Int J Legal Med (2000) 113 : 229–235

ORIGINAL ARTICLE

W. Weinmann · M. Renz · S. Vogt · S. Pollak

Automated solid-phase extraction and two-step derivatisation for simultaneous analysis of basic illicit drugs in serum by GC/MS

Received: 23 June 1998 / Received in revised form: 14 August 1999

Abstract A combination of automated solid-phase extraction (SPE) and subsequent two-step derivatisation has been developed for the simultaneous analysis of basic drugs of abuse and cocaine metabolites in serum samples. Substances included in this procedure are morphine, codeine, methadone, cocaine, benzoylecgonine, methylecgonine, amphetamine, methamphetamine, MDMA, MDEA and MDA. SPE with mixed-mode cartridges (RP-C8 and cation-exchange) was fully automated with a Zymark RapidTrace SPE robot. GC/MS analysis was performed after derivatisation with a new two-step reaction by trifluoroacetic anhydride and 2,2,3,3,3-pentafluoropropanol. High recoveries (> 85%) with high reproducibility (CV 1.1–3.8%) were found for all drugs. High correlation coefficients ($r > 0.998$) were obtained due to the addition of deuterated standards prior to extraction. Experience obtained over 2 years of applying this method to drug analysis in serum is discussed.

Key words Drugs of abuse · Quantitative analysis · Automation · Solid-phase extraction · GC/MS · Derivatisation

Introduction

A bill amending § 24a of the German traffic law („Strassenverkehrsgesetz“ [1]) dealing with driving under the influence of drugs (DUID) has been passed by the German Parliament (Bundestag) and came into force in August 1998. The new regulation states that driving a vehicle after drug consumption will be prosecuted if certain substances (drugs or metabolites) can be detected in blood, even if there are no obvious effects on the driving ability. However, a driver has to be conspicuous in some way otherwise he cannot be forced to give a blood sample. For the realisation of this new law, specific and sensitive methods for the quantitative analysis of drugs of abuse and the main cocaine metabolite (morphine, benzoylecgonine, tetrahydrocannabinol, amphetamine and the 3,4-methylenedioxyamphetamine derivatives MDMA and MDEA) in blood are necessary. Furthermore, these methods have to be standardised to achieve the same limits of quantitation and detection in all forensic laboratories which are certified to perform quantitative analysis for drugs.

For drug screening in serum and urine, immunoassays are commonly used first, followed by a specific analysis for the immunological positive drugs by GC/MS. Since sample material is limited in most forensic cases, a simultaneous GC/MS analysis for several different drugs using only small amounts of sample is desirable. Instead, several methods are commonly used [2–10], each of which is specific for only one or two classes of drugs. Combinations of solid phase extraction (SPE) and derivatisation methods for GC/MS analysis for opiates [9,10], opiates, cocaine and cocaine metabolites [2,3] have been described. For derivatisation either perfluoropropylation [2,3], propylation [9,10] or silylation [8] have been used. Although derivatisation of opiates, amphetamine, benzoylecgonine and methylecgonine was achieved by the first method applying pentafluoropropanol and perfluoropropionic anhydride, the designer-drugs MDMA and MDEA were not included in this procedure.

To reduce the number of extraction steps, a manual SPE method for the simultaneous extraction of basic drugs of abuse, i.e. morphine, codeine, methadone, cocaine, benzoylecgonine, methylecgonine amphetamine, methamphetamine and 3,4-methylenedioxyamphetamine derivatives (MDMA, MDEA and MDA) has been developed in our laboratory [11–13]. Solid-phase extraction is often advantageous for subsequent GC/MS analysis when compared to liquid-liquid extraction due to its high specificity resulting in less matrix co-extraction. However, if performed manually, SPE lacks robustness due to the vari-

W. Weinmann (✉) · M. Renz · S. Vogt · S. Pollak
Institut für Rechtsmedizin,
Klinikum der Albert-Ludwigs-Universität, Albertstrasse 9,
D-79104 Freiburg, Germany
e-mail: weinmann@sun11.ukl.uni-freiburg.de,
Tel.: +49-761-2036856, Fax: +49-761-2036858

ability of many parameters such as flow-rates for sample application, washing and elution, grade of drying between single steps, batch-to-batch homogeneity, sample viscosity, influences of matrix and last but not least due to the operator (lack of lab-to-lab reproducibility). With automation, flow rates and drying parameters can be standardised. Inhomogeneity of packing density of the cartridge-bed which might be due to transportation, can be reduced by pressing the cartridge-bed with a plunger prior to use. Some parameters such as batch-to-batch reproducibility of the cartridges and variations of sample matrix cannot be eliminated but their effects on extraction can be minimised by adding internal stable isotope standards for subsequent GC/MS analysis.

