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High-temperature solid-phase microextraction procedure for the detection of drugs by gas chromatography–mass spectrometry

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Abstract

High-temperature headspace solid-phase microextraction (SPME) with simultaneous (''in situ'') derivatisation (acetylation or silylation) is a new sample preparation technique for the screening of illicit drugs in urine and for the confirmation analysis in serum by GC–MS. After extraction of urine with a small portion of an organic solvent mixture (e.g., 2 ml of hexane–ethyl acetate) at pH 9, the organic layer is separated and evaporated to dryness in a small headspace vial. A SPME-fiber (e.g., polyacrylate) doped with acetic anhydride–pyridine (for acetylation) is exposed to the vapour phase for 10 min at 200°C in a blockheater. The SPME fiber is then injected into the GC–MS for thermal desorption and analysis. After addition of perchloric acid and extraction with *n*-hexane to remove lipids, the serum can be analysed after adjusting to pH 9 as described for urine. Very clean extracts are obtained. The various drugs investigated could be detected and identified in urine by the total ion current technique at the following concentrations: amphetamines (200 μg/l), barbiturates (500 μg/l), benzodiazepines (100 μg/l), benzoylecgonine (150 μg/l), methadone (100 μg/l) and opiates (200 μg/l). In serum all drugs could be detected by the selected ion monitoring technique within their therapeutic range. As compared to liquid–liquid extraction only small amounts of organic solvent are needed and larger amounts of the pertinent analytes could be transferred to the GC column. In contrast to solid-phase extraction (SPE), the SPME-fiber is reusable several times (as there is no contamination by endogenous compounds). The method is time-saving and can be mechanised by the use of a dedicated autosampler.

Keywords: Solid-phase microextraction; Drugs

1. Introduction

Screening for drugs in biological fluids (e.g., urine and serum) with gas chromatography–mass spectrometry (GC–MS) commonly needs sample preparation to concentrate the pertinent analytes and to remove the interfering compounds from the matrix. For this purpose liquid–liquid extraction (LLE) or solid-phase extraction (SFE) were usually applied in the past. However, these methods need either large quantities of organic solvents, in the case of LLE, or are time-consuming and the materials used are not reusable and expensive, in the case of SFE. Solid-phase microextraction (SPME), first introduced by Arthur and Pawliszyn [1], is a new technique which uses a fused-silica fiber coated with polymers (e.g., polyacrylate) for sampling analytes directly from the matrix. The SPME-fiber is mounted in a syringe-like device for protection. By pressing the syringe plunger, the fiber is lowered either into the liquid

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sample (immersion technique) or into the headspace (headspace technique) for a fixed period of time. Afterwards the fiber is withdrawn into the needle and transfered to the injector of the gas chromatograph for thermal desorption and analysis.

The method has been mainly used for the analysis of aqueous samples of environmental pollution. In the last few years, the technique was applied for the detection of individual drugs or groups of components in biological fluids (urine, serum) (e.g., amphetamine [2–6], ethanol [7], malathione [8], valproate [9], antihistaminics [10], organophosphates [11], phencyclidine [12], trizylic antidepressants [13,14], steroids [15], benzodiazepines [16], local-anesthetics [17], anoretic drugs [18], designer drugs [19], barbiturates [20], chlorophenoles [21], methadone [22], phenothiazines [23], cocaine [24], pethidine and opiates (morphine, codeine, diamorphine) [25–27].

There are difficulties in analysing polar, high-boiling point substances like opiates and benzodiazepines from biological samples. Because of the physical properties (high-boiling point, low-volatility) of these analytes, the headspace technique has many constraints (low concentration of the analytes in the gas phase). On the other hand, the immersion technique in complex aqueous matrices like serum is restricted, e.g., by the contamination of the SPME-fiber by endogenous biomolecules (e.g., proteins).

In this manuscript measurement procedures are described for the screening of drugs in urine and confirmation analysis of suspected substances in serum, which overcome the above mentioned shortcomings by extracting the analytes with the SPME-fiber from the headspace over a dry sample extract at high temperatures (100–200°C) with simultaneous in situ derivatisation.

2. Experimental

2.1. Chemicals

Pharmaceutical quality substances (drugs) were obtained from commercial suppliers. Derivatising agents [acetic anhydride–pyridine and *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide (MSTFA)] were obtained from Supelco (Deisenhofen, Germany). All other chemicals were purchased from Merck (Darmstadt, Germany) in analytical quality.

2.2. Solutions

Methanolic stock solutions of the pharmaceutical substances were prepared at a concentration of 1 g/l and stored at 4°C. Standard solutions were prepared by diluting the stock solutions with methanol 1:99 (v/v) (final concentration=10 mg/l) and stored at 4°C.

The urine and serum were obtained from the clinical laboratory of the Medical University of Hanover (Hanover, Germany) and examined to be drug free before use. Spiked urine and spiked serum samples were prepared by adding the standard solutions to drug-free urine or serum.

The control materials (urine samples) were obtained from the United Kingdom External Quality Assessment Scheme (Cardiff, UK) and the Referenzinstitut für Bioanalytik der Deutschen Gesellschaft für Klinische Chemie (Bonn, Germany). Urine from patients was obtained from the clinical laboratory of the Medical University of Hanover (Hanover, Germany).

2.3. Material

A Hewlett-Packard (Waldbronn, Germany) 5890 series II plus gas chromatograph coupled with a 5972 mass-sensitive detector (MSD) and a MS-Windows workstation were used for gas chromatographic analysis. Chromatography was performed with a 30 m×0.25 mm I.D., HP 5 capillary column (Hewlett-Packard) and helium as carrier gas with a constant inlet pressure of 21 kPa.

