Journal of Chromatography, 369 (1986) 365–377
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 969

PROFILING OF IMPURITIES IN ILLICIT AMPHETAMINE SAMPLES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY USING COLUMN SWITCHING

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(Received July 23rd, 1986)

SUMMARY

A simple high-performance liquid chromatographic method, suitable for routine profiling of impurities in illegally produced amphetamine, has been developed. Amphetamine is dissolved in acetonitrile–citrate buffer (pH 3) (2:8) and injected directly without further sample pre-treatment. The impurities are enriched on-line on a C_8 extraction column, while amphetamine and polar diluents are washed out with water. After washing for 1.5 min, a six-port valve is switched and an acetonitrile–0.2 *M* butylamine in water (pH 8) gradient elutes the impurities from the extraction column on to a C_{18} analytical column where they are separated. The compounds are monitored by UV detection at 220 and 254 nm. The total extraction and analysis time is 30 min. The method allows automated extraction and analysis to be performed.

INTRODUCTION

Illegal amphetamine samples often contain traces of by-products and intermediates from the illicit manufacture of the drug. The demand for an intimate knowledge of the composition of these impurities has increased in recent years, as detailed impurity profiles can provide valuable information concerning illegal methods of manufacture[1,2]. In addition, the presence or absence of impurities can aid in identifying drug samples of common origin[3,4]. Both capillary gas chromatographic (GC)[5,6] and high-performance liquid chromatographic (HPLC) methods[7] have proved to be useful in giving detailed impurity profiles suitable for the comparison of illicit amphetamine samples.

When different samples are compared, it is important to obtain an analytical method that causes minimum changes to the sample composition. One means of obtaining this is to use chromatographic systems that make direct injection of the sample possible.

On-line extraction columns and column switching in HPLC have recently been extensively performed for the direct injection of plasma samples[8–10]. The method is

also a powerful technique for the enrichment of trace impurities in environmental samples[11,12].

In this work, a column switching procedure was used for the isolation and enrichment of impurities in illegally produced amphetamine. A sample solution of amphetamine is injected directly on to an on-line extraction column and the main constituents, amphetamine and water-soluble diluents, are washed out with water. Less polar trace impurities from the synthesis, often present in amounts of less than 1%, are enriched on the column and are later eluted and separated.

An off-line liquid–solid extraction method with Bond Elut columns has earlier been described for the extraction of impurities in amphetamine[13]. However, the present on-line extraction procedure can easily be automated and is suitable for the routine screening of impurities in illegal samples.

In several countries in Europe amphetamine is most frequently synthesized by the Leuckart procedure[5], samples produced by other procedures being only occasionally encountered. A typical Leuckart profile has been compared with impurity profiles of amphetamine synthesized by three other routes. The contents of some of the impurities in illegal seizures have been determined.

EXPERIMENTAL

Chemicals

HPLC-grade water was obtained by purifying distilled water in a Milli-Q (Millipore, Bedford, MD, U.S.A.) filtration system. HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, U.K.). Analytical-reagent grade butylamine and dichloromethane were obtained from Fluka (Buchs, Switzerland) and Riedel de Häen (Hannover, F.R.G.), respectively. Citrate and phosphate buffer–Titrisol (pH 3 and 7) and orthophosphoric acid were provided by E. Merck (Darmstadt, F.R.G.).

Samples of seized amphetamine were obtained from the Forensic Laboratory Department, National Bureau of Crime Investigation (Oslo, Norway). Standards of N,N-di(β-phenylisopropyl)amine sulphate, 4-methyl-5-phenylpyrimidine, 2-benzyl-2-methyl-5-phenyl-2,3-dihydropyrid-4-one and a mixture of the high-boiling pyridines described by Van der Ark *et al.*[14] mainly consisting of 2,6-dimethyl-3,5-diphenylpyridine and amphetamine synthesized by reductive amination with Raney nickel as catalyst[15,16] were obtained as a gift from the Forensic Science Laboratory of the Ministry of Justice, Rijswijk, The Netherlands. Amphetamine synthesized by the nitrostyrene route[17] were kindly supplied by the National Laboratory of Forensic Science, Linköping, Sweden. Amphetamine were synthesized by the aluminium method[18] for research purposes at the Department of Chemistry, University of Oslo, Norway.