Two solid-phase extraction robots have been used successfully for forensic applications, the Hewlett Packard PrepStation [8, 14] and the Zymark RapidTrace SPE robots [15,16]. When using a RapidTrace module, samples are prepared manually in the same way as for manual SPE and placed into a sample rack with ten positions for the samples and ten positions for the eluates. The extraction steps are performed automatically at constant programmable flow rates and samples are extracted serially. The eluates have to be evaporated off-line under a stream of nitrogen and have to be derivatised off-line prior to GC/MS analysis.

Our aim was to develop a robust automated SPE method with a Zymark RapidTrace module for the simultaneous extraction of basic drugs of abuse (opiates, amphetamine and 3,4-methylenedioxyamphetamine derivatives, cocaine and cocaine metabolites) from serum and a new two-step derivatisation procedure which allows simultaneous GC/MS analysis of these drugs.

Table 1 List of m/z-values of derivatised drugs used for mass-spectrometric analysis. Mass-to-charge ratios used for quantitation are printed in *bold letters*. (*TFA* trifluoroacetylated, *PFP* pentafluoropropionylester)

Derivatised drug	m/z non deuterated	m/z deuterated	Gas chromatographic retention time
Amphetamine-TFA	**91**	**92**	5.3 min
	118	**122**	
	140	**144**	
Methamphetamine-TFA	**110**	**113**	6.1 min
	118	120	
	154	**158**	
Ecgonine-methylester-TFA	182	185	6.4 min
	264	**267**	
	295	**298**	
MDA-TFA	135	136	7.5 min
	162	**167**	
	275	**280**	
MDMA-TFA	**110**	**113**	8.3 min
	154	**158**	
	289	**294**	
MDEA-TFA	140	141	8.5 min
	168	**173**	
	303	**308**	
MBDB-TFA[a]	110	113	8.7 min
	168	**172**	
	303	**308**	
Benzoylecgonine-PFP	82	85	11.1 min
	300	**303**	
	421	**424**	
Methadone	72	72	11.5 min
	223	**226**	
	294	**297**	
Cocaine	82	85	12.6 min
	182	**185**	
	303	**306**	
Morphine-TFA_2	**311**	**314**	13.0 min
	364	**367**	
	477	**480**	
Codeine-TFA	**282**	**285**	13.7 min
	395	**398**	
MAM-TFA[b]	311	314	14.5 min
	364	**367**	
	423	**426**	

[a] MBDB (*N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine), [b] MAM (6-monoacetylmorphine): both were included in the derivatisation procedure, but extraction has not yet been validated for these

Materials and methods

All drug standards were purchased from Promochem/Radian (Wesel, Germany). For derivatisation, trifluoroacetic anhydride and 2,2,3,3,3-pentafluoropropanol (purity 99%) were purchased from Sigma (Deisenhofen, Germany). Phosphate buffer (0.1 M) was prepared from NaH_2PO_4 and adjusted to pH 6.0 with 1 M NaOH. All solvents were of analytical grade.

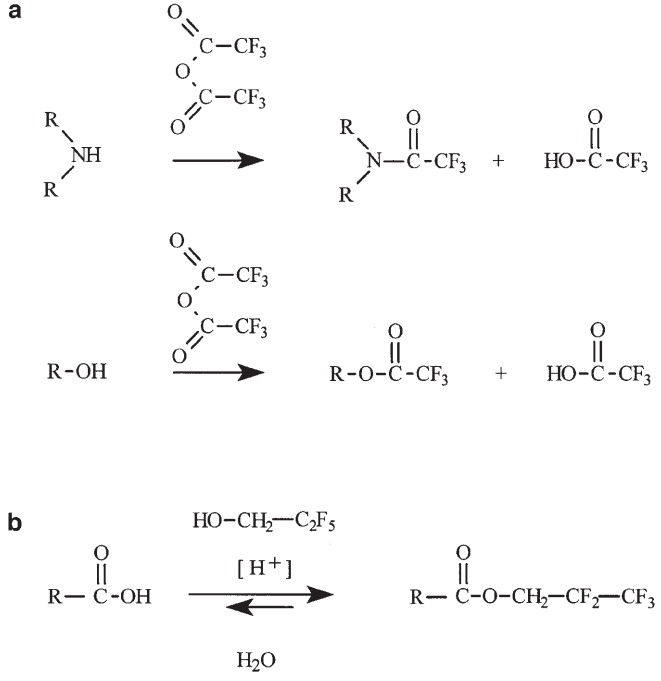

Fig. 1 a, b Reaction scheme of the two-step derivatisation procedure. Amino- and hydroxy-groups are trifluoroacetylated in the first step with trifluoroacetic anhydride (**a**). By addition of 2,2,3,3,3,-pentafluoropropanol, carboxylic groups are esterified in the second step (**b**)

Drug mixtures of 1, 2, 5, 10, 25, 50, 100, 250, 500 and 1000 ng/ml, respectively, of all non-deuterated drugs and 50 ng/ml deuterated standards were prepared in methanolic solution. The solvent was evaporated and drugs were derivatised and analysed by GC/SIM-MS (SIM: selected ion monitoring). Using the m/z values from Table 1, the calibration for all drugs was found to be linear in this range with good correlation coefficients ($r > 0.998$).