For the screening test in urine, the GC was set up as follows: initial oven temperature 70°C for 4 min, from 70 to 160°C at a rate of 20°C/min, from 160 to 250°C at a rate of 5°C/min and finally from 250 to 300°C at a rate of 20°C/min. The final temperature was held for 11 min.

MS conditions for screening tests in urine were as follows: total ion current mode, detector temperature: 280°C, MS scan parameters: 50–550 amu with 1.2 scans/s, solvent delay: 10 min.

GC conditions for confirmation test of drugs in serum as follows: initial oven temperature 70°C for 4

min, from 70 to 300°C at a rate of 20°C/min. The final temperature was held for 10 min.

MS conditions were as follows: (a) total ion current mode, detector temperature: 280°C, MS scan parameters: 50–550 amu with 1.2 scans/s, solvent delay: 10 min and (b) selected ion monitoring (SIM) mode, detector temperature: 280°C. Qualifier ions (SIM) as described for the single analytes below (see Table 1).

In all cases the injector was operated at 250°C in splitless mode. After a desorption time of the SPME-fiber for 3 min in the injector, the GC run was started by opening the purge vent. After every analysis the SPME-fiber (polyacrylate) was cleaned by thermal desorption at 320°C for 45 min in the injector of a gas chromatograph.

2.4. Mass-spectrometric reference spectra

All substances in the drug-screening process were identified by computer based spectrometric library search using ''K. Pfleger, H.H. Mauerer, A. Weber: Electronic spectrometric library for drugs, poisons and pesticides (2. edition, Hewlett-Packard, Waldbronn, Germany)''. The matching quality measured by the computer-program (MS Chem Station, version c.02.00, Hewlett-Packard, Waldbronn, Germany) had to be greater than 95% for identification. As an additional parameter to check the identity of the pertinent analyte its retention index was measured and compared to the one given in the spectrometric library.

The SPME-device (manual sampling holder, polyacrylate (PA) coated fiber (film-thickness: 85 μm)) was purchased from Supelco (Deisenhofen, Germany). The blockheater was obtained from Liebisch (Bielefeld, Germany).

2.5. Sample preparation

2.5.1. Acid-hydrolysis

An 3-ml aliquot of urine and 300 μl of concen-

Table 1
Experimental conditions for substances investigated with confirmation test in serum[a]

Analytes	Qualifier ions SIM modus	Solvent for liquid–liquid extraction	SPME-extraction temperature (°C)	Derivatisation
Amitriptyline	277, 215, 202	hex.	150	underivatised
Amphetamine	177, 86, 91	hex.	100	acan–pyr
Benzoylecgonine	361, 240,105	dca–ipa (8:2, v/v)	150	MSTFA
Cocaine	303, 182, 82	dca–ipa (8:2, v/v)	150	underivatised
Codeine	371, 178, 146	dca–ipa (8:2, v/v)	150	MSTFA
Diphenhydramine	227, 73, 58	hex.	120	underivatised
Imipramine	280, 234, 58	hex.	150	underivatised
MDA	221, 162, 135	hex.	100	acan–pyr
MDE	249, 162, 72	hex.	100	acan–pyr
MDMA	235, 162, 58	hex.	100	acan–pyr
Meprobamate	–	hex	120	underivatised
Methadone	309, 294, 72	hex.	150	underivatised
Methamphetamine	191, 100, 58	hex.	100	acan–pyr
Methaqualone	250, 235, 91	hex.	120	underivatised
Metohexitale	–	hex–eac.(1:1, v/v)	200	underivatised
Methylphenidate	–	hex.	120	underivatised
Morphine	429, 236, 196	dca–ipa (8:2, v/v)	150	mstfa
Normethadone	–	hex.	150	underivatised
Pentobarbital	–	hex–eac.(1:1, v/v)	200	underivatised
Pethidine	247, 172, 71	hex–eac.(1:1 v/v)	150	underivatised
Phencyclidine	243, 242, 200	hex.	120	underivatised
Prolintane	–	hex.	120	underivatised
Secobarbital	–	hex–eac.(1:1, v/v)	200	underivatised
Tramadol	263, 135, 58	hex–eac.(1:1, v/v)	150	underivatised

[a] SIM=Selected ion monitoring; dca=dichlormethane; ipa=isopropylalcohol; hex=hexane; eac=ethyl acetate; acan–pyr=acetic anhydride–pyridine.

trated hydrochloric acid were heated up to 120°C in a sealed vial for 20 min. After cooling down the hydrolysate was adjusted to pH 9.

2.6. Drug screening of urine

A sample of 3 ml of urine, 2 ml of the organic solvent mixture [hexane–ethyl acetate, 1:1 (v/v)] and 2.0 g of the salt mixture (1.4 g of $NaHCO_3$–1.4 g of Na_2CO_3–15.1 g of NaCl) were vortexed for 5 min at pH 9. After centrifugation (3220 *g*, 4°C, 5 min) the organic phase was evaporated to dryness (nitrogen) in a 2-ml headspace vial. The vial was sealed and heated to 200°C in a block-heater (see Fig. 1). A SPME-fiber doped with the derivatising agent (acetic anhydride–pyridine) was exposed to the headspace of the vial for 10 min (in situ on fiber derivatisation). After thermal absorption of the analytes, the fiber was transfered to the GC-injector for analysis.

In the screening procedure for opiates and cocaine metabolites, the aqueous phase was vortexed (5 min) with 2 ml of dichlormethane. After centrifugation (3220 *g*, 4°C, 5 min) the organic phase was evaporated to dryness (nitrogen) in a 2-ml headspace vial. A 2-μl aliquot of the derivatising agent (MSTFA) was added. The vial was sealed and heated to 200°C in a block-heater. A SPME-fiber was exposed to the headspace of the vial for 10 min (in situ derivatisation). After thermal absorption of the analytes, the fiber was transfered to the GC-injector for analysis.