HPLC and column switching

Apparatus. An SP 8700 (Spectra-Physics, San Jose, CA, U.S.A.) gradient pump was used in combination with a Model 709 (LDC/Milton Roy, Riviera Beach, CA, U.S.A.) isocratic pump with a Model 7000 (Rheodyne, Berkely, CA, U.S.A.) six-port switching valve to perform the column switching. A Spectromonitor III variable-wavelength UV detector (LDC) was used for detection at 220 and 254 nm. Chromatograms were recorded on an SP 4270 integrator (Spectra-Physics). The injector was a Model 7120 (Rheodyne) with 100, 250 and 500 μl sample loops.

TABLE I

TIME SCHEDULE, SWITCHING

Time after injection (min)	*Event*
0	Sample is injected on to the extraction column with water as mobile phase (switching valve is in position 1)
1.5	Washing with water is finished. Switching valve: switching from position 1 to 2. Gradient started. Printer started. Desorption of trace impurities from extraction column, separation on analytical column starts
16.5	Reset of switching valve: position 2 to 1. Re-equilibration of extraction column with water before next injection
26.5	Elution and separation from analytical column are finished
30	Analytical column re-equilibrated with initial gradient. New injection.

Mobile phases and columns

MPLC™ cartridge columns (Brownlee Labs., Santa Clara, CA, U.S.A.) were used for both extraction and separation of the impurities. For the extraction and enrichment 7 μm C_2, C_4 and C_8 (15 $\times$ 3.2 mm I.D.) cartridges were evaluated. A 5 μm Spheri 5 C_{18} analytical column (100 $\times$ 4.6 mm I.D.) with a 5 μm C_{18} (30 $\times$ 3.2 mm I.D.) guard column were used for the separation of impurities. Measurements were repeated on two different analytical columns to ensure that no irreversible changes occured during the method development with the first column.

The washing procedure was performed with HPLC-grade water with a flow-rate of 1.0 ml/min. The following gradient was used for the desorption and separation of the impurities: (A) 0.2 *M* butylamine in water, the pH being adjusted to 8.0 with orthophosphoric acid; (B) 20% (v/v) of A in acetonitrile. The gradient was programmed linearly from 30 to 100% B in 20 min, then isocratic 100% B for 5 min. The flow-rate was 1.0 ml/min and the analyses were carried out at ambient temperature.

At the end of the day, the system was washed with 75% acetonitrile in water for about 30 min.

Samples. Samples were prepared at a concentration of 50 mg/ml in acetonitrile–citrate buffer (pH 3) (2:8). If necessary, the solutions were placed on an ultrasonic bath for 15 min.

Scheme of column-switching events. The scheme of switching events is given in Table I and the positions of the switching valve are shown in Fig. 1.

Quantitation. Quantitative estimates of the N-formylamphetamine (N-f-A), 4-methyl-5-phenylpyrimidine (4-me) and N,N-di(β-phenylisopropyl)amine (di-iso-an) contents were based on peak-height measurements. The content was calculated as the weight percentage of the impurity in "uncut" amphetamine sulphate. Contents of less than 0.003, 0.001 and 0.006% of N-f-A, 4-me and di-iso-an, respectively, were not quantified.

