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Automated determination of 'Ecstasy' and amphetamines in urine by SPME and capillary gas chromatography after propylchloroformate derivatisation

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Abstract

The determination of amphetamines and their methylenedioxylated analogs in urine by propylchloroformate derivatisation and automated solid-phase microextraction is described. The urine sample was adjusted to pH 10.8 and added propylchloroformate reagent and an internal standard. Derivatisation resulted in water-stable carbamates which were automatically extracted by solid-phase microextraction. A fiber coated with polydimethylsiloxane was inserted into the urine matrix and agitated for 16 min. The fibre with the extracted carbamates was injected into the heated split-splitless injection port of the gas chromatograph where the analytes were evaporated at 300°C, separated on a methylsilicone capillary column and detected by either a nitrogen–phosphorous detector or by mass spectrometry. The method was shown to be highly reproducible and robust with respect to variations in the urine matrices. The detection limits were 5 ng ml^{-1} of methamphetamine, MDMA and MDEA and 15 ng ml^{-1} of amphetamine and MDA in urine. The method is a solvent free, automated alternative to traditional methods for determination of the amphetamine and their methylendioxylated analogs in urine.

Keywords: Ecstasy; Amphetamines; Gas chromatography; Aqueous derivatisation; SPME

1. Introduction

Due to the widespread abuse of amphetamine, methamphetamine and the designer drugs 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), 3,4-methylenedioxyamphetamine (MDA) and 3,4-methylenedioxyethamphetamine, drug testing for amphetamines is routinely done in forensic toxicology. Immunological methods such as EMIT (enzyme multiplied immunoassay technique) is widely used for screening while capillary gas chromatography (GC) [1–6], GC-mass spectrometry (GC-MS) [1,3–10], high-performance liquid chromatography (HPLC) [11–14] and capillary electrophoresis (CE) [15,16] have been used for the determination of amphetamines in urine. GC and GC-MS methods are often preferred for quantitative determination and confirmation [1].

Most laboratories prefer liquid–liquid extraction for sample preparation as these compounds

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are effectively extracted into an organic solvent from an alkaline urine sample. Most GC and GC-MS methods for the amphetamines include a derivatisation step after extraction with reagents such as trichloroacetic anhydride, trifluoroacetic anhydride, pentafluoropropionic anhydride and heptafluorobutyric anhydride [9,10,17–19] to reduce the volatility and to improve the chromatography of the compounds. The sample preparation procedures prior to GC includes several steps such as addition of organic solvent for extraction, mixing, centrifugation, collection of organic phase, evaporation of extraction solvent, addition of derivatisation reagent, heating, evaporation of excess reagent and dissolution of the residue in a solvent for injection into the GC. These procedures are difficult to automate and when analysing a large number of samples the high amount of organic solvent used in the extraction process is a health and safety issue.

Solid-phase microextraction (SPME) integrates sampling, extraction, concentration and sample introduction into a single step and offers a simple, solvent free alternative to traditional methods for sample preparation and in addition SPME is easy to automate. SPME was originally developed for environmental analysis and has been successfully applied in the analysis of volatile drugs in biological samples [20–33]. In SPME the solid phase is a non-volatile polymeric sorbent coated on a silica fibre mounted in a syringe-like device for protection [34]. SPME is based on the partitioning of organic compounds between an aqueous sample and the organic polymer phase. Several polymeric phases of varying polarity are commercially available for SPME, e.g. polydimethylsiloxane (PDMS) and polyacrylate. The amount of analytes extracted depends on the partition coefficient between the fiber coating and the sample matrix.

The major problems associated with SPME-GC of amphetamines and their methylenedioxylated derivatives are associated with the polar nature of the amino groups. Derivatisation of these polar groups into less polar analogues will increase the coating/water partition coefficient and improve the SPME efficiency. The alkylchloroformate derivatisation of amines in aqueous environment was extensively studied in the early 1980s [35–37]. The reaction of amines with alkylchloroformates in biological media has been reported to be rapid and resulting in water stable carbamates thus allowing the direct derivatisation of amphetamines in the biological sample [4,6]. We recently reported a method for the determination of amphetamine and methamphetamine in urine based on aqueous alkylchloroformate derivatisation and SPME [31]. The aim of this study was to demonstrate the potential and robustness of SPME and aqueous alkylchloroformate derivatisation for determination of the designer drugs MDA, MDMA and MDEA in addition to amphetamine and methamphetamine in urine.

2. Experimental

2.1. Chemicals

Amphetamine sulphate and methamphetamine hydrochloride were supplied by Norsk Medisinaldepot (Oslo, Norway). Methoxyphenamine was obtained from Sigma (St. Louis, MO). 3,4-methylenedioxyamphetamine hydrochloride (MDA), 3,4-methylenedioxymethamphetamine hydrochloride (MDMA) and 3,4-methylenedioxyethamphetamine hydrochloride (MDEA) were generous gifts from the National Institute of Forensic Toxicology (Oslo, Norway) and the Hormone Laboratory, Aker University Hospital (Oslo, Norway). Propyl-, and butylchloroformate were purchased from Aldrich (Milwaukee). Potassium hydrogen carbonate, potassium carbonate, sodium chloride, hexane and chloroform were supplied by E. Merck (Darmstadt, Germany). Deionized water was obtained from a Milli-Q water-purification system (Millipore, Bedford, MA).

2.2. Preparations of standards

Stock standard solutions (1 mg ml^{-1}) of amphetamine, methamphetamine and methoxyphenamine (internal standard, I.S.) were prepared in methanol. Stock standard solutions of MDA, MDMA and MDEA (100 μg ml^{-1}) were prepared in methanol. Spiked urine samples with