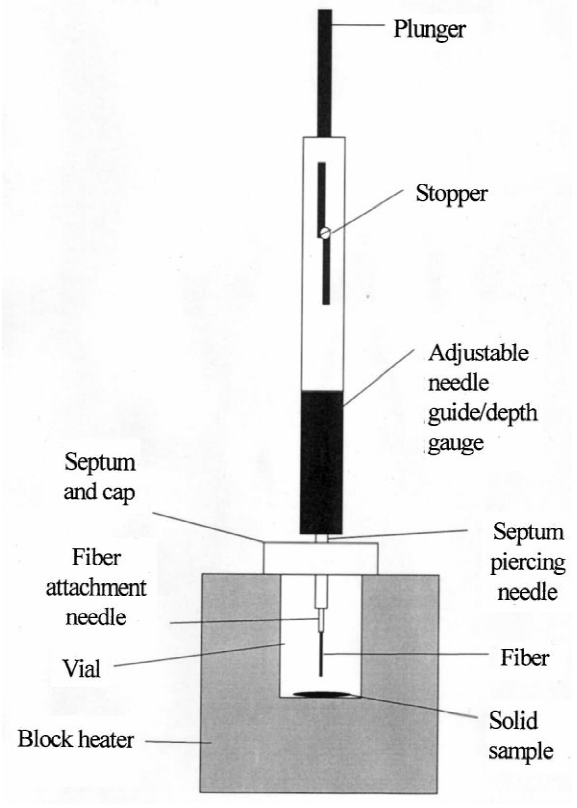

Fig. 1. SPME high-temperature headspace sampling.

2.7. Confirmation analysis of serum

A 2-ml aliquot of the serum was mixed with 100 μl of 70% perchloric acid and 2 ml of *n*-hexane to remove endogenous substances, e.g., proteins and fatty acids. After centrifugation (3220 *g*, 20°C, 5 min) the aqueous phase was adjusted to pH 9 and vortexed (5 min) with 2 ml of an organic solvent or organic solvent mixture depending on the substance which should be analysed (see Table 1).

After centrifugation (3220 *g*, 4°C, 5 min) the organic phase was evaporated to dryness (nitrogen) in a 2-ml headspace vial. SPME headspace extraction with in situ derivatisation took place as described for urine (derivatising agent and SPME extraction temperature depending on the substance which should be analysed, see Table 1).

3. Results and discussion

A screening method for drugs in biological fluids implies the problem of concentrating a huge variety of pertinent analytes with very different chemical–physical properties (polarity, boiling-point) and the need for removing interfering substances from a complex matrix. When SPME is used for sample preparation, there are two ways to address these problems. Using the SPME-headspace technique, the analytes are enriched by the fiber in the gas phase of an aqueous sample. This technique is adequate for extracting substances that are very volatile, like amphetamines, alcohols and organic pesticides. The SPME-fiber is not in direct contact with the matrix, so that only a few interfering substances are co-extracted. For substances with high molecular

weights and low-volatilities like opiates (morphine, codeine and heroin), cocaine or barbiturates the SPME-immersion technique may be used. As the SPME-fibers are non-selective by their nature, many endogenous components of the biological matrix are co-extracted (especially in serum or blood), which decreases the analytical specificity and sensitivity. Furthermore, many of the interfering macromolecules are difficult to desorb from the SPME-fibers and may cause difficulties in cleaning the fiber or irreversible contamination.

To overcome these problems, the analytes are extracted from the headspace of a solid, dry matrix by the SPME-fiber using higher temperatures (100–200°C), so that the less volatile substances can also be analysed without direct contact between the fiber and the matrix. Simultaneous derivatisation (e.g., acetylation, silylation or methylation) during the SPME-extraction can additionally improve the sensitivity of the procedure by converting the polar analytes into their less polar derivatives, thus increasing their volatility and the partition coefficients between the fiber-coating and headspace. Fig. 2 shows a chromatogram of 2 ml of spiked urine analysed by this technique.

The concentrations of the analytes in the headspace were high enough to recover all substances by the SPME-polyacrylate fiber at 200°C. Compared to direct-injection of the solvent-extract, larger amounts of the pertinent analytes could be transferred to the GC column by high-temperature SPME headspace extraction (concentration and purification process).

3.1. Effect of temperature and extraction time

SPME-extraction is an exothermic equilibration process [28], so that an increase of the sampling temperature will reduce the analyte recovery because the fiber-coating–headspace-partition coefficient is getting smaller. On the other hand the headspace–sample-partition coefficient of the analyte increases with higher sampling temperature, resulting in a higher concentration of the analyte in the headspace and a shorter equilibrium time.

Fig. 3 shows the influence of different temperatures and sampling times on the SPME-extraction recoveries. Samples of 300 ng of each substance (methamphetamine, methadone, cocaine, morphine and flunitrazepam) from the standard solutions were filled into 2-ml headspace vials and were evaporated to dryness by nitrogen. Afterwards they were absorbed by an SPME-polyacrylate fiber using high-temperature headspace extraction with in situ on fiber derivatisation (acetic anhydride–pyridine) at different temperatures and times in a blockheater.