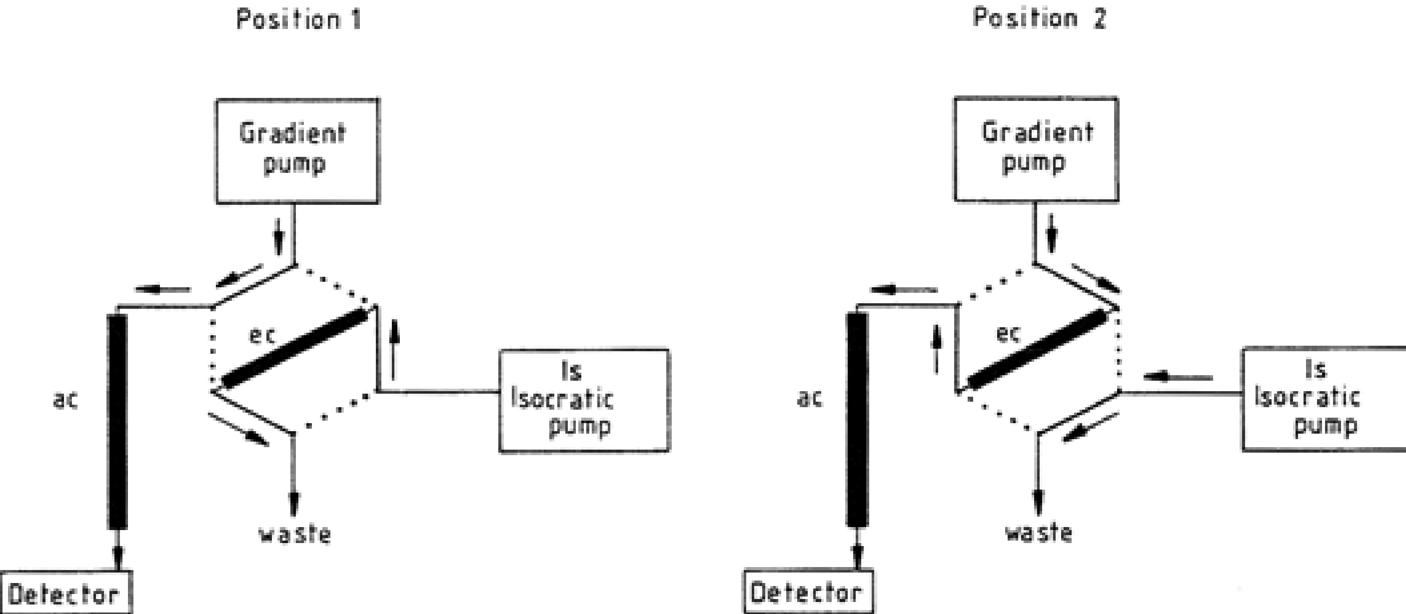


Fig. 1. Switching configuration for on-line pre-concentration and clean-up. ec = Extraction column; ac = analytical column; is = injection system.

Bond Elut extraction

The off-line liquid–solid sample preparation procedure with Bond Elut columns has been described elsewhere[13].

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 to check the standards and to identify the impurities eluted from the HPLC column. The eluates from ten injections were concentrated under a stream of nitrogen at 35°C, then extracted with dichloromethane. The dichloromethane extract was evaporated to dryness under a stream of nitrogen at 35°C. The residue was dissolved in 50 µl of acetonitrile and 1 µl of the solution was injected into the gas chromatograph. The experimental conditions for the GC–MS analysis have been described earlier[7].

RESULTS AND DISCUSSION

Extraction and pre-concentration of trace impurities

The impurity profile of an illegal amphetamine sample (sample 1) obtained by on-line pre-concentration on a C_8 reversed phase column is given in Fig. 2. The method allows a 100 µl sample solution (50 mg/ml) to be injected directly, as the dominating component, amphetamine, is efficiently removed during the washing procedure. Water-soluble diluents such as glucose and sucrose, which are often added during the drug distribution process in concentrations varying from 10 to 90%, are also washed out of the column. However, the less polar trace impurities originating from the synthesis are retained and enriched on the extraction column.

Structures, names and abbreviations of amphetamine and the impurities from sample 1 that are identified by GC–MS are given in Table II. The sample, which contained 90% amphetamine and was diluted with glucose, was synthesized by the Leuckart procedure. Several impurities associated specifically with this method were detected and identified (N-f-A, 4-me and di-iso-ad)[2]. The characteristic difference in