For calibration with serum samples, drug-free serum was spiked with non-deuterated drug standards to obtain drug concentrations of 1, 2, 5, 10, 25 and 50 ng/ml. Then, 50 µl of a deuterated drug mixture (0.2 ng/µl) were added to each 1 ml serum sample to yield a concentration of 10 ng/ml deuterated standards. For higher ranges (50–1000 ng/ml drug concentration), 25 µl of a deuterated drug mixture (2 ng/µl) was added to a 1-ml serum sample. Prior to SPE samples were made up to 2.5 ml with phosphate buffer (pH 6.0), centrifuged (4000 U/min, 3 min) and the supernatant was transferred into 13 × 100 mm glass vials and placed into the sample rack of the SPE robot.

Solid phase extraction was performed with an SPE robot (RapidTrace, Zymark, Idstein, Germany). For all extractions mixed-mode SPE cartridges (Chromabond Drug, 3 ml / 200 mg, Macherey-Nagel, Düren, Germany) based on cation exchange and lipophilic (RP-C8) interactions were used.

The SPE eluates were evaporated under a gentle stream of nitrogen in a thermostatic aluminium block heater at 40 °C and derivatised using a two-step reaction (Fig. 1): firstly trifluoroacetylation by incubation with trifluoroacetic anhydride (100 µl, 30 min/60 °C), and secondly, without evaporation of the anhydride, esterification of benzoylecgonine by addition of pentafluoropropanol (70 µl) and further incubation (30 min/60 °C). Finally, the reaction mixture was evaporated to dryness under a stream of nitrogen at 40 °C. All evaporation steps were stopped immediately after evaporation of the solvent to avoid evaporation of the volatile amphetamine derivatives. For safety of laboratory personnel evaporation of solvents and toxic reagents was performed under a hood. After redissolving in 30–100 µl ethyl acetate, 1 µl was injected in the splitless mode into a GC/MS (MD 800/250, CE Instruments, Egelsbach, Germany) with 70eV-EI ionisation and a DB5-ms capillary column (J&W, 30 m × 0.25 mm, 0.25 µm film thickness). The column temperature was initially held at 80 °C for 1 min, increased to 200 °C at 20 °C/min, to 240 °C at 5 °C/min, to 310 °C at 30 °C/min and finally held at 310 °C for 6 min (injection port temperature 250 °C, scan width 0.2 amu, dwell times 80 ms). M/z values of ions used for quantitation in the SIM-mode are listed in Table 1. A total ion chromatogram (scan range: 50–550 amu) of a mixture of derivatised drugs (200 ng each) is shown in Fig. 2.