The recoveries of the analytes first increased until

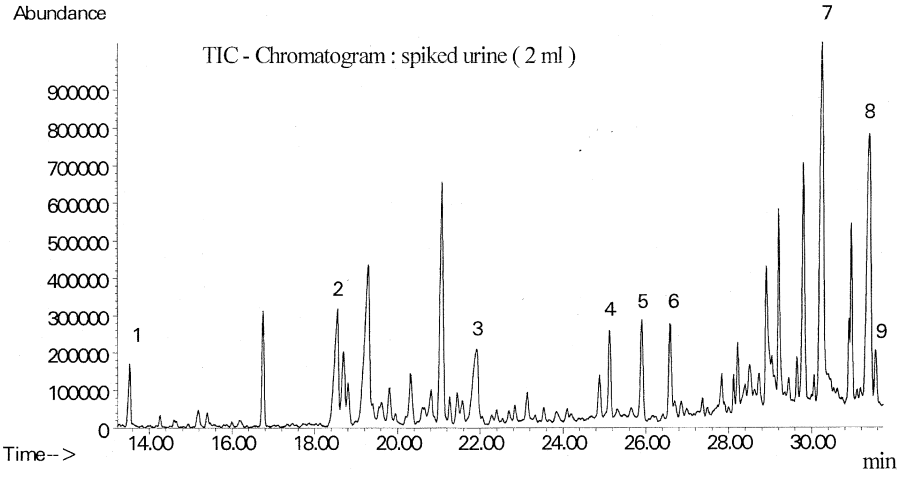

Fig. 2. TIC-chromatogram of spiked urine (2 ml) containing (1) acetylated amphetamine (300 mg/l), (2) secobarbital (100 μg/l), (3) phenobarbital (500 μg/ml), (4) methadone (150 μg/l), (5) propoxyphene (400 μg/l), (6) imipramine (150 μg/l), (7) acetylated codeine (300 μg/l), (8) flunitrazepam (300 μg/l) and (9) diacetylated morphine (300 μg/l). Derivatising agent: acetic anhydride–pyridine.

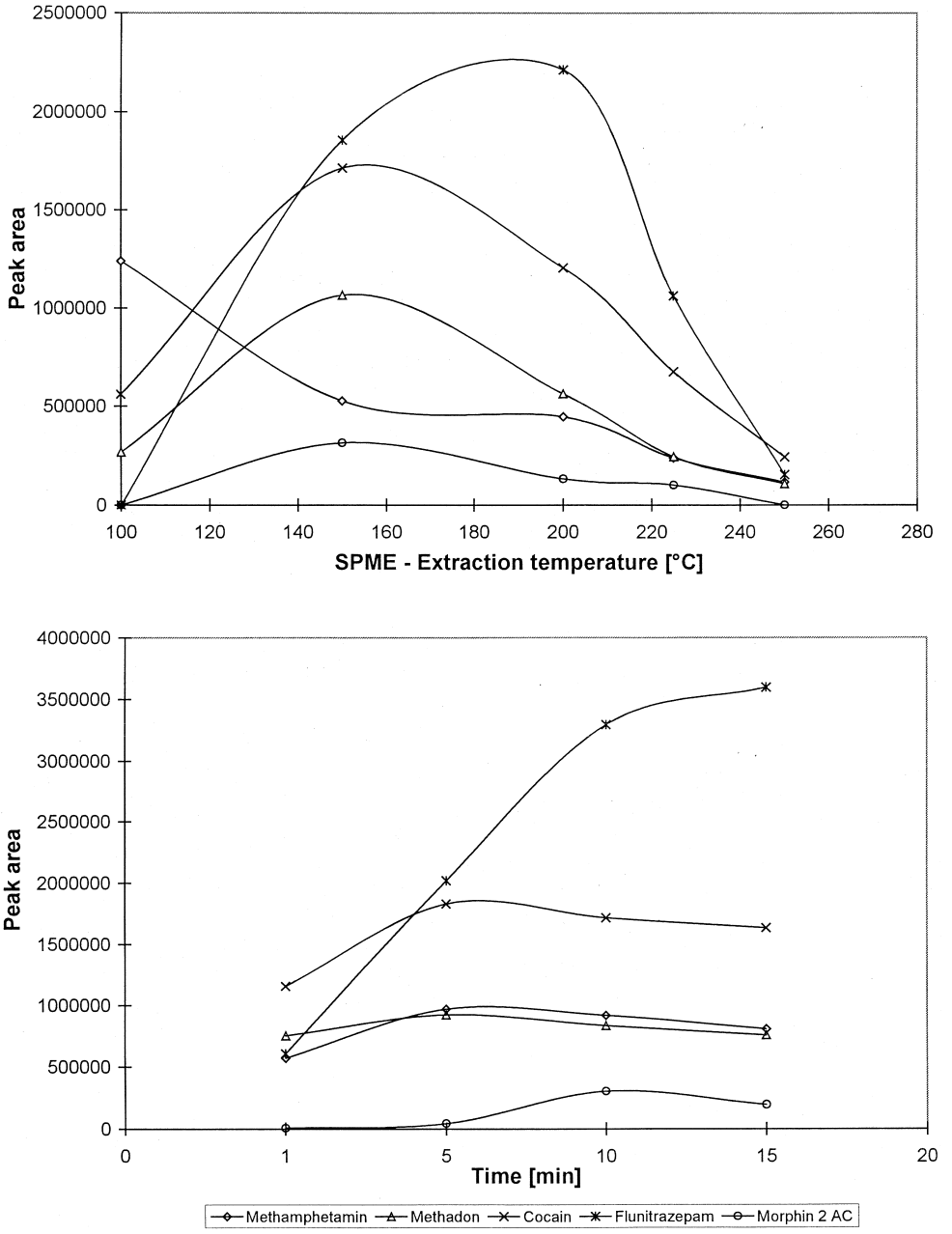

Fig. 3. Effect of SPME-extraction temperature (top) and extraction time (bottom) (in situ acetylation, 300ng each substance). AC: Acetylated.

an optimum was reached and then declined. Every substance tested has its own optimal SPME-extraction temperature that is related to its volatility. Volatile substances like amphetamine (100°C) have a much lower optimum temperature than substances with a high-boiling point like flunitrazepam (200°C). Choosing a SPME-extraction temperature and a time for screening drugs have to be a compromise be-

Table 2
Substances investigated with the SPME drug screening procedure in urine

Analytes	EU-cut off level (μg/l) [30]	Lowest concentration examined: TIC (μg/l)	Lowest concentration examined: SIM (μg/l)
Amphetamines	300	200	10
Designer drugs (MDA, MDMA, MDE)	–	200	10
Anoretic drugs	–	300	–
Barbiturates	–	500	–
Benzodiazepines	–	100	–
Cocaine and metabolites	300	150	10
Opiates (diamorphine, morphine, codeine)	300	200	20
Methadone	–	100	30
Tramadol	–	200	–
Phencyclidine	–	100	–
Antihistaminics (diphenhydramine)	–	300	10
Tricyclic antidepressants	–	200	20

tween the different optimum temperatures and extraction times of the single analytes. For the screening process in urine we selected 200°C and 10 min.