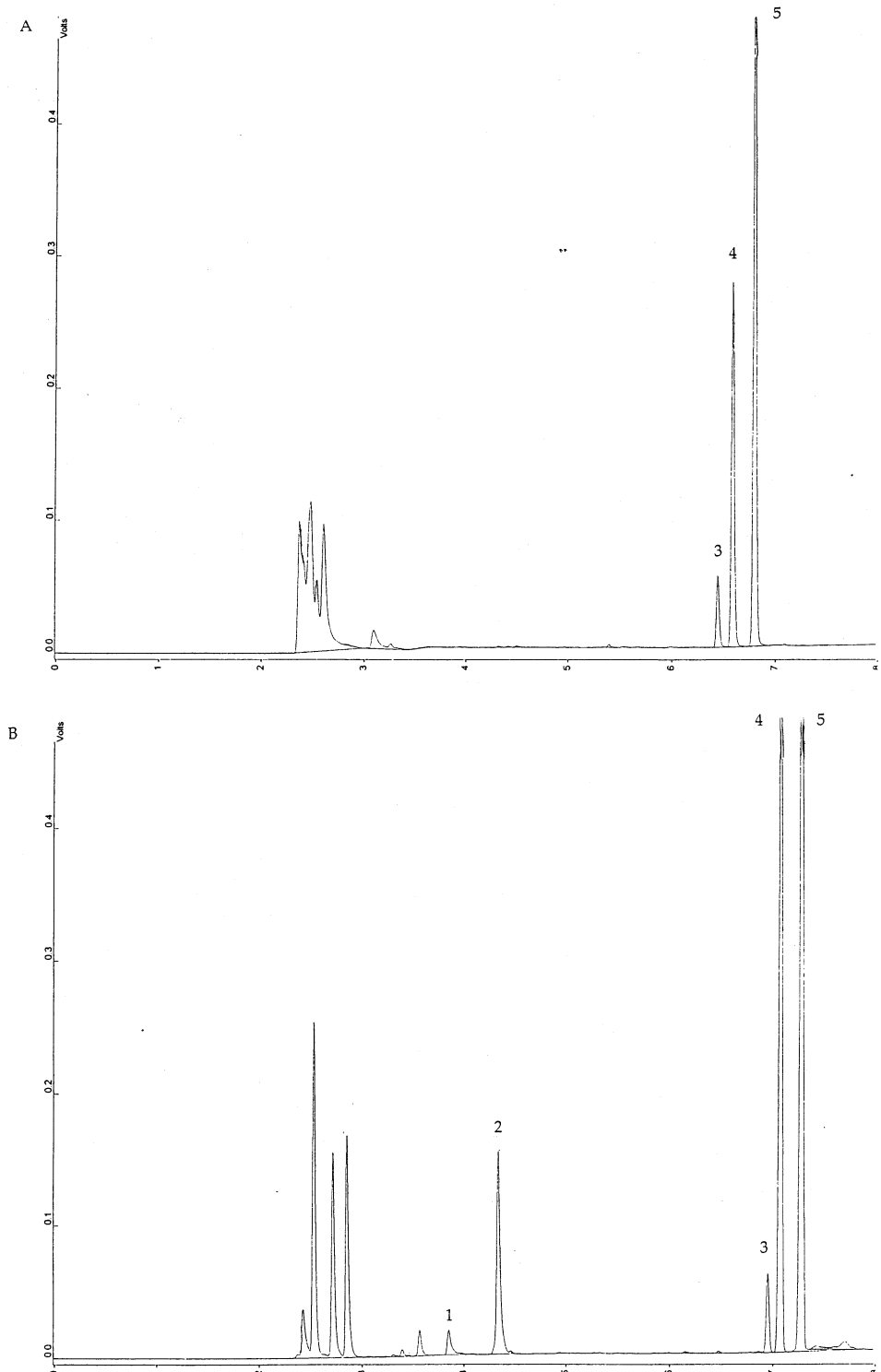

Fig. 1. Chromatograms of MDA, MDMA and MDEA after (A) propyl- and (B) butylchloroformate derivatisation and SPME. Peaks: 1, underivatised MDA; 2, underivatised MDEA; 3, derivatised MDA; 4, derivatised MDMA; 5, derivatised MDEA.

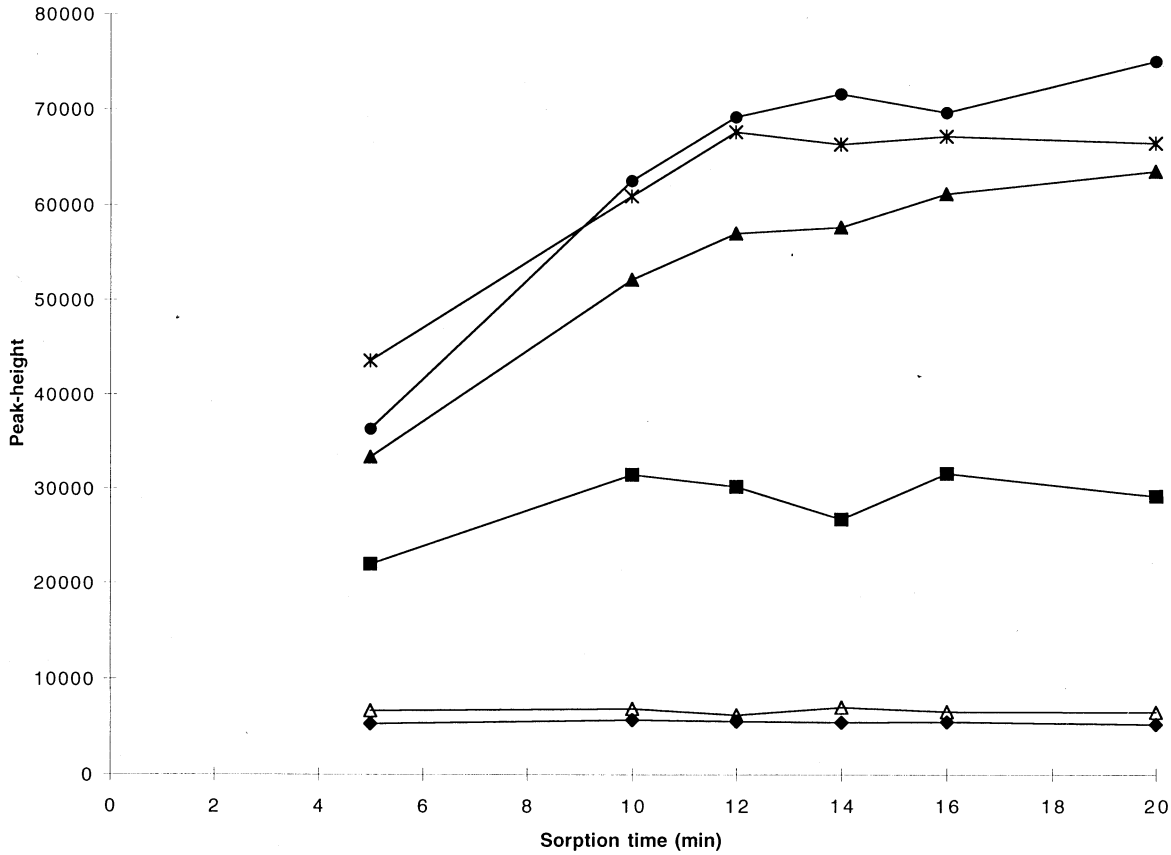

Fig. 2. SPME sorption curves for amphetamine (◆), methamphetamine (△) and methoxyphenamine (I.S.) (■), MDA (▲), MDMA (*) and MDEA (●) in urine after derivatisation propylchloroformate.

amphetamine, methamphetamine, MDA, MDMA and MDEA (0.1–10 μg ml^{-1}) were prepared from the stock solutions. The spiked urine samples were prepared freshly prior to analysis.