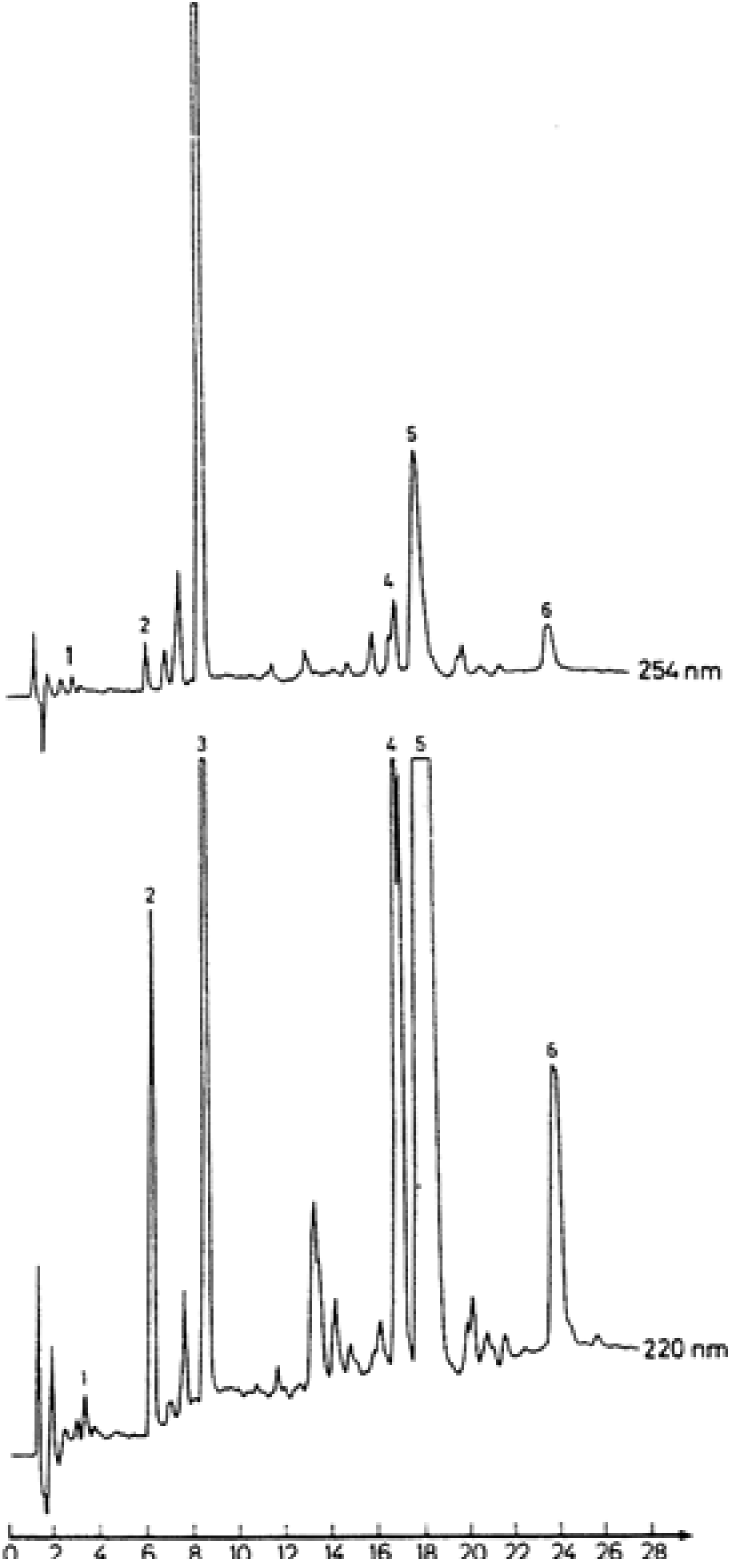


Fig. 2. HPLC impurity profile of a Leuckart-synthesized sample (sample 1) obtained by direct injection of 100 µl of a 50 mg/ml sample solution. Detection by UV absorption at 254 and 220 nm. Peaks: 1 = amphetamine; 2 = N-formylamphetamine; 3 = 4-methyl-5-phenylpyrimidine; 4 = N,N-di(β-phenylisopropyl)formamide; 5 = N,N-di(β-phenylisopropyl)amine; 6 = N,N-di(β-phenylisopropyl)methylamine.

absorbance at 254 and 220 nm of N-f-A, di-iso-ad, di-iso-an and di-iso-mn is demonstrated in Fig. 2.

A more detailed impurity profile is obtained by the on-line extraction method than with the off-line Bond Elut procedure described earlier[13], as a higher sample volume is injected. When only small sample amounts are available, the whole sample can be utilized for one injection to provide the highest possible sensitivity.

Several reversed-phase extraction columns were evaluated for the extraction of impurities. Short columns (2–5 mm) and large particle size (30–50 µm) packing

TABLE II

AMPHETAMINE AND IMPURITIES IN ILLEGALLY SYNTHESIZED AMPHETAMINE

Compounds as discussed in the text.

Formula	*Abbreviation*	*Name*
$C_6H_5-CH_2-CH(CH_3)-NH_2$		Amphetamine
$C_6H_5-CH_2-CH(CH_3)-NH-CHO$	N-f-A	N-Formylamphetamine
4-methyl-5-phenylpyrimidine (CH_3, N, N)	4-me	4-Methyl-5-phenylpyrimidine
$(C_6H_5-CH_2-CH(CH_3))_2N-CHO$	di-iso-ad	N,N-Di(β-phenylisopropyl)formamide
$(C_6H_5-CH_2-CH(CH_3))_2NH$	di-iso-an	N,N-Di(β-phenylisopropyl)amine
$(C_6H_5-CH_2-CH(CH_3))_2N-CH_3$	di-iso-mn	N,N-Di(β-phenylisopropyl)methylamine

materials are usually recommended for plasma samples, but for the trace enrichment of impurities in amphetamine samples, longer columns (1.5 cm) with small particle size (7 μm) packing material were chosen. These "high-capacity" extraction cartridges were preferred because of the large variation in the contents of impurities in different samples. Problems with clogging of the columns or increased back-pressure were not observed.