3.2. Derivatisation

Derivatisation in SPME enhances the partition coefficient of the coating/gas phase (headspace), thus increasing the extraction efficiency and method sensitivity. The derivatising process can take place before, simultaneously (in situ) or after the SPME-extraction [29]. It is also described as derivatisation in the matrix, in the SPME-fiber coating or in the GC-injector port [28].

Because of the time saved by simultaneous (in situ) derivatisation, this procedure was used for drug screening in urine. Common derivatising agents like MSTFA for silylation, acetic anhydride–pyridine for acetylation and trimethylammonium hydroxide (TMAH) for methylation were selected. The pertinent mass spectra are very often shown in commercial mass spectra libraries, thus supporting a computer assisted identification of the analytes in urine.

In the case of acetylation, the derivatisation takes place on the SPME-fiber. Prior to the SPME-extraction of the analytes, the polyacrylate fiber was doped with the derivatising agent acetic anhydride–pyridine in the headspace of a separate 2-ml vial in a blockheater at 70°C for 5 min. This procedure improved the recoveries as compared to the addition of the derivatising mixture to the dried sample during the SPME-extraction as well as the signal-to-noise ratio.

Amphetamines (amphetamine and methamphetamine) and designer drugs (MDA, MDMA and MDE) are completely derivatised by this procedure.

Table 3
Results of 24 external quality assessment programs investigated with the SPME drug screening procedure in urine

Analytes	Appearance in quality ass. prog. (*N*)	Correct identification (*N*)	Correct identification (%)
Amphetamines, anoretic drugs, designer drugs	17	17	100
Barbiturates	10	10	100
Cocaine and metabolites	9	9	100
Benzodiazepines	14	14	100
Non-opiate narcotics (e.g., methadone, tramadol)	12	10	83
Opiates (e.g., diamorphine, morphine, codeine)	24	22	92
Phencyclidine	9	9	100
Propoxyphene	2	2	100
Tricyclic antidepressants	4	4	100
Methaqualone	2	2	100

Opiates, e.g., codeine, were not completely derivatised by this procedure, therefore, for this group of analytes as well as for benzoylecgonine, silylation with MSTFA was preferred.

MSTFA (2 μl) was transfered directly into the headspace vial containing the dried sample extract because complete silylation on a SPME-fiber doped with MSTFA could not be achieved.