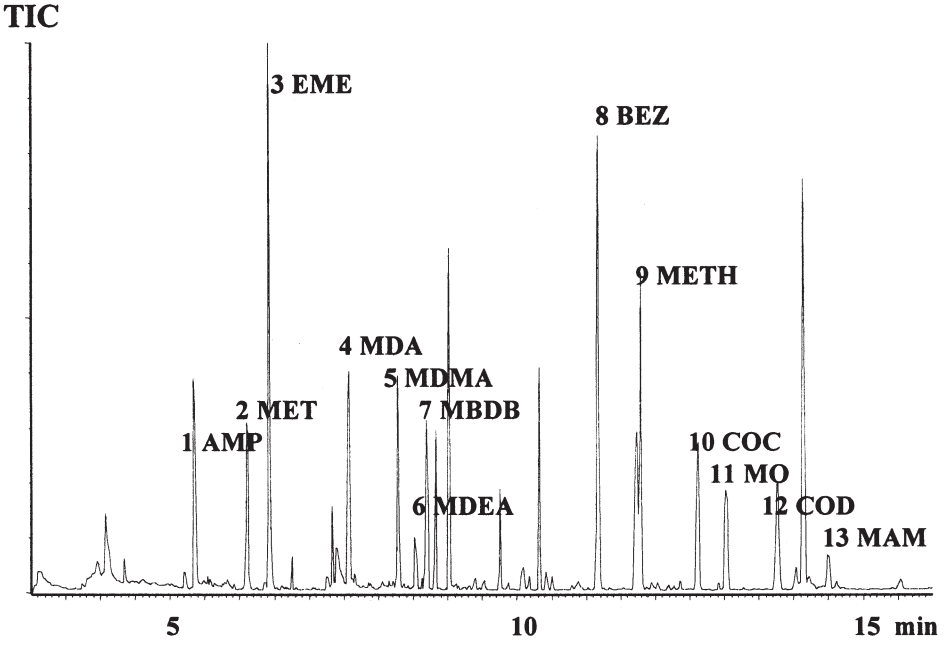

Fig. 2 GC/MS-analysis of a derivatised drug mixture. TIC: total-ion-current of full-scan analysis of derivatives: numbers indicate chromatographic signals of TFA-derivatives of amphetamine (AMP), methamphetamine (MET), ecgonine-methylester (EME), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB), morphine (MO), codeine (COD), 6-monoacetylmorphine (MAM); PFP derivative of benzoylecgonine (BEZ) and underivatised drugs methadone (METH) and cocaine (COC)

Results

An existing method for simultaneous derivatisation of amphetamine, opiates, cocaine and cocaine metabolites with a mixture of perfluoropropionic anhydride and pentafluoropropanol [2,3] gave only low derivatisation yields for MDEA, MDMA and methamphetamine. Steric hindrance of these secondary amines might be the reason for the low yields when using a mixture of reagents. Different procedures were tested to achieve quantitative derivatisation of all basic drugs. For optimising the derivatisation procedure, each drug was derivatised in separate experiments and the reaction products were analysed in full-scan mode by GC/MS. Instead of the simultaneous incubation with anhydride and alcohol the derivatisation was performed in two steps: firstly by incubation with anhydride (60 °C, 30 min), secondly by addition of pentafluoropropanol and further incubation (60 °C, 30 min) for esterification of the carboxylic function of benzoylecgonine. With these reaction conditions no educts were detectable by GC/MS in full-scan mode, and furthermore, morphine was derivatised quantitatively to its doubly trifluoroacetylated derivative. With these reaction conditions quantitative derivatisation was obtained for all drugs tested. With shorter derivatisation times in the first step, however, morphine was only partly derivatised to its mono- and bis-trifluoroacetylated derivatives. For the mono-trifluoroacetylated morphine derivatives, a signal with major ions at m/z 268 and m/z 381 with a slightly increased retention time (+ 0.4 min) compared to bis-trifluoroacetylated morphine was detected in full-scan analysis. Reaction schemes and gas chromatographic separation of the derivatives are shown in Figs. 1 and 2.

The manual SPE method for basic drugs which has already been validated in our laboratory was transferred directly onto the SPE robot using the same extraction cartridges, sample and solvent volumes and same flow-rates as for the manual procedure (method 1, see Table 2). Optimisation of the automated extraction was performed in four steps. Extraction yields were controlled by analysing serum samples with 20 ng/ml drug mixture three times with all methods. Deuterated standards were added to the SPE eluate prior to derivatisation and GC/MS analysis.

Method 1, which was closest to the manual method, is shown in Table 2. The flow rate for sample application was increased to 1 ml/min (step 3) and the volume of water for rinsing was reduced to 1 ml (step 4), resulting in a second more rapid method. For method 3 the flow rates for rinsing (steps 4–6) were doubled. In the original manual method drying of the column before elution was performed by centrifugation and suction of air through the cartridge by a vacuum manifold. With the SPE robot the exchange of solvent is performed by flushing the cartridge with nitrogen gas at a pressure of 3 bar (step 7). This step is not sufficient to totally dry the column but sufficient to remove the solvent of the preceding rinsing step (methanol) from the column bed. The drying time of 3 min (methods 1, 2 and 3) was reduced to 1 min (method 4).

Recoveries of all tested methods varied by less than 10% and were higher than 80% for all drugs tested. The calculated time for one extraction could be decreased from 18 min (method 1) to 9 min (method 4). The fastest extraction procedure (method 4) was then used for further experiments.

The recovery of SPE method 4 and the reproducibility of extraction and GC/MS analysis were determined for all drugs listed in Table 3. For testing recoveries 1-ml aliquots of serum containing 20 ng/ml of each drug were extracted by SPE and deuterated standards were added to the eluates prior to derivatisation. The recoveries for all drugs were higher than 85% (see Table 3).

For determination of run-to-run reproducibility seven 1-ml aliquots of serum with 20 ng/ml of each drug were spiked with deuterated drugs (20 ng/ml each) and analysed. The concentrations found and the coefficients of variation are shown in Table 4.