C_2, C_4 and C_8 extraction columns from Brownlee Labs. were compared by connecting them directly to the detector. With water as the mobile phase the difference in retention of amphetamine and the early eluting impurity in Leuckart-synthesized amphetamine, N-f-A, was measured. The experiment showed that N-f-A was most efficiently retained on the C_8 column, while amphetamine was washed out. A washing period of 1.5 min with water at 1.0 ml/min gave reproducible peak heights of the impurities [the switching valve is switched to position II 1.5 min after injection (Fig. 1)]. The switching valve was reset to position I (Fig. 1) after 15 min of elution

and the extraction column was equilibrated with water for 13.5 min before the next injection (30 min working cycle).

No significant difference was noted in extra peak broadening between back- and forward-flush elution. The forward-flush mode was chosen in order to maintain the protective filter aspect of the pre-column. Extra-column band broadening for the foreward-flush mode was less than 10%.

Efficient clean-up was obtained for concentrations up to 100 mg/ml amphetamine solutions and for the injection of 100–500 μl sample solutions. In general, 100 μl of the sample at a concentration of 50 mg/ml were injected.

Development of mobile phase for efficient desorption and separation of impurities

Several mobile phases were investigated to obtain the best desorption from the extraction column and separation of the impurities on the analytical column. When the acetonitrile–water gradient described previously[13] was used, a washing procedure was required after ten injections because of baseline drift. A decrease in efficiency of