2.3. Sample pretreatment

An aliquot of urine sample (1200 μl) was placed in a 2 ml autosampler vial. The urine was added methoxyphenamine (I.S.) (5 μg ml^{-1}) and 300 μl of a solution consisting of 2.5 M K_2CO_3/$KHCO_3$ buffer (pH 10.8) and 0.5 g of NaCl (final concentration 5.5 M). The mixture was agitated and added 8 μl propylchloroformate and vortexed for 10 s. The vial was loaded into the GC autosampler for automated SPME and injection.

2.4. Automated solid-phase microextraction (SPME) and capillary GC analysis

Automated SPME was performed by Varian 8200 CX GC Autosampler (Varian, Walnut Creek, CA) equipped with a polydimethylsiloxane coated fibre (film thickness 100 μm) (Supelco, Bellefonte, PA). Before use, the PDMS-coated fibers were conditioned at 250°C for 1/2 h under nitrogen, and the fibers were checked for impurities prior to GC analysis. The derivatised designer drugs and amphetamines were microextracted by immersion of the PDMS fibre in the urine sample. The fiber was agitated for 16 min during enrichment to enhance partitioning of propylderivatives onto the fiber. The capillary gas chromatograph was equipped with

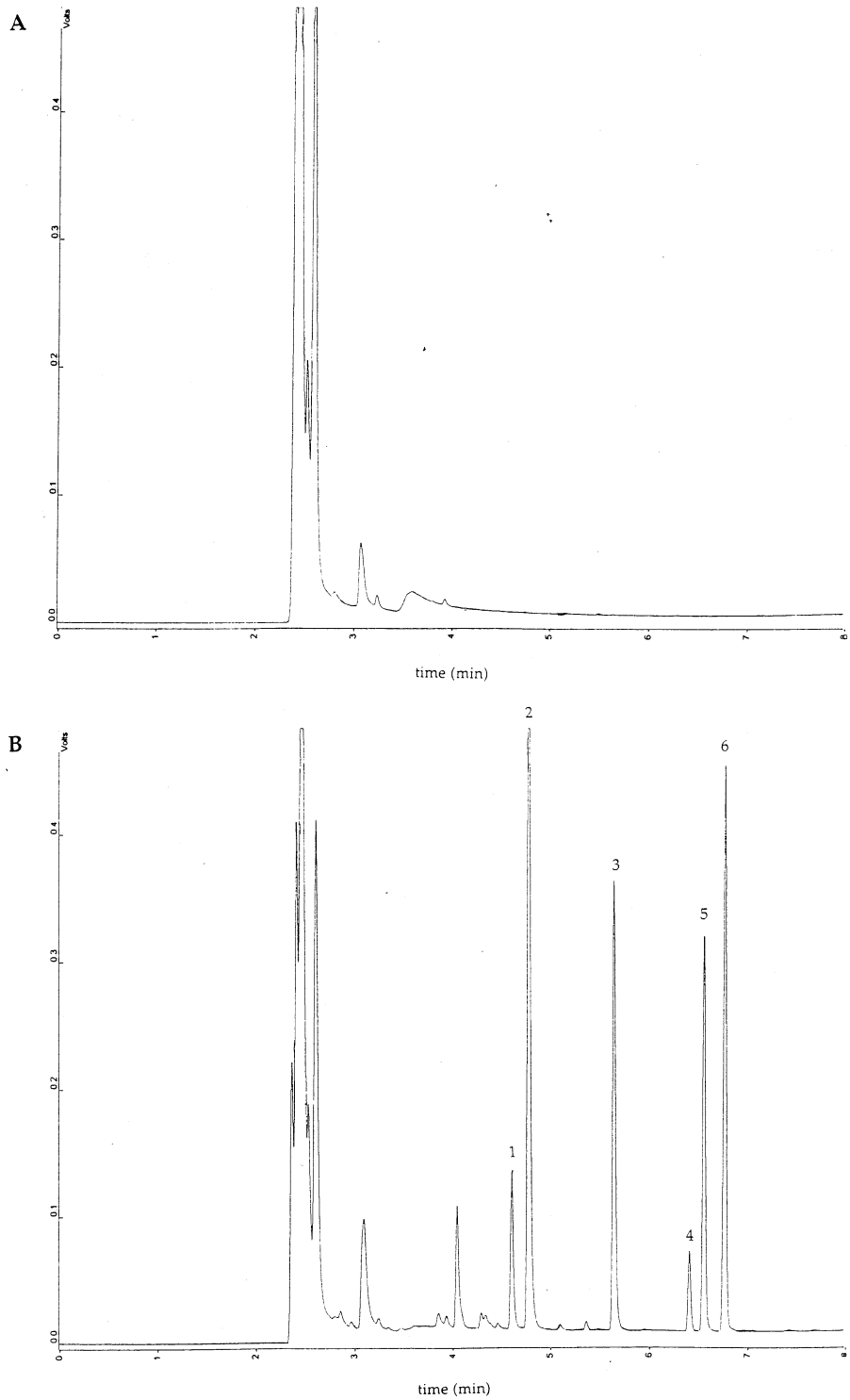

Fig. 3. Chromatograms of (A) a drug-free urine sample and (B) a urine sample with amphetamine, methamphetamine, MDA, MDMA and MDEA (7 μg ml^{-1}) and methoxyphenamine (5 μg ml^{-1}, I.S.) after derivatisation with propylchloroformate. Peaks: 1, amphetamine; 2, methamphetamine; 3, methoxyphenamine (I.S.); 4, MDA; 5, MDMA and 6, MDEA. For chromatographic conditions see text.