The range of linearity, limits of detection (LOD) and limits of quantitation (LOQ) were determined. Therefore a method using linear regression of seven calibration standards was used [17] with an α-error of 1% (α = 1%) and a relative confidence interval of 33% (k = 3). This method is also suggested by the German Industrial Norm (DIN 32645) and has recently been used for the determination

Table 2 Original extraction scheme (method 1) and optimised extraction scheme (method 4) of automated SPE. Parameters changed during method development are printed in *bold letters*. *DIA* Dichloromethane/2-propanol/25% aqueous ammonia, 80 : 20 : 2 (v/v/v)

	Step	Source	Volume (ml) or time (min)[a]		Flow-rate (ml/min)	
			Method 1	Method 4	Method 1	Method 4
1	Condition	Methanol	2.0	2.0	2.0	2.0
2	Condition	Phosphate	2.0	2.0	2.0	2.0
3	Load	Sample	3.0	3.0	**0.5**	**1.0**
4	Rinse	Water	**2.0**	**1.0**	**1.0**	**2.0**
5	Rinse	0.1 M acetic acid	1.0	1.0	**1.0**	**2.0**
6	Rinse	Methanol	2.0	2.0	**1.0**	**2.0**
7	Dry	Nitrogen	**(3.0)**	**(1.0)**		
8	Collect	DIA	1.5	1.5	**1.0**	**2.0**
9	Dry	Nitrogen	(0.1)	(0.1)		
10	Purge-cannula	Methanol/water	4.0	4.0	30.0	30.0
11	Purge-cannula	Methanol/water	4.0	4.0	30.0	30.0

[a] Time for drying steps

Table 3 Recovery and coefficient of variation (CV) of three extractions of serum samples containing 20 ng/ml drugs: deuterated standards were added after SPE

Drug	Average concentration found (n = 3)	CV (n = 3)	Recovery (%)
Amphetamine	17.4 ± 1.0 ng/ml	5.9%	86.8
Methamphetamine	18.0 ± 0.9 ng/ml	5.0%	90.1
MDEA	19.4 ± 1.5 ng/ml	7.7%	97.1
MDA	18.1 ± 1.0 ng/ml	5.4%	90.6
MDMA	18.0 ± 1.0 ng/ml	5.7%	90.2
Cocaine	18.5 ± 0.2 ng/ml	1.1%	92.5
Methylecgonine	20.2 ± 1.5 ng/ml	7.4%	101.1
Benzoylecgonine	18.6 ± 1.1 ng/ml	5.7%	93.2
Codeine	17.1 ± 0.5 ng/ml	2.7%	85.6
Morphine	18.6 ± 0.8 ng/ml	4.5%	92.6
Methadone	19.6 ± 1.0 ng/ml	5.0%	98.3

of LOQs and LODs for the quantitation of cannabinoids in serum by GC/MS [18]. LODs and LOQs were determined using the method with linear regression of two ratios of ion-pairs for each substance (printed in bold letters in Table 1). Drug concentrations were 0, 1, 2, 5, 10, 25 and 50 ng/ml. From two LOD values and two LOQ values, the higher LOD values and the lower LOQ values are shown in Table 4. The third ion-pairs required for identification of the substances which are quantified (see Table 1), fulfilled the quality criteria for drug identification at the LOD [19]. For higher drug concentrations (50–1000 ng/ml) 50 ng of deuterated drug standards was added as internal standard. The calibration functions of two ion pairs for each drug were found to be linear in this range with high correlation coefficients $r > 0.998$.

Selected ion chromatograms obtained by analysis of forensic case serum samples, proficiency test serum samples or spiked serum samples are shown in Fig. 3.

There is one part of the extraction where specimens come into contact with the same surface, i.e. the cannula for removing the sample from the test-tube. This is important, because serum contains proteins which might adsorb to surfaces and drugs can be bound by their active residues. To evaluate the potential of carry-over from one sample to the next, serum samples spiked with very high concentrations of drugs were extracted followed by the extraction of blank serum samples, which were then analysed for carry-over of drugs. The following concentrations of morphine were used: (a) 100 µg/ml and (b) 10 µg/ml. The blank serum after (a) contained 66 ng/ml morphine (0.066% carry-over) whereas in the blank serum after (b) no morphine could be detected (< 1 ng/ml). The test with a 100 µg/ml and a 10 µg/ml sample was repeated with method 4 (see Table 2) with an additional "purge-cannula" step (step11). In the following blank serum 25 ng/ml morphine (0.025% carry-over from the 100 µg/ml sample) was detected but no carry-over was found with the 10 µg/ml sample. When adding a third "purge-cannula" step, however, no carry-over was detected even after the 100 µg/ml sample. Although the concentrations used to test for carry-over were artificially high, these preliminary tests show the necessity of separate series for analysis of serum samples and urine samples, because urine samples may contain by more than 1000 times higher drug concentrations than serum samples. To avoid carry-over when analysing serum samples, two to three purge cannula steps were found to be sufficient for specific analysis with high sensitivity.