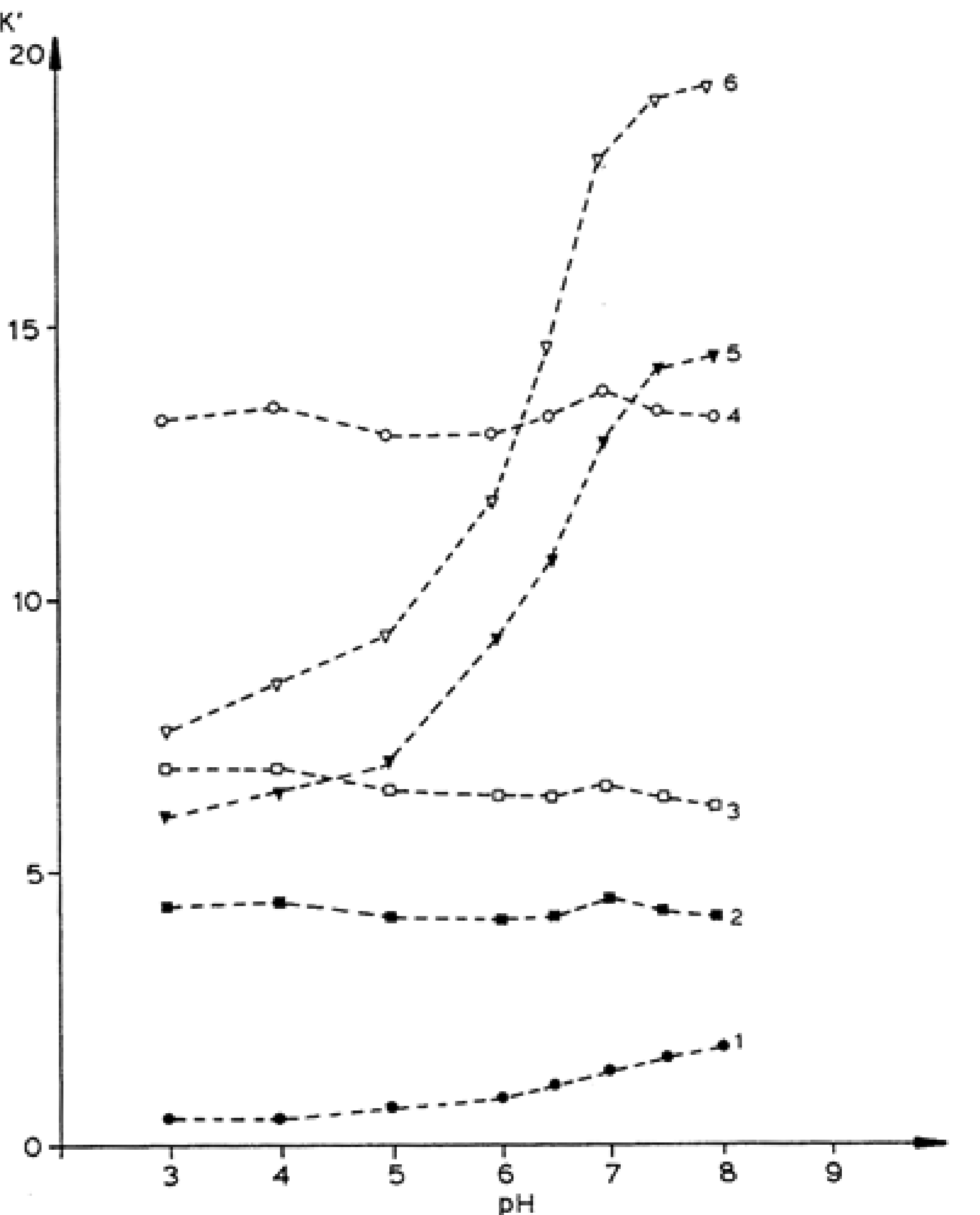


Fig. 3. Influence of the pH of the mobile phase on the capacity ratios (k') of amphetamine and impurities. 1 = Amphetamine; 2 = N-formylamphetamine; 3 = 4-methyl-5-phenylpyrimidine; 4 = N,N-di(β-phenylisopropyl)formamide; 5 = N,N-di(β-phenylisopropyl)amine; 6 = N,N-di(β-phenylisopropyl)methylamine.

the analytical column after a few injections was also observed. As most of the impurities in illicit amphetamine samples are weakly basic[1], this phenomenon was thought to be due to adsorption effects of some of the basic amine impurities to uncovered silanol groups. The addition of an amine[19,20] or a quaternary ammonium compound[21] to the mobile phase has proved to be an effective method of masking the silanol groups on the packing material. With addition of the amine modifier, butylamine, more than 100 samples could be injected on to the same extraction column without an increase in baseline drift or column back-pressure. No memory effects were observed when a sample blind was injected after every ten samples. Extra peak broadening and tailing of some of the components were also reduced.

The mobile phase was optimized to separate most of the impurities commonly encountered in Leuckart-synthesized amphetamine, as this method still seems to be the most popular in Europe. The pH of the mobile phase was varied by the addition of orthophosphoric acid and the capacity ratios (k') of amphetamine, N-f-A, 4-me, di-iso-ad, di-iso-an and di-iso-mn were measured. The k' values of di-iso-an and di-iso-mn showed a drastic increase when the mobile phase pH was raised from 3 to 8, as shown in Fig. 3, whereas the k' values of the other compounds were almost constant in the pH range 3–8. In the pH range 5–6.5 di-iso-an gave broad peaks and extensive tailing.

The UV responses of some compounds were strongly dependent on pH. Measurements with sample 1 showed that the peak areas of di-iso-an and di-iso-mn were reduced by about the half on going from pH 8 to 6. The final mobile phase with the addition of orthophosphoric acid to give pH 8.0 gave the best separation and sensitivity and minimum peak tailing and baseline drift at 220 nm.

TABLE III

REPRODUCIBILITY OF CAPACITY RATIOS (k') OF PEAKS FROM SAMPLE 1

t_0 = 1.17 min.

Compound	*Variation**	*k' (mean, n=8)*	*Standard deviation*	*Relative standard deviation (%)*
Amphetamine (peak 1)	A	1.7	0.01	0.6
	B	1.8	0.05	2.3
N-Formylamphetamine (peak 2)	A	4.2	0.02	0.5
	B	4.2	0.07	1.7
4-Methyl-5-phenylpyrimidine (peak 3)	A	6.2	0.03	0.5
	B	6.1	0.05	0.8
N,N-Di(β-phenylisopropyl)formamide (peak 4)	A	13.3	0.04	0.3
	B	13.3	0.04	0.3
N,N-Di(β-phenylisopropyl)amine (peak 5)	A	14.3	0.07	0.5
	B	14.4	0.08	0.6
N,N-Di(β-phenylisopropyl)methylamine (peak 6)	A	19.3	0.10	0.5
	B	19.3	0.13	0.7

* A = run-to-run variations; B = day-to-day variations with eight different batches of mobile phase.

The reproducibility of the k' values of the identified impurities from sample 1 is shown in Table III. The relative standard deviations are less than 1% on a run-to-run basis (A), which are equivalent to the relative standard deviations when Bond Elut-extracted samples were injected without using the column switching system. The reproducibility based on day-to-day variations was less than 3.0% for eight different batches of mobile phase (B).

The guard column lasts for about 1–2 months of continuous use and the analytical column has been used for about 6 months without a significant decrease in efficiency.

Preparation of sample solutions

Several solvents were evaluated for the dissolution of amphetamine samples. A screening of buffers in the pH range 2–9 showed that amphetamine was most efficiently washed out of the extraction column when injected in an acidic solution. The trace impurities from the synthesis, although some of them were probably protonated at acidic pH, were retained on the column. Variation of pH from 2–7 did not have a significant influence on the recovery, but at alkaline pH the intensities of several peaks decreased.