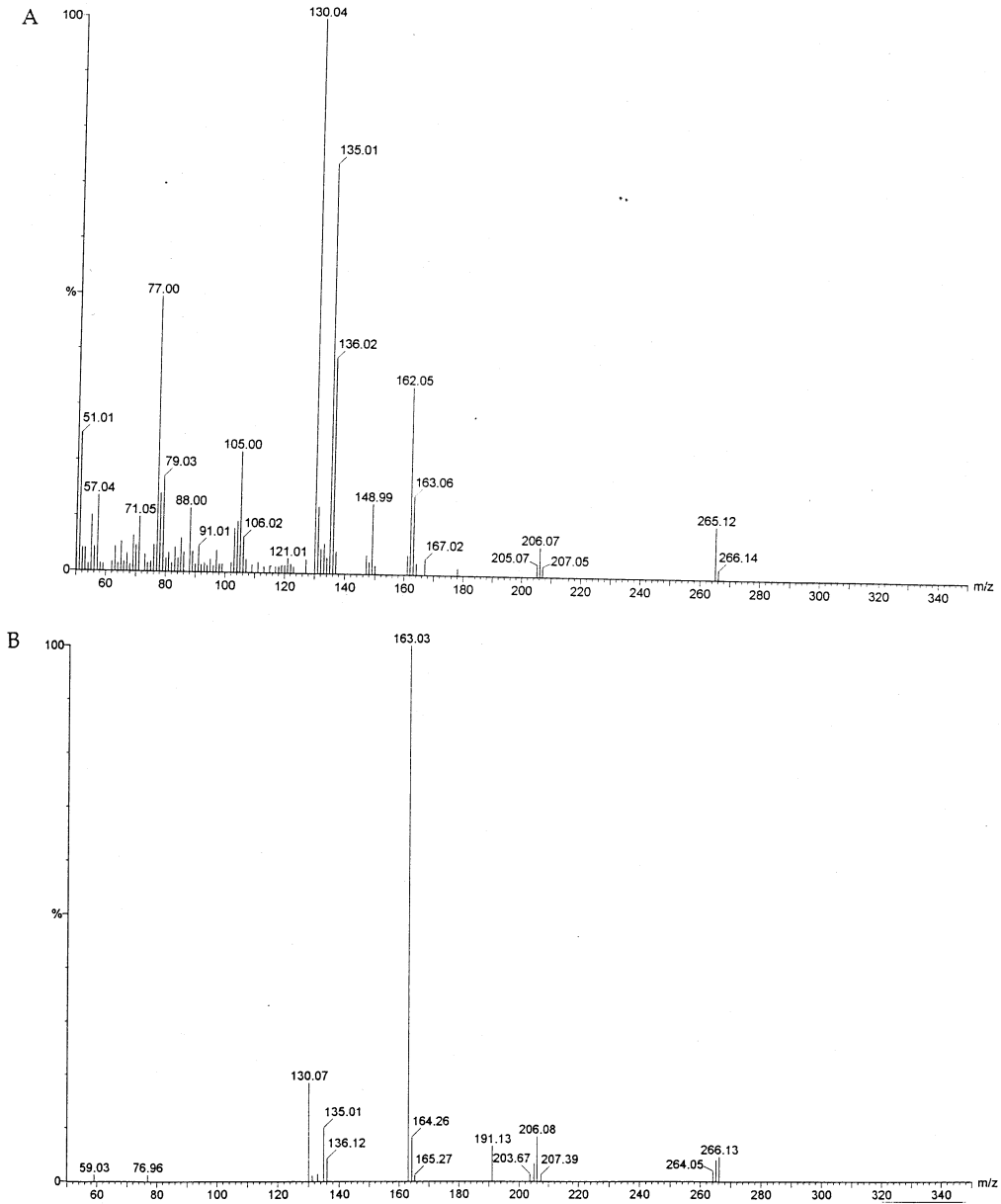

Fig. 4. Mass spectra of MDA, (A) electron impact, (B) chemical ionisation after derivatisation with propylchloroformate.

a 30 m × 0.25 mm I.D. SPB-1™ (polymethylsilicon) column (0.25 μm film thickness, Supelco, Bellefonte, PA) and a nitrogen-phosphorus detector (NPD). The temperature of the injector and the detector was set at 300 and 250°C, respectively. Helium was used as the carrier gas at a flow-rate of 1 ml min^{-1} (180°C). The detector gases were hydrogen (4.2 ml min^{-1}) and air (171 ml min^{-1}). Helium was used as make-up gas at a flow-rate of 6.8 ml min^{-1}.