A second possibility of carry-over is during injection of derivatised extracts into the GC system, especially by the injection syringe and the injection-port. This was accounted for by five syringe washing steps prior to injection and five steps after injection from two different solvent vials. A blank solvent was injected between injections of samples.

Discussion

Over a 2-year-period of application of this method described above to serum, we found that there are some important rules to obey. First, the sample has to be free of particles to avoid blocking of the cartridge. This can be achieved by centrifugation of the sample before SPE. To avoid precipitation of protein in the cannula, it must not be purged with pure organic solvents after serum application, but aqueous buffer or a mixture of water and organic solvents have to be used instead. Plugging of the waste line

Table 4 Reproducibility and limits of detection *(LOD)* and limits of quantitation *(LOQ)*

Drug (TFA or PFP derivative)	Average concentration (n = 7)	CV[a]	LOQ[b]/ng/ml	LOD[b]/ng/ml
Amphetamine	20.1 ± 0.5 ng/ml	2.7%	4.9	1.5 ng/ml
Methamphetamine	19.7 ± 0.4 ng/ml	1.8%	1.8	1.4 ng/ml
MDEA	20.3 ± 0.7 ng/ml	3.7%	9.6	2.9 ng/ml
MDA	21.1 ± 0.7 ng/ml	3.1%	1.0	0.4 ng/ml
MDMA	19.8 ± 0.5 ng/ml	2.7%	10.4	3.2 ng/ml
Cocaine	20.9 ± 0.4 ng/ml	2.0%	9.5	5.2 ng/ml
Methylecgonine	19.8 ± 0.4 ng/ml	1.8%	5.3	4.2 ng/ml
Benzoylecgonine	18.6 ± 0.4 ng/ml	2.2%	3.3	1.3 ng/ml
Morphine	18.9 ± 0.2 ng/ml	1.1%	4.5	2.0 ng/ml
Codeine	18.6 ± 0.4 ng/ml	2.4%	3.4	3.0 ng/ml
Methadone	19.8 ± 0.7 ng/ml	3.8%	44	16.8 ng/ml

[a] Coefficients of variation of repeated extractions of serum samples containing 20 ng/ml of each drug followed by GC/MS analysis

[b] LOQ and LOD were determined by a method using linear regression of seven calibration strandards