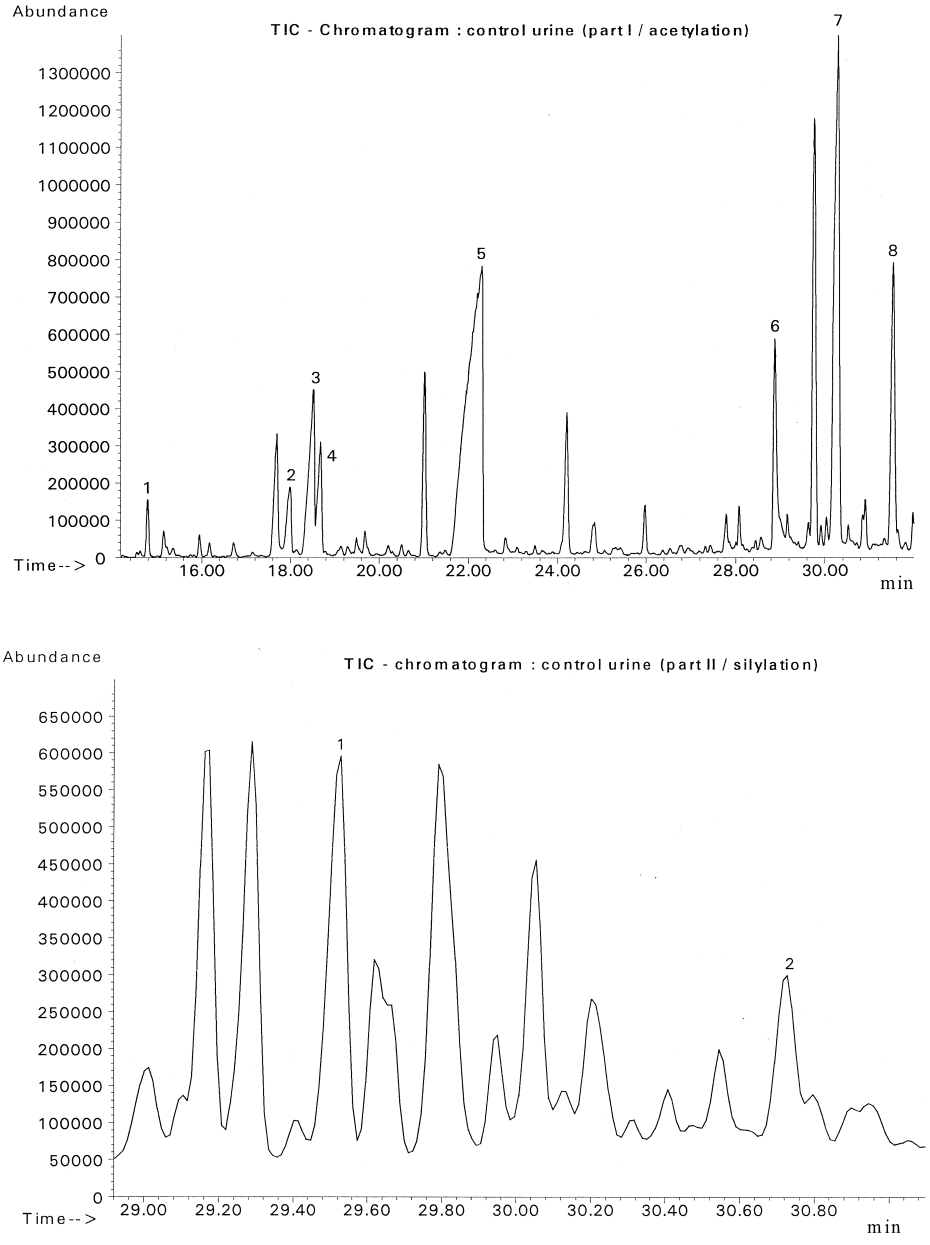

Fig. 4. (Top): TIC-chromatogram of control urine (3 ml) part 1 (acetylation) containing: (1) acetylated methamphetamine, (2) acetylated paracetamol, (3) secobarbital, (4) diacetylated ephedrine, (5) phenobarbital, (6) codeine, (7) acetylated codeine and (8) diacetylated morphine. (Bottom): TIC-chromatogram of reference urine (3 ml), part 2 (silylation) containing: (1) codeine-TMS, (2) diamorphine-TMS. TMS: Trimethylsilyl.

3.3. Results of the drug screening in urine

Using the SPME high-temperature headspace extraction technique, the analytes that could be detected in urine are shown in Table 2.

The substances in the drug screening process were identified by computer assisted spectrometric library search. The matching qualitity, measured by the computer-program, had to be greater than 95% to accept the pertinent analytes as identified. As an

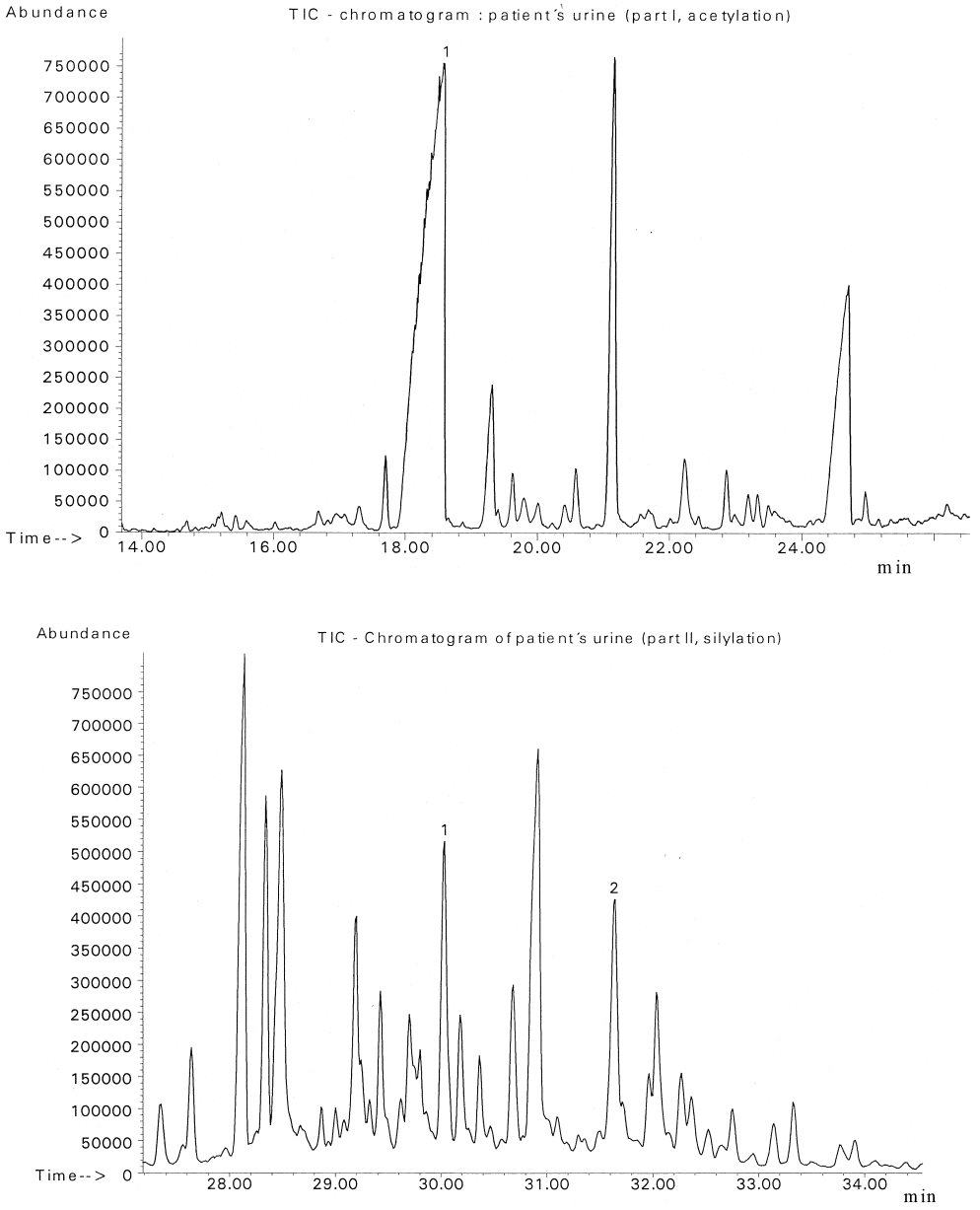

Fig. 5. (Top): TIC-chromatogram of a patient's urine (acid hydrolysis, SPME extraction with in situ acetylation), part 1: (1) paracetamol-AC. AC: Acetyl. (Bottom): TIC-chromatogram of a patient's urine (acid hydrolysis, in situ silylation), part 2: (1) morphine-2TMS and (2) metoclopramide. TMS: Trimethylsilyl.

additional parameter to check the identity of the analyte, its retention index was measured and compared to the one given in the used spectrometric library. Only small amounts (2–4 ml) of organic solvent are required and very clean extracts are obtained. The procedure was evaluated with reference material (urine) from external quality assessment programs and authentic material.