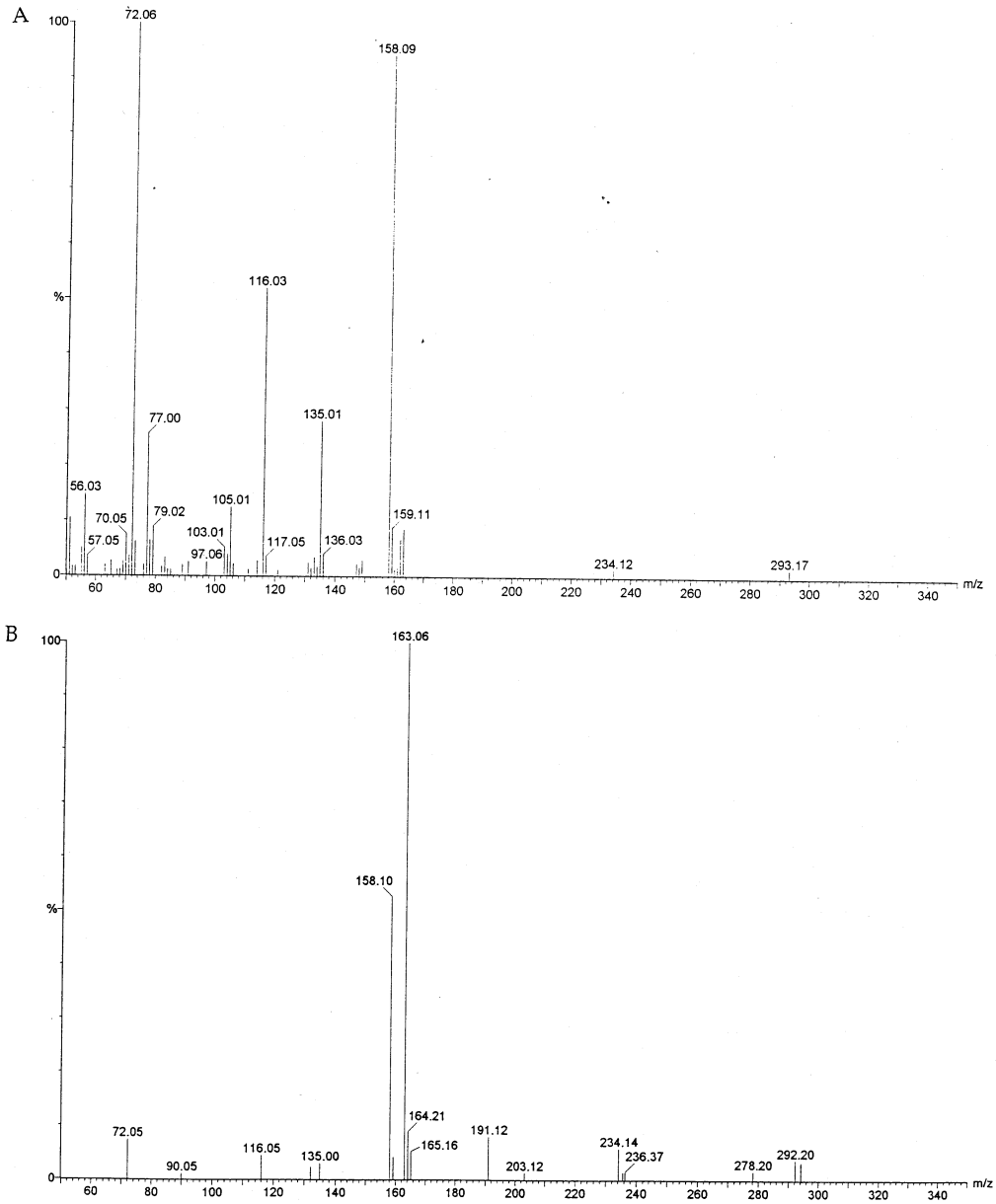

Fig. 5. Mass spectra of MDEA, (A) electron impact, (B) chemical ionisation after derivatisation with propylchloroformate.

The extracted analytes were thermally desorbed from the PDMS coating into the heated (300°C) splitless injector and into the capillary GC system for chromatographic separation and detection. After the desorption was complete (1 min) the SPME fibre was removed from the injection port and the split vent was opened. Chromatographic separation was achieved by temperature programming. The chromatograms were recorded by Varian Star Chromatography Workstation, version 4.5. A new sample could be injected every 17 min and a total of 85 samples could be analysed in 24 h.

Table 1
Absolute amount of amphetamine, methamphetamine, methoxyphenamine (I.S.), MDA, MDMA and MDEA sorbed from a urine sample spiked with 1 μg ml^{-1} of each compund, expressed as ng on the PDMS SPME fibre

Amphetamine	17
Methamphetamine	81
Methoxyphenamine (I.S.)	64
MDA	11
MDMA	117
MDEA	69

2.5. Liquid/liquid extraction and determination of amount extracted

A urine sample (200 μl) spiked with amphetamine, methamphetamine, MDMA, MDA, MDEA and methoxyphenamine (I.S.) (20 μg ml^{-1}) was added 200 μl hexane-chloroform (3:1) after alkalisation with 50 μl 0.25 M K_2CO_3/$KHCO_3$ buffer (adjusted to pH 12). For derivatisation the mixture was added 4 μl propylchloroformate. The sample was vortexed for 1 min. The aqueous bottom phase was removed and the organic phase was centrifuged twice at 3000 rpm for 5 min and the remaining aqueous phase was removed. The organic phase was concentrated to 50 μl under a stream of nitrogen, and 1 μl was injected into either a GC/NPD or a GC/MS. The amount of amphetamine, methamphetamine, MDMA, MDA, MDEA and methoxyphenamine (I.S.) extracted was determined by comparison of peak-heights obtained by liquid–liquid extraction and peak-heights obtained by SPME. For separation of the derivatised amphetamines and designer drugs, the temperature was held at 180°C for 1 min and then increased by 20°C min^{-1} to 300°C, and held constant for 1 min.

2.6. Capillary GC-MS analysis

The mass spectrometer was a Fisons MD 800 (Micromass, Manchester, UK) connected to a Fisons GC 8065 equipped with a HP-1 methylsilicon capillary column (12 m × 0.2 mm I.D., 0.33 μm film thickness). The MS-instrument was operated in the electron impact mode at 70 eV or in the positive chemical ionisation mode using methane as the reagent gas and scanned in

Table 2
Intra-assay variations after derivatisation with propylchloroformate and SPME, expressed as the mean of the parallel samples ± SD and relative standard deviation (RSD), ($n = 6$)

Drug	Concentration added (μg ml^{-1})	Measured concentration mean ± SD) (μg ml^{-1})	RSD (%)	Bias (%)
Amphetamine	1.00	0.99 ± 0.10	9.8	−1.0
	4.00	4.04 ± 0.25	6.2	+1.0
	7.00	7.57 ± 0.58	7.7	+8.1
Methamphetamine	1.00	0.94 ± 0.02	2.2	−6.0
	4.00	4.32 ± 0.07	1.7	+8.0
	7.00	7.61 ± 0.20	2.6	+8.7
MDA	1.00	0.90 ± 0.05	5.1	−10.0
	4.00	4.10 ± 0.24	5.9	+2.5
	7.00	7.34 ± 0.47	6.4	+4.9
MDMA	1.00	0.97 ± 0.02	1.8	−3.0
	4.00	4.21 ± 0.08	1.9	+5.3
	7.00	7.47 ± 0.13	1.8	+6.7
MDEA	1.00	1.04 ± 0.07	7.1	+4.0
	4.00	4.32 ± 0.07	1.6	+8.0
	7.00	7.62 ± 0.41	5.4	+8.9

Table 3
Inter-assay variations after derivatisation with propylchloroformate and SPME, expressed as the mean of the parallel samples ± SD and relative standard deviation (RSD), ($n = 6$)

