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Automated headspace solid-phase dynamic extraction for the determination of amphetamines and synthetic designer drugs in hair samples☆

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Abstract

The technique of automated headspace solid-phase dynamic extraction (SPDE) coupled with gas chromatography–mass spectrometry was evaluated for the determination of amphetamines and synthetic designer drugs in hair samples. Headspace SPDE is a novel method for the solventless extraction of organic compounds in aqueous samples. In a so-called inside needle capillary absorption trap a hollow needle with an internal coating of polydimethylsiloxane is used as extraction and preconcentration medium. Sampling is performed on the solution headspace by passing the gas through the device actively by a syringe. Analytes present in the sample are sorbed onto the deposited stationary phase. The syringe needle is placed into the injection port of a GC and rapid heating of the metal needle induces the desorption of analytes. For the determination of amphetamine, methamphetamine, 3,4-methylendioxyamphetamine (MDA), 3,4-methylendioxymethamphetamine, 3,4-methylendioxyethylamphetamine (MDEA), 3,4-methylendioxyphenyl-2-butanamine and *N*-methyl-1-(3,4-methylendioxyphenyl)-2-butanamine in human hair samples, 10 mg of hair were hydrolysed with sodium hydroxide. After absorption of analytes for an on-coating derivatization procedure the SPDE needle was directly placed into the headspace of a second vial containing *N*-methyl-bis(trifluoroacetamide). A validation procedure revealed absolute analyte recoveries between 10.2 and 16.7%. Linearity was obtained from 0.1 to 20 ng/mg with coefficients of correlation between 0.992 and 0.999. Intra- and inter-day precision were determined at two different concentrations and resulted in ranges between 1.4 and 4.1% (intra-day) and 4.2–14.6% (inter-day). Limits of detection between 0.03 ng/mg (MDA) and 0.19 ng/mg (MDEA) were achieved. Results indicated that SPDE is a rapid and sensitive method for the analysis of biological samples. Compared to solid-phase microextraction we found a higher extraction rate coupled with a faster automated operation.

Keywords: Headspace analysis; Solid-phase dynamic extraction; Extraction methods; Hair; Forensic analysis; Amphetamines; Butanamines

1. Introduction

During the past few years, solid-phase microextraction (SPME), discovered and developed by Zhang and Pawliszyn [1], has emerged as a versatile solvent-free alternative to conventional liquid–liquid

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extraction and solid-phase extraction procedures. SPME in conjunction with gas chromatography–mass spectrometry (GC–MS) analysis has been employed for a variety of organic compounds, especially for volatile and semi-volatile agents using the headspace technique. The main disadvantages of SPME are the fragility of the fused-silica and the unprotected stationary phase coating on the outer surface of the fibre when extended through the syringe needle. The limited flexibility regarding surface area and film thickness is another problem of SPME. There were several efforts to overcome these disadvantages. All attempts aimed at developing a device with the coating on the interior of a needle or capillary instead of a fibre. The advantages are greater capacity, higher extraction speed and stability of the device. A technique using internally coated hollow needles was described by Murphy [2]. In 1997, an inside needle capillary adsorption trap (INCAT) technique was developed [3] which has been used for analysis of complex mixtures of volatile organic compounds [4] and the sampling of benzene, toluene, ethyl benzene and xylene compounds [5]. An SPME–LC system known as in-tube SPME using an open tubular fused-silica capillary column was developed by Eisert and Pawliszyn [6]. Several applications have been described [7–16].

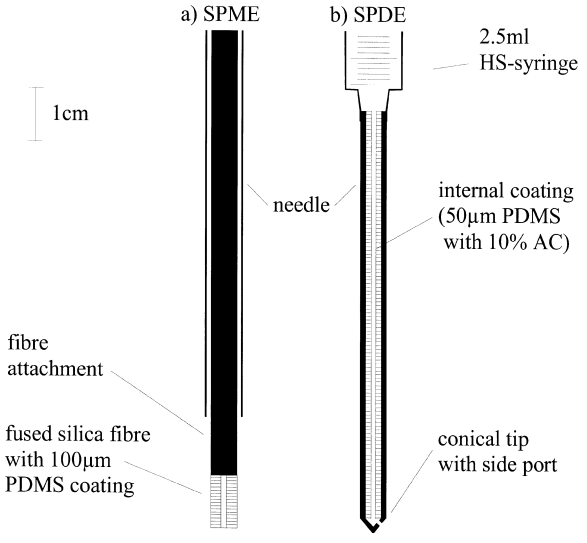

Fig. 1. Schematic representation of the SPME device (a) in comparison to the SPDE device (b). The volume of stationary phase (hatched) is significantly increased.