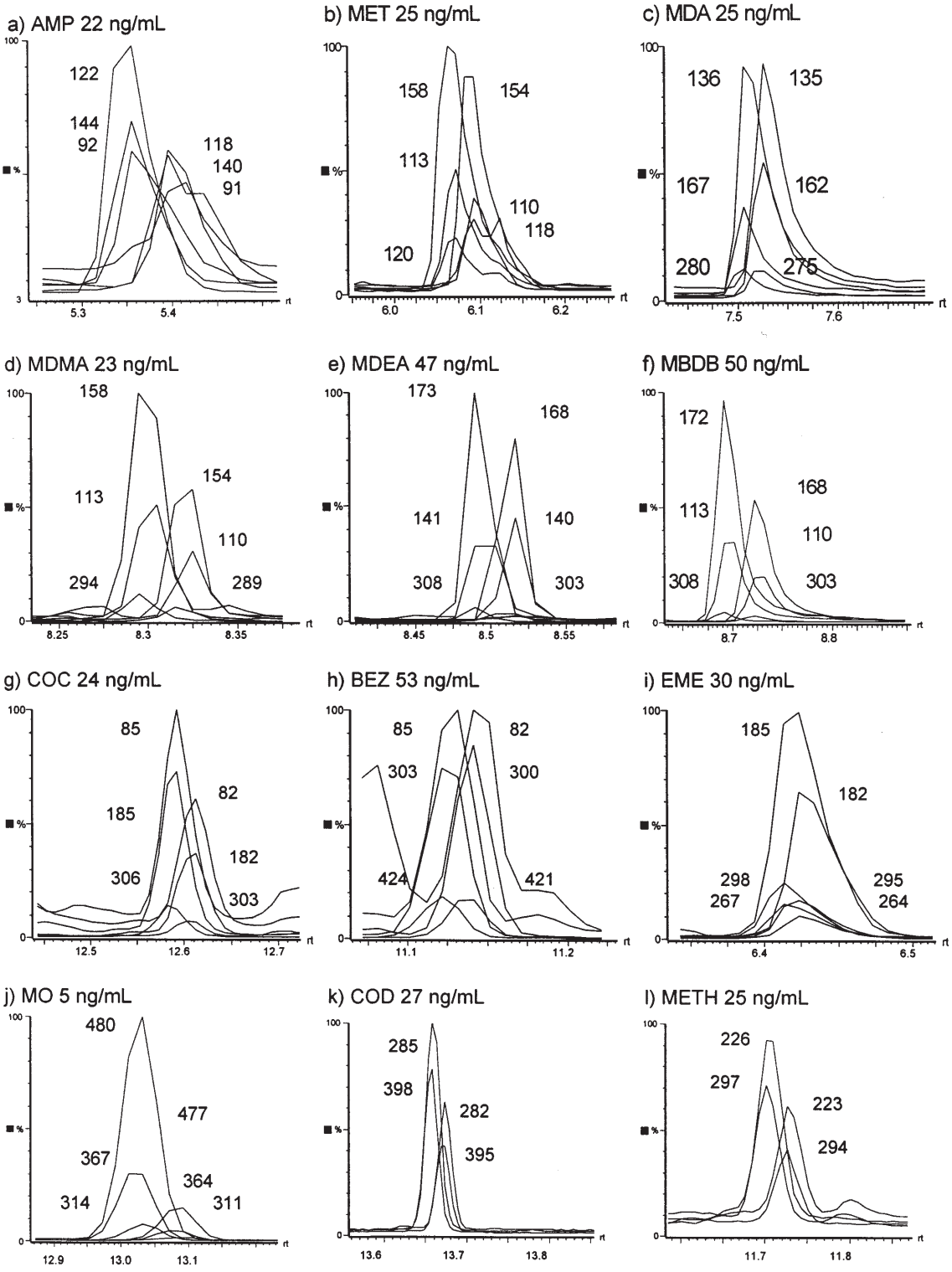

Fig. 3 Ion chromatograms obtained by analysis of serum samples from forensic cases (**a, j, k**), of proficiency test serum samples (**d, e, g, h, i**) and of spiked serum samples (**b, c, f, l**)

of the cannula or of the cartridge makes the SPE robot stop the extraction procedure because the back pressure becomes too high. The operation of the SPE robot requires tests for carry-over when setting-up a procedure. Only little effort has been made to investigate the reason for carry-over in SPE robots but we found that carry-over could be reduced by additional "purge cannula" steps. Similar results were also obtained by Diamond et al., who used the SPE robot for drug analysis in urine samples [15].

Derivatisation steps were optimized in preliminary experiments by varying the reaction times in both steps and using full-scan GC/MS-analysis. Quantitative derivatisation was found with the method shown here for all tested drugs. With shorter derivatisation times in the first step,

morphine was only partly bis-trifluoroacetylated – part was only mono-trifluoroacetylated. Amphetamine derivatives could be derivatised quantitatively using a shorter reaction time of 15 min in the first step.

The possibility of evaporation of amphetamine or amphetamine derivatives in the evaporation steps after SPE or after derivatisation has to be considered. To avoid losses of amphetamine or its TFA derivative a temperature of 40 °C was used for solvent evaporation and the evaporation process was stopped just after the solvent had evaporated. It is not possible to determine the recoveries of the whole derivatisation and evaporation process by GC/MS, because the reaction mixtures cannot be analysed without evaporation and redissolving. However, even at low concentrations of amphetamine, a high degree of linearity of the calibration curve and high ion abundances were found, showing that losses were negligible during the evaporation process using a 40 °C block temperature.

The procedure for basic drugs described here has been successfully used for the quantitation of drugs of abuse by direct injection into an ion spray/triple-quadrupole-MS/MS-system [13], as well as for the extraction of tricyclic antidepressants and neuroleptics from serum and urine samples [16] and subsequent analysis by GC/MS. For the extraction of acidic, neutral and basic drugs from serum, a manual method with collection of two fractions [20] has been automated for subsequent GC/MS and HPLC-analysis. For GC/MS quantitation of cannabinoids (THC, 11-OH-THC, THC-COOH) in serum and urine a SPE method using C18-cartridges [8] has been automated and validated [18].

With automation of solid phase extraction a rather complex manual method, which, due to several possibilities of variations of many manual steps and non controlled parameters, is liable to yield irreproducible extraction results depending on the laboratory personnel performing it, has been replaced by a programmable procedure. High reproducibility and extraction yields could be achieved by use of the SPE robot for the extraction of basic drugs from serum samples. A benefit of the new two-step derivatisation method is the possibility of the simultaneous quantitation of several different basic drugs (opiates, cocaine and its metabolites, amphetamine and designer amphetamines) with only one GC/MS analysis. Consumption of sample as well as costs and time for a drug screening analysis can be reduced by this simultaneous procedure. A specific GC/MS analysis for drugs of abuse is an important prerequisite for the realisation of the new German traffic law (§ 24 a StVG) which requires the specific analysis of blood or serum for these basic drugs or metabolites and tetrahydrocannabinol [18] for the prosecution of driving under the influence of drugs cases.