Drug	Concentration added (μg ml^{-1})	Measured concentration (mean ± SD) (μg ml^{-1})	RSD (%)	Bias (%)
Amphetamine	1.00	0.87 ± 0.04	4.6	−13.0
	4.00	3.93 ± 0.29	7.5	−1.8
	7.00	7.14 ± 0.38	5.3	+2.0
Methamphetamine	1.00	1.10 ± 0.02	1.9	+10.0
	4.00	4.29 ± 0.03	0.8	+7.3
	7.00	7.18 ± 0.09	1.2	+2.6
MDA	1.00	0.89 ± 0.06	7.1	−11.0
	4.00	4.33 ± 0.48	11.1	+8.3
	7.00	7.22 ± 0.56	7.8	+3.1
MDMA	1.00	0.91 ± 0.02	2.4	−9.0
	4.00	4.12 ± 0.08	1.9	+3.0
	7.00	7.38 ± 0.15	2.0	+5.4
MDEA	1.00	1.12 ± 0.05	3.7	+12.0
	4.00	4.57 ± 0.10	2.1	+14.3
	7.00	7.48 ± 0.38	5.0	+6.9

the mass range from 50 to 300 (ion source temperature 275°C).

2.7. Validation of the method

The calibration graphs in the concentration range 0.1–10.0 μg ml^{-1} for the determination of amphetamine, methamphetamine, MDMA, MDA and MDEA were based on peak-height measurements versus peak-height of the I.S. The limit of detection was determined at a signal-to-noise ratio of 3 ($S/N = 3$).

2.8. Analysis of different urine matrices

The robustness of the method was determined by analysis of 15 different urines spiked with 1.0 and 7.0 μg ml^{-1} of amphetamine, methamphetamine, MDA, MDMA and MDEA. The urine samples were collected from eight individuals and represented urine collected in the morning and throughout the day. The pH and the concentration of creatinine in the different urine samples were determinated as a measure of salt content in the urine samples.

3. Results and discussion

3.1. Derivatisation

Derivatisation of small polar compounds prior to capillary GC and GC-MS offers several advantages. The chromatography is improved and a shift in mass spectra towards higher masses is an advantage for the identification. In SPME-GC derivatisation can be performed in the sample matrix, in the fibre coating or in the GC injection port [38]. For analysis of polar compounds such as the amphetamines the conversion of polar amino groups into less polar analogues increase their partitioning to the fiber coating, thus enhancing the amount of drug extracted and thereby improve the sensitivity of the method. Derivatisation in urine prior to SPME was therefore preferred. Amphetamine and methamphetamine have previously been derivatised with propylchloroformate in urine and extracted with an organic solvent prior to GC analysis [4,6] and derivatised with butylchloroformate prior to SPME-GC [25]. In this investigation both propyl- and butylchloroformate were studied. Chromatograms of the

Table 4
Intra-assay variations after derivatisation with propylchloroformate and SPME, expressed as the mean of the parallel samples ± SD and relative standard deviation (RSD), ($n = 15$)

Drug	Concentration added (μg ml^{-1})	Measured concentration (mean ± SD) (μg ml^{-1})	RSD (%)	Bias (%)
Amphetamine	1.00	0.87 ± 0.07	7.8	−13.0
	7.00	7.80 ± 0.51	6.5	+11.4
Methamphetamine	1.00	0.90 ± 0.02	2.6	−10.0
	7.00	6.80 ± 0.50	7.3	−2.9
MDA	1.00	0.92 ± 0.09	9.9	−8.0
	7.00	7.20 ± 0.58	8.0	+2.9
MDMA	1.00	1.10 ± 0.07	5.9 + 10.0	
	7.00	7.40 ± 0.58	7.9	+5.7
MDEA	1.00	0.90 ± 0.02	1.9	−10.0
	7.00	7.22 ± 0.43	6.0	+3.1

MDA, MDMA and MDEA after propyl- (a) and butylchloroformate (b) derivatisation are shown in Fig. 1. All the analytes were completely derivatised with propylchloroformate as no traces of underivatised compounds were detected with GC-MS. However, derivatisation of MDA and MDEA with butylchloroformate was found to be incomplete (Fig. 1) which may be due to lower solubility of the reagent. Increasing the amount of butylchloroformate reagent resulted in significantly lower extraction efficiency of the derivatives. Variation in the reaction condition such as increasing the temperature and changing the pH had no effect on the derivatisation. Propylchloroformate was therefore preferred as derivatisation reagent. The reaction occurred rapidly for all analytes and the sample vials could be placed in the GC autosampler immediately after the reagent had been mixed with the urine sample. The propylchloroformate derivatives were stable in the matrix as no change in quantitative results could be detected between samples analysed immediately and samples analysed after 24 h of storage in the GC autosampler.

3.2. SPME

The non polar fibre coating PDMS was found to be the most efficient as well as the most robust in the extraction of the derivatised drugs compared to the other commercially available SPME fibre coatings. The addition of sodium chloride to the urine had previously been found to further enhance the partitioning of the analytes onto the PDMS coating [25]. The SPME sorption curves of amphetamine, methamphetamine, MDA, MDMA and MDEA in urine after derivatisation with propylchloroformate are shown in Fig. 2. A sorption time of 16 min was chosen as the partitioning had reached equilibrium and the limit of detection was more than sufficient for use in forensic toxicology. The sample were automatically agitated during sorption. At these conditions 85 samples could be automatically analysed within 24 h. A decrease in reproducibility and enrichment was observed when the fibres were used for more than 150 extractions from urine samples. The SPME fibres were replaced every 100 samples. The lifetime of the fibres were enhanced by immersion of the fibre in pure water when not in use and by placing water samples in between the urine samples to be analysed.