Some of the impurities were more soluble in a mixture of water and acetonitrile than in pure water. Acetonitrile at concentrations of 10–50% was therefore added to the buffer to improve the solubility of these compounds. The addition of acetonitrile gave higher recoveries of di-iso-ad, di-iso-an and di-iso-mn and lower recoveries of N-f-A and 4-me. At an intermediate concentration of acetonitrile (20%), the loss of N-f-A and 4-me was negligible, whereas higher intensities of di-iso-an, di-iso-ad and di-iso-mn were obtained. Table IV shows the reproducibility of peak-height ratios of impurities from sample 1 (peak height of impurities relative to the 4-me peak) dissolved in citrate buffer (pH 3)–acetonitrile (8:2). The relative standard deviations of the peak-height ratios were less than 4% and are acceptable for the comparison of samples.

To avoid stability problems of the impurities in the solvent, it is advisable to

TABLE IV

REPRODUCIBILITY OF PEAK-HEIGHT RATIOS OF IMPURITIES FROM SAMPLE 1

$h_{N\text{-}f\text{-}A}$ = peak height of N-formylamphetamine (peak 2); $h_{4\text{-}me}$ = peak height of 4-methyl-5-phenylpyrimidine (peak 3); $h_{di\text{-}iso\text{-}ad}$ = peak height of N,N-di(β-phenylisopropyl)formamide (peak 4); $h_{di\text{-}iso\text{-}an}$ = peak height of N,N-di(β-phenylisopropyl)amine (peak 5); $h_{di\text{-}iso\text{-}mn}$ = peak height of N,N-di(β-phenylisopropyl)methylamine (peak 6).

Peak-height ratio	*Value (mean, n = 8)*	*Standard deviation*	*Relative standard deviation (%)*
$h_{N\text{-}f\text{-}A}/h_{4\text{-}me}$	0.435	0.011	2.5
$h_{di\text{-}iso\text{-}ad}/h_{4\text{-}me}$	0.761	0.022	2.9
$h_{di\text{-}iso\text{-}an}/h_{4\text{-}me}$	1.450	0.056	3.9
$h_{di\text{-}iso\text{-}mn}/h_{4\text{-}me}$	0.370	0.003	0.8

Fig. 4. HPLC impurity profile of two separately analysed samples (A and B) from the same seizure synthesized by the Leuckart method (sample 2). The sample (100 μl sample solution at a concentration of 50 mg/ml) was injected directly. Detection by UV absorption at 220 nm. Peaks as in Fig. 1.

prepare the sample solutions immediately prior to the analysis. If necessary, centrifugation instead of filtration should be performed. An investigation with filtration of sample solutions through Millex HV-4 filters (Millipore) indicated adsorption of some of the components on the filter.

Screening of amphetamine samples

A screening of a series of twenty amphetamine samples seized in Norway in 1985 and 1986 was performed. Wide variations in the purity of the samples and the

TABLE V

RETENTION OF COMMON DILUENTS IN AMPHETAMINE SEIZURES

Diluent	*Capacity ratio (k')*
Caffeine	0.7
Ephedrine chloride	1.5
Phenazone	1.8
Procaine chloride	4.8

addition of diluents were observed. When monitoring at 220 nm, detailed impurity profiles suitable for the comparison of samples were obtained.

The analyses showed that most of the samples had been synthesized by the Leuckart procedure. Fig. 4 shows the impurity profiles of two different samples from a seizure synthesized by this method. The comparison of the two profiles monitored at 220 nm demonstrates the good reproducibility of the method. This sample contained 70% amphetamine and was diluted with glucose (sample 2).

The k' values of commonly used diluents in Norwegian seizures are given in Table V. Most of the diluents elute in or near the front and will not interfere with the trace impurity profile. Glucose and sucrose will not be detected. The k' values of two impurities identified in Leuckart seizures, 2-benzyl-2-methyl-5-phenyl-2,3-dihydropyrid-4-one and 2,6-dimethyl-3,5-diphenylpyridine, were 15.9 and 9.8, respectively. Other high-boiling pyridines described by Van der Ark *et al.*[14] are expected to elute near 2,6-dimethyl-3,5-diphenylpyridine because of structural similarity.