References

1. Deutscher Bundestag (1998) Gesetz zur Änderung des Strassenverkehrsgesetzes vom 28. April 1998. Bundesgesetzblatt I/25: 810–811
2. Moeller MR, Fey P, Wennig R (1993) Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program. Forensic Sci Int 63: 185–206
3. Weinmann W, Renz M, Pelz C, Brauchle P, Vogt S, Pollak St (1998) Mikromethode zur simultanen Quantifizierung von Benzoylecgonin, Amphetamin, Codein und Morphin in Blutserum mit GC/MS. Blutalkohol 35: 195–203
4. Daldrup T, Mußhoff F (1995) Forensische Analytik: Drogen und Arzneimittel. In: Günzler H, Bahadir AM, Borsdorf R, Danzer K, Fresenius W, Huber W, Lüderwald I, Schwedt G, Tölg G, Wisser H (eds) Analytiker Taschenbuch 13. Springer, Berlin Heidelberg New York, pp 210–233
5. Mulé SJ, Casella GA (1988) Confirmation of marijuana, cocaine, morphine, codeine, amphetamine, methamphetamine, phencyclidine by GC/MS in urine following immunoassay screening. J Anal Toxicol 12: 102–107
6. Yinon J (1995) Forensic applications of mass spectrometry. CRC Press, Boca Raton
7. Foltz RL (1992) Recent applications of mass spectrometry in forensic toxicology. Int J Mass Spectr Ion Processes 118/119: 237–263
8. Temme O, Daldrup T, Mußhoff F (1997) Erste Erfahrungen mit der HP-PrepStation: Adaption manueller Methoden zur Festphasenextraktion von Cannabinoiden, Opiaten und Cocain. In: Pragst F (ed) GTFCh-Symposium Moderne Meßverfahren im Rahmen der Toxikologisch-Forensischen Begutachtung. Dr. Dieter Helm, Heppenheim, pp 40–50
9. Mußhoff F, Daldrup T (1993) Evaluation of a method for simultaneous quantification of codeine, dihydrocodeine, morphine, and 6-monoacetylmorphine in serum, blood, and postmortem blood. Int J Legal Med 106: 107–109
10. Geier A, Bergemann D, von Meyer L (1996) Evaluation of a solid-phase extraction procedure for the simultaneous determination of morphine, 6-monoacetylmorphine, codeine and dihydrocodeine in plasma and whole blood by GC/MS. Int J Legal Med 109: 80–83
11. Weinmann W, Bohn C (1996) Quantitation of drugs of abuse by SPE and GC/MS using microliter-amounts of serum. In: Proceedings of the 44th American Society of Mass Spectrometry conference on mass spectrometry and allied topics, 8–15 May, Portland, Oregon, p 977
12. Weinmann W, Bohnert M (1998) Lethal monointoxication by overdosage of MDEA. Forensic Sci Int 91: 91–101
13. Weinmann W, Svoboda M (1998) Fast screening for drugs of abuse by solid-phase extraction combined with flow-injection ion spray-tandem mass spectrometry. J Anal Toxicol 22: 319–328
14. Harzer K (1996) Nachweis von Betäubungsmitteln im Serum. Labor Praxis 5: 44–48
15. Diamond FX, Vickery WE, de Kanel J (1996) Extraction of benzoylecgonine (cocaine metabolite) and opiates (codeine and morphine) from urine samples using the Zymark RapidTrace™. J Anal Toxicol 20: 587–591
16. Vogt S, Renz M, Rickli R, Briellmann T, Weinmann W (1997) Automatische Festphasenextraktion zum Nachweis von Drogen und Psychopharmaka aus Blutserum und Vollblut. Toxichem + Krimtech 64/3: 96–101
17. Funk W, Damman V, Donnevert G (eds) (1995) In: Quality assurance in analytical chemistry, VCH Verlagsgesellschaft, Weinheim, pp 32–40
18. Bromberger A, Goerke R, Weinmann W (1999) Bestimmungsgrenzen für den Nachweis von Cannabinoiden in Serum. Rechtsmedizin 9: 131–137
19. SOFT/AAFS (1997): Forensic Toxicology Laboratory Guidelines, Internet version at http://www.soft-tox.org/docs/guidelines.pdf
20. Chen XH, Franke JP, Wijsbeek J, de Zeeuw RA (1992) Isolation of acidic, neutral, and basic drugs from whole blood using a single mixed-mode solid-phase extraction column. J Anal Toxicol 16: 351–355