3.3. Capillary GC analysis

A satisfactory separation was achieved after propylchloroformate derivatisation of the designer drugs and the amphetamines within 10 min. The propylchloroformate derivatised drugs were less volatile than the underivatised drugs

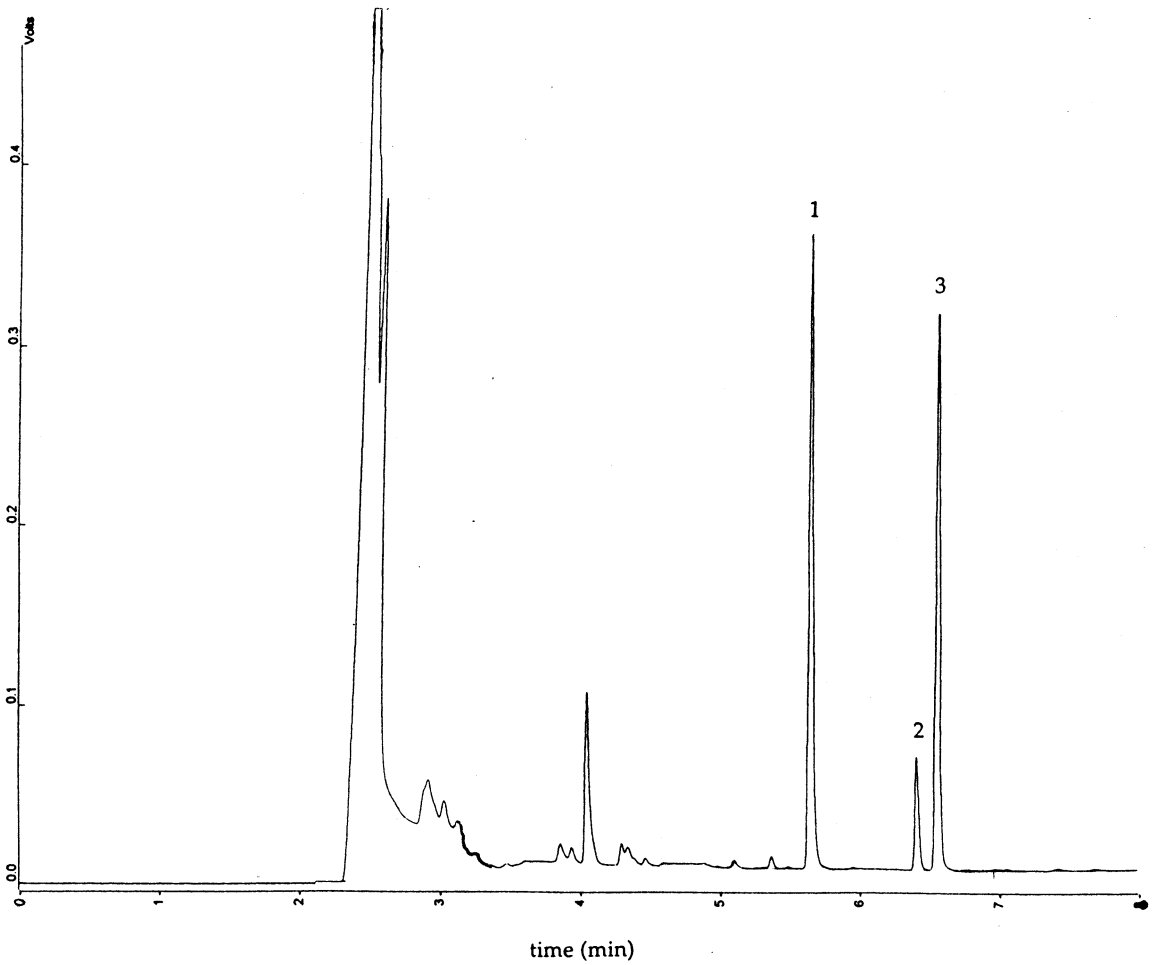

Fig. 6. Chromatogram of a real urine sample. Peaks: 1, metoxyphenamine (I. S.); 2, MDA and 3, MDMA. For chromatographic conditions see text.

and an increase in their retention time compared to the underivatised drugs was observed. Due to the high volatility of the underivatised drugs interference with volatile matrix components may be a problem as they elute early in the chromatogram and coelution with matrix components may occur. Interference from matrix compounds was not observed after propylchloroformate derivatisation. Chromatograms of a urine blank and a urine spiked with amphetamine, methamphetamine, MDA, MDMA and MDEA after propylchloroformate derivatisation are shown in Fig. 3.

3.4. GC-MS analysis

Mass spectra of the propylchloroformate derivatives of MDA and MDEA in the electron impact mode and the chemical ionisation mode over the mass range 50–400 are shown in Figs. 4 and 5. Owing to low abundance of molecular ions for the propylchloroformate derivative in the electron impact mode misidentification may occur from structurally related compounds [6]. Chemical ionisation may eliminate the possibility of misinterpretation. The mass spectra of the propylchloroformate derivatives of amphetamine,

methamphetamine and MDMA were in concurrence with previously published mass spectra [4,25]

3.5. Determination of the amount extracted

The absolute amount of amphetamine, methamphetamine, MDA, MDMA and MDEA extracted from the urine sample (1.2 ml) after derivatisation with propylchloroformate was determined by comparison with syringe injection of an aliquot of the organic phase after liquid–liquid extraction (Table 1). After propylchloroformate derivatisation and SPME, 1.4–9.8% of the initial amount of the designer drugs and the amphetamines were extracted by the PDMS coated fibre. The small amount of drugs extracted renders the method vulnerable to changes in, e.g. fiber coating and an internal standard was found to be absolutely necessary.

3.6. Linearity and sensitivity

The calibration graphs were linear in the concentration range 0.1–10 μg ml^{-1} of amphetamine, methamphetamine, MDA, MDMA and MDEA, with correlation coefficients $r = 0.9998$ or better after derivatisation with propylchloroformate. The limit of detection in urine at a signal-to-noise ratio of 3 ($S/N = 3$) was 5 ng ml^{-1} for methamphetamine, MDMA and MDEA and 15 ng ml^{-1} for amphetamine and MDA, respectively. The sensitivity was equivalent to the sensitivity of existing methods based on GC and GC-MS [4] and was sufficient for use in forensic toxicology.

3.7. Specificity

The specificity of the method has been demonstrated by the representative chromatogram of urine shown in Fig. 3. Additional blank human urine from several individuals have been tested and showed no significant interference at the retention times of the compounds of interest.

3.8. Precision and accuracy

The relative standard deviation (RSD) of the slopes from the calibration curves were no greater than 5%. The intra- and inter-assay validation data are shown in Tables 2 and 3. Both RSD and Bias for intra- and inter assay were $\leq 14.3\%$.