The results of 24 external quality assessment programs in which the new SPME-procedure was tested is shown in Table 3 (United Kingdom External Quality Assessment Scheme: 16, Referenzinstitut

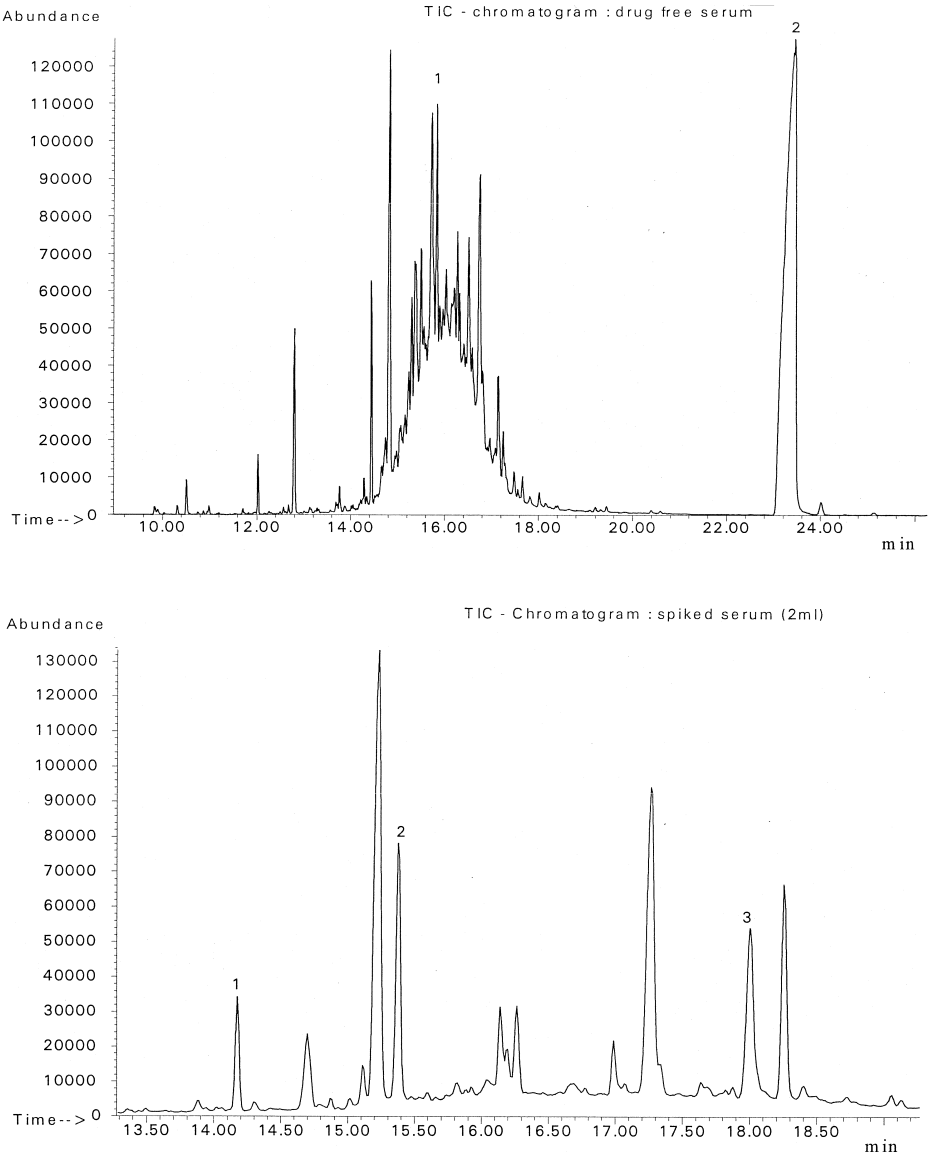

Fig. 6. (Top): TIC-chromatogramm of a SPME extraction of drug-free serum (evaporated solvent extract) with a polyacrylate fiber at 200°C for 10 min, (1) fatty acids (e.g., palmitine acid, linolene acid and stearine acid) and (2) cholesterole. (Bottom): TIC-chromatogram of spiked serum (2 ml) containing: (1) pethidine, (2) tramadol and (3) codeine, (300 μg/l each) after removing interfering substances.

Table 4
Substances investigated with the confirmation test in serum (TIC)[a]

Analytes	Lowest concentration examined: TIC (μg/l)
Amitriptyline	200
Amphetamine	200
Benzoylecgonine	150
Cocaine	200
Codeine	200
Diphenhydramine	300
Imipramine	200
MDA/MDMA/MDE	200
Meprobamate	300
Methadone	100
Methamphetamine	200
Methaqualone	300
Methohexital	500
Methylphenidate	300
Morphine	200
Normethadone	100
Pentobarbital	500
Pethidine	200
Phencyclidine	100
Prolintane	300
Propylhexedrine	300
Secobarbital	500
Tramadol	200

[a] TIC=Total ion current.

für Bioanalytik der Deutschen Gesellschaft für Klinische Chemie: 6).

Fig. 4 shows the TIC chromatogram of a control material (Deutsche Gesellschaft für Klinische Chemie: A I/97) containing the following substances: methamphetamine (1.0 mg/l), codeine (2.0 mg/l), morphine-3β-D-glucuronide (2.0 mg/l), diamorphine (0.5 mg/l), ephedrine (2.0 mg/l), secobarbital (0.5 mg/l) and phenobarbital (5.0 mg/l). Morphine glucuronide could be detected after acid hydrolysis of the urine.

The TIC-chromatograms of the investigation of a patient's urine containing paracetamol, metoclopamide and morphine using this procedure is shown in Fig. 5.

3.4. Results of confirmation analysis in serum

The SPME high-temperature headspace extraction of a dried sample extract is also applicable to drug detection in complex matrices like serum.

Applying the SPME procedure to an evaporated solvent extract of serum, endogenous substances like fatty acids (e.g., linolene, palmitine and stearine acid) and cholesterol interfered with the pertinent

Table 5
Substances investigated with the confirmation test in serum (SIM)[a]

Analytes	Therapeutic range: serum (μg/l) [31]	Lowest concentration examined: SIM (μg/l)
Amitriptyline	50–300	20
Amphetamine	20–100	10
Benzoylecgonine	–	10
Cocaine	100–300	10
Codeine	30–250	20
Diphenhydramine	100–1000	10
Imipramine	50–300	20
MDA	–	10
MDE	–	10
MDMA	–	10
Methadone	80–300	30
Methamphetamine	10–50	10
Methaqualone	1000–3000	10
Morphine	10–100	20
Pethidine	100–1000	30
Phencyclidine	–	10
Tramadol	100–1000	30

[a] SIM=Selected ion monitoring.

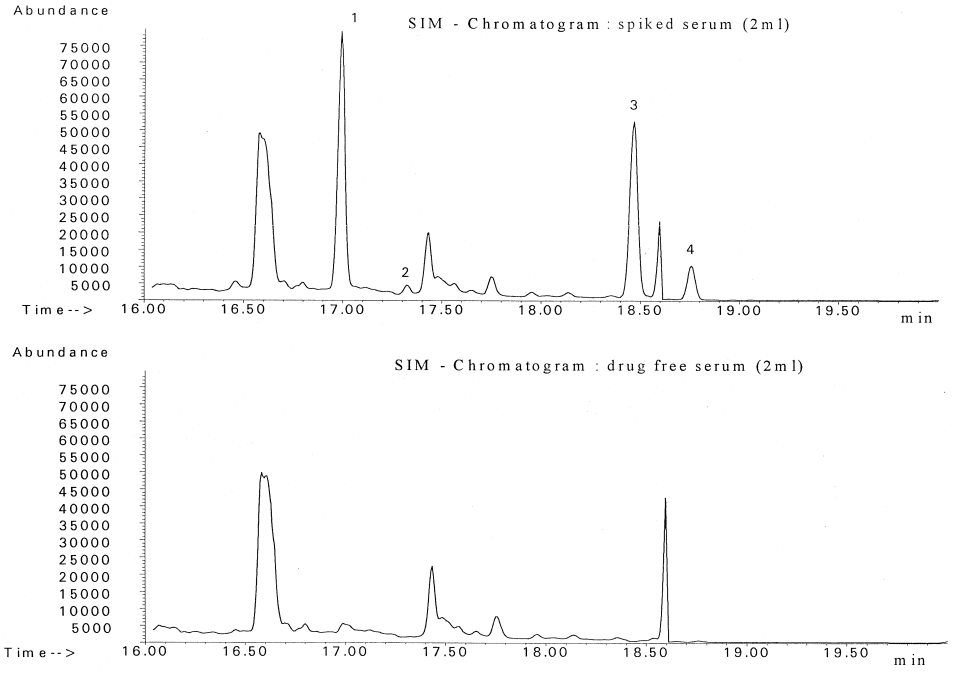

Fig. 7. SIM-chromatogram (part) of spiked serum (top) and drug free serum (bottom): Simultaneous detection of (1) cocaine (5 μg/l), (2) benzoylecgonine-TMS (10 μg/l), (3) codeine-TMS (10 μg/l) and (4) morphine-2TMS (10 μg/l) in 2 ml of serum. TMS: Trimethylsilyl, SIM: selected ion monitoring.

analytes (see the upper section of Fig. 6). However, after addition of perchloric acid and pre-extraction with *n*-hexane to remove the lipids, the serum can be analysed after adjusting to pH 9 as described for urine (see the lower section of Fig. 6: TIC-chromatogram of a serum (2 ml) spiked with pethidine, tramadol and codeine.).

The analytes investigated by the total ion current mode (TIC) are shown in Table 4. Fig. 7 shows the simultaneous detection of cocaine, benzoylecgonine, codeine and morphine from 2 ml of spiked serum in SIM-mode. Table 5 lists drugs that were detectable in serum at concentrations within their therapeutic range [31], which demonstrates the high sensitivity of the procedure.

There was no irreversible contamination of the SPME–polyacrylate fiber from the endogenous substances, which allowed their repeated use (30–50 times).

3.5. Quantitative determination in urine and serum

The SPME high-temperature headspace extraction was also used for the quantitative determination of opiates (morphine and codeine) in urine and serum (morphine, ethylmorphine and codeine). The results will be presented in a separate publication.

4. Conclusion

High-temperature headspace analysis of a dried sample extract using SPME for absorption, in situ derivatisation and transfer of the volatiles to the gas chromatograph is a reliable and time-saving method for drug screening in urine and confirmation analysis in serum. As compared to traditional liquid–liquid extraction only small amounts of organic solvent are

required and larger amounts of the pertinent analytes could be transferred to a GC column.

In contrast to solid-phase extraction (SPE) the SPME-fiber is reusable several times. By using a dedicated autosampler system the procedure can be mechanised.

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