The results of the determination of N-f-A, 4-me and di-iso-an in eight different Leuckart-synthesized samples are shown in Table VI. The contents of N-f-A and 4-me were less than 1%, while di-iso-an showed concentrations up to 2.4%. This is

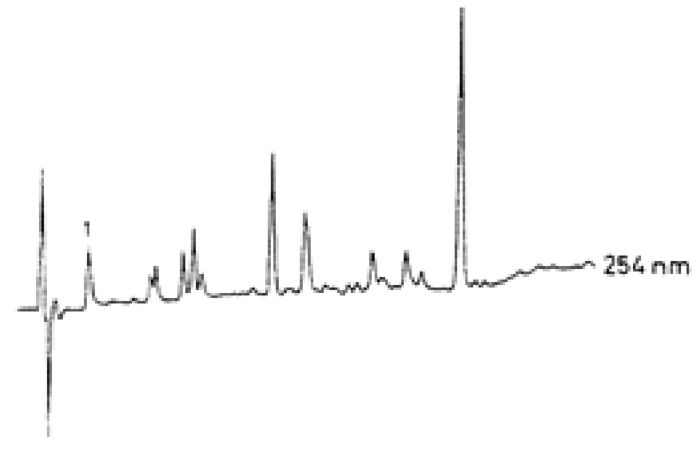
TABLE VI

QUANTITATIVE HPLC DATA FOR N-f-A, 4-me AND di-iso-an IN EIGHT AMPHETAMINE SAMPLES SYNTHESIZED BY THE LEUCKART METHOD

Sample No.	*N-f-A content* (%, w/w)*	*4-me content* (%, w/w)*	*di-iso-an content* (%, w/w)*
1	0.3	0.09	1.9
2	0.7	0.3	2.4
3	–**	0.1	0.9
4	–**	0.02	0.7
5	0.03	0.2	0.6
6	0.2	0.1	0.9
7	0.02	0.1	2.0
8	0.3	0.1	1.3

* These values represent concentrations calculated as % (w/w) of the impurity in "uncut" amphetamine sulphate.

** Quantitation not possible.



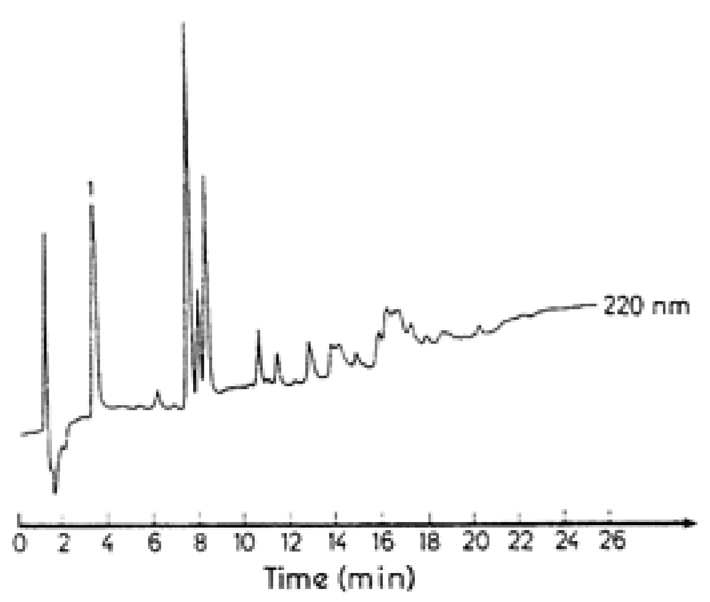


Fig. 5. HPLC impurity profile of amphetamine synthesized by the nitrostyrene route obtained by direct injection of 100 μl of a 100 mg/ml sample solution. Detection by UV absorption at 254 and 220 nm. Attenuation at 254 nm is double that at 220 nm. Peaks: 1 = amphetamine; others not identified.

in agreement with the findings of Huizer *et al.*[22], who showed that di-iso-an was often the main impurity in Leuckart-synthesized samples produced in The Netherlands in recent years.

A typical Leuckart profile was compared with the impurity profiles of amphetamine synthesized by the nitrostyrene route, reductive amination with Raney nickel as catalyst and the aluminium method. A significant difference in impurity profiles between these manufacturing methods was observed, and these samples contained less impurities than the Leuckart-synthesized samples. Fig. 5 shows the chromatogram of a sample synthesized by the nitrostyrene route detected at 254 and 220 nm.

CONCLUSIONS

By using a C_8 on-line extraction column and column switching in HPLC, automated enrichment, isolation and separation of impurities in illegally produced amphetamine can be performed. The method is suitable for routine impurity screening of illegal amphetamine samples.

ACKNOWLEDGEMENTS

The forensic science laboratories in Oslo (Norway), Linköping (Sweden) and Rijswijk (The Netherlands) are gratefully acknowledged for supplying amphetamine samples and standards of the impurities. Mr. H. Dugstad (Department of Chemistry, University of Oslo, Norway) is thanked for performing the amphetamine syntheses.

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