The solid-phase dynamic extraction (SPDE) developed by Chromtech (Idstein, Germany) in 2000 is the first commercially available inside-needle device for headspace analysis using GC–MS. Stainless steel needles (8 cm) coated with a 50-µm film of polydimethylsiloxane (PDMS) and 10% of activated carbon are used. A diagram of a SPDE device in comparison to a SPME fibre is given in Fig. 1. The volume of the stationary phase of the SPDE needle is approximately 5.99 mm^3 compared to a 100-µm PDMS SPME fibre with 0.94 mm^3. SPDE was successfully applied to the analysis of pesticides in water by Lipinski [17].

Our first validated method was developed for the analysis of amphetamines and synthetic designer drugs in hair samples of drug abusers.

2. Experimental

2.1. Reagents and materials

The following chemicals were purchased from Promochem (Wesel, Germany) as methanolic standard solutions: amphetamine, [$^2H_5$]amphetamine (amphetamine-d_5), methamphetamine, [$^2H_{11}$]methamphetamine (methamphetamine-d_{11}), 3,4-methylendioxyamphetamine (MDA), [$^2H_5$]3,4-methylendioxyamphetamine (MDA-d_5), 3,4-methylendioxyethylamphetamine (MDEA), [$^2H_5$]3,4-methylendioxyethylamphetamine (MDEA-d_5), 3,4-methylendioxymethamphetamine (MDMA), [$^2H_5$]3,4-methylendioxymethamphetamine (MDMA-d_5), 3,4-methylendioxyphenyl-2-butanamine (BDB), *N*-methyl-1-(3,4-methylendioxyphenyl)-2-butanamine (MBDB), [$^2H_5$]1, 2-dideutero-*N*-trideuteromethyl-1-(3, 4-methylendioxyphenyl)-2-butanamine (MBDB-d_5). The compounds were deuterated at the side chain (methamphetamine-d_{11} also at the phenyl ring). The solutions were stored at 8 °C and used after dilution to the required concentrations. *N*-Methyl-bis(trifluoroacetamide) (MBTFA) was obtained from Macherey–Nagel (Düren, Germany).

The SPDE equipment (syringes with attached SPDE needles and SPDE gas station) was kindly donated by Chromtech. The needles (50 mm×0.8 mm, I.D. 0.53 mm, conical needle tip with side port)

were coated by the manufacturer with 50 μm PDMS containing 10% of activated carbon (AC). The needles were attached to 2.5-ml gas-tight syringes with a side port for gas flushing (Hamilton, Darmstadt, Germany). Gas station and syringe were connected to the nitrogen gas supply for flushing regulated by the autosampler. The gas station is used to acquire a defined volume of nitrogen before desorption. The side port of the syringe could not be used for desorption, because it has no pressure regulator. The syringe adapter heater was set at 50 °C.

2.2. GC–MS method

An Agilent model 6890 Series Plus gas chromatograph in combination with a model 5973 N mass spectrometer and a CTC-Combi-PAL-Autosampler including an incubator oven with six heated vial positions and shaker (Agitator) were used for analysis (Chromtech). Data acquisition and analysis were carried out using standard software supplied by the manufacturer. All steps of the SPDE methods were fully automated, controlled by the CTC-Combi-PAL software with custom-made macros. Substances were separated on a fused-silica capillary column (DB-5MS, 30 m×0.25 mm I.D., film thickness 0.25 μm, J&W Scientific, Cologne, Germany). The temperature program was as follows: 90 °C hold for 1 min, 8 °C/min up to 210 °C, hold for 2 min, 30 °C/min up to 280 °C, hold for 5 min. The temperatures for the injection port, ion source, quadrupole and interface were set at 250, 230, 150 and 280 °C, respectively. The splitless injection mode was used and helium with a flow-rate of 1.0 ml/min was used as carrier gas. The inlet nut was modified to accommodate the SPDE needles with a diameter of 0.8 mm. A 1.5 mm I.D. headspace insert liner (Supelco, Deisenhofen, Germany) and a conventional septum were used.

To determine the retention times and characteristic mass fragments, electron impact (EI) mass spectra of the analytes were recorded by total ion monitoring. For quantitative analysis, the chosen diagnostic mass fragments were monