12</sup>, di-iso was identified. The GC-MS analysis of this compound showed two separate peaks due to stereoisomerism, with mass spectra identical with N,N-di( $\beta$ -phenylisopropyl)formamide<sup>15</sup>. This impurity makes an important contribution to the Leuckart HPLC profile, because of the high UV absorption at 220 nm.

Formamide, one of the precursors of the Leuckart route, eluted at the solvent front. Benzyl methyl ketone, a precursor of several routes of amphetamine synthesis, had a  $t_R$  of 5.09 min. The high-boiling pyridines<sup>16</sup> eluted after 13 min and were confirmed by GC-MS.

#### *Analysis of seized drugs*

Street drugs are usually encountered as mixtures with excipients and other drugs. The influence of common diluents on the impurity profile was therefore investigated. Sugars, such as glucose, lactose and sucrose, gave no extra peaks. Ephedrine chloride and caffeine eluted at the front and did not interfere with the impurity profile. Phenazone and procaine chloride eluted with early-eluting compounds ( $t_R$  2.67 and 2.77 min, respectively).

A series of seized amphetamine samples has been analysed by the new procedure, and the seizures synthesized by the Leuckart route were easily recognized. Fig. 4 shows the impurity profile of a seizure monitored at 254 and 220 nm. Although the sample only contained 20% amphetamine and was diluted with glucose, caffeine and phenazone, a typical Leuckart profile was obtained.



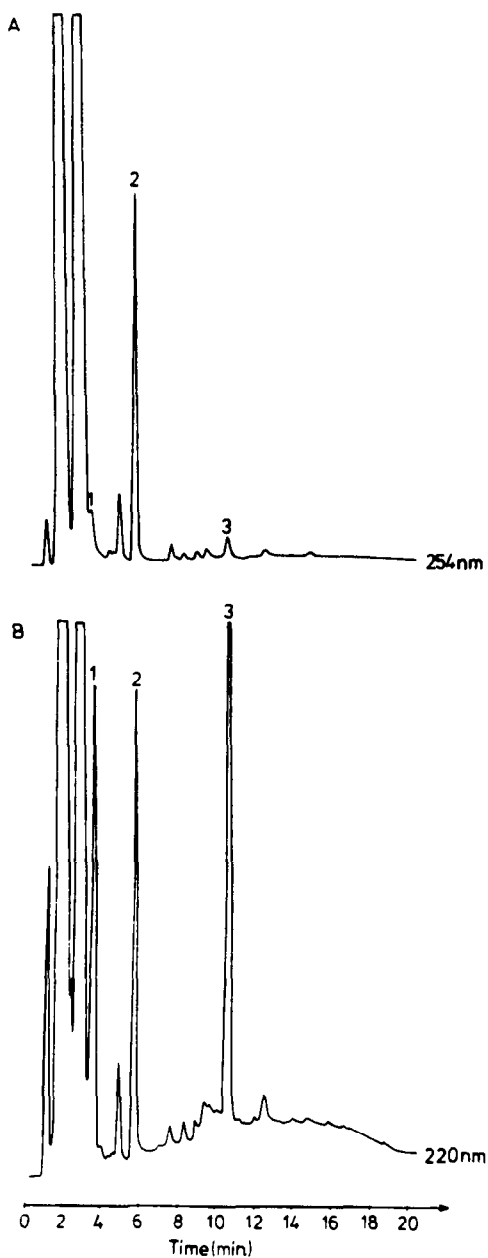


Fig. 4. HPLC impurity profile of a strongly diluted seizure; diluents were glucose, phenazone and caffeine. The sample was prepared by Bond Elut extraction. Detection by UV absorption at 254 nm (A) and 220 nm (B). Peaks as in Fig. 1.

## CONCLUSIONS

The liquid–solid extraction on C<sub>8</sub> Bond Elut cartridges is a rapid and reliable method for the sampling of impurities in illicit amphetamine. The advantages of the new procedure are minimal handling time and no need for concentration of the extracts. By HPLC analysis with UV detection at 220 and 254 nm, characteristic Leuckart impurity profiles are obtained.

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