3.9. Analysis of different urine matrices

SPME is sensitive towards matrix variations and changes in salt concentrations and pH may greatly affect analyte recovery and reproducibility [32]. Urine is a highly variable matrix as diet and liquid intake vary urine ionic strength and pH to a great degree. Testing of accuracy and precision in different urine matrices should therefore be included in the validation of a SPME method. In order to overcome the difficulty with a variable urine matrix in this procedure a high ionic strength buffer and salt was added to the urine samples prior to SPME to eliminate matrix differences. The pH of the 15 collected urine samples were in the range 5.6–7.1 and the concentrations of creatinine were in the range 1.6–18.0 mM thus representing very low and very high urine salt concentrations. The intra-assay relative standard deviations were between 1.9 and 9.9% ($n = 15$) for the determination of amphetamine, methamphetamine, MDA, MDMA and MDEA in different urine samples as shown in Table 4. The results were found to be in concurrence with the results shown in Table 2. The method was found to be highly reproducible and robust towards natural variations in the sample matrix.

3.10. Application

The SPME-GC method was successfully applied in the analysis of real urine samples. Chromatogram from the analysis of a real urine sample containing MDA and MDMA is shown in Fig. 6.

4. Conclusions

A method based on the aqueous propylchloroformate derivatisation and SPME was developed for the determination of the designer drugs MDA, MDMA and MDEA and amphetamine and methamphetamine in urine. The method is simple and involves only addition of the derivatisation reagent to buffered urine prior to automated

SPME. Propylchloroformate derivatisation and SPME was found to be highly reproducible and robust towards variations in the urine samples. The method has sufficient sensitivity to be used in forensic toxicology and is a solvent free alternative to traditional methods.

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References

[1] F. Centini, A. Masti, I.B. Comparini, Forensic Sci. Int. 83 (1996) 161.
[2] P.G.M. Zweiphenning, A.H.C.M. Wilderink, P. Horsthuis, J.P. Franke, R.A. De Zeeuw, J. Chromatogr. A 674 (1994) 87.
[3] M. Yashiki, T. Koijma, T. Mikazaki, N. Nagasawa, Y. Iwasaki, K. Hara, Forensic Sci. Int. 76 (1995) 169.
[4] R. Meatherall, J. Anal. Toxicol. 19 (1995) 316.
[5] J. Jonsson, R. Kronstrand, M. Hatanpaa, J. Forensic Sci. 41 (1996) 148.
[6] A. Dasgupta, A.P. Hart, J. Forensic Sci. 42 (1997) 106.
[7] M. Katagi, H. Nishioka, K. Nakajima, M. Nishikawa, H. Tsuchihashi, M. Takino, K. Yamaguchi, Jpn. J. Toxicol. Environ. Health 41 (1995) 148.
[8] E.M. Thurman, M.J. Pedersen, R.L. Stout, T. Martin, J. Anal. Toxicol. 16 (1992) 19.
[9] C.L. Hornbeck, R.J. Czarny, J. Anal. Toxicol. 13 (1989) 114.
[10] R.J. Czarny, C.L. Hornbeck, J. Anal. Toxicol. 13 (1989) 257.
[11] R. Herráez-Hernández, P. Campíns-Falco, A. Sevillano-Cabez, J. Chromatogr. B 679 (1997) 69.
[12] O. Al-Dirbashi, N. Kuroda, S. Akiyama, K. Nakashima, J. Chromatogr. B 695 (1997) 251.
[13] P. Campíns-Falco, A. Sevillano-Cabez, C. Molíns-Legua, M. Kuhlmann, J. Chromatogr. B 687 (1996) 239.
[14] M. Katagi, H. Nishioka, K. Nakajima, H. Tsuchihashi, K. Makino, J. Chromatogr. B 676 (1996) 35.
[15] D.P. Bogan, M. Thornes, M. teigmeier, E.A. Schafer, R. O'Kennedy, Analyst 121 (1996) 243.
[16] T. HyštylŠinen, H. Sirén, M.-L. Riekkola, J. Chromatogr. A 735 (1996) 439.
[17] J.B. Jones, L.D. Mell, J. Anal. Toxicol. 17 (1993) 447.
[18] R.W. Taylor, S.D. Le, S. Philip, N.C. Jain, J. Anal. Toxicol. 13 (1989) 293.
[19] H. Gjerde, I. Hasvold, G. Pettersen, A.S. Christophersen, J. Anal. Toxicol. 17 (1993) 65.
[20] T. Kumazawa, X.P. Lee, M.C. Tsai, H. Seno, A. Ishii, K. Sato, Jpn. J. Forensic Toxicol. 13 (1995) 25.
[21] M. Krogh, K. Johansen, F. Tønnesen, K.E. Rasmussen, J. Chromatogr. B 673 (1995) 299.
[22] H. Seno, T. Kumazawa, A. Ishii, M. Nishikawa, H. Hattori, O. Suzuki, Jpn. J. Forensic Toxicol. 13 (1995) 211.
[23] T. Kumazawa, X.P. Lee, K. Sato, H. Seno, A. Ishii, O. Suzuki, Jpn. J. Forensic Toxicol. 13 (1995) 182.
[24] N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, T. Kojima, Forensic Sci. Int. 78 (1996) 95.
[25] X.P. Lee, T. Kumazawa, K. Sato, O. Suzuki, Chromatographia 42 (1996) 135.
[26] K. Ameno, C. Fuke, S. Ameno, H. Kinoshita, I. Ijiri, J. Can. Soc. Forensic Sci. 29 (1996) 43.
[27] A. Ishii, H. Seno, T. Kumazawa, K. Watanabe, H. Hattori, O. Suzuki, Chromatographia 43 (1996) 331.
[28] T. Kumazawa, H. Seno, X. P. Lee, A. Ishii, O. Suzuki, K. Sato, Chromatographia 43 (1996) 393.
[29] F. Degel, Clin. Biochem. 29 (1996) 529.
[30] F. Asakawa, F. Jitsunari, C. Jinko, S. Suna, N. Takeda, T. Kitamado, J. Occup. Health 38 (1996) 258.
[31] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Chromatogr. B 701 (1997) 29.
[32] B.J. Hall, J.S. Brodbelt, J. Chromatogr. A 777 (1997) 275.
[33] S. Ulrich, J. Martens, J. Chromatogr. B 696 (1997) 217.
[34] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[35] N.-O. Ahnfelt, P. Hartvig, Acta Pharm. Suec. 17 (1980) 307.
[36] K.-E. Karlsson, Thesis, University of Uppsala, 1981.
[37] N.-O. Ahnfelt, Thesis, University of Uppsala, 1982.
[38] L. Pan, J. Pawliszyn, Anal. Chem. 69 (1997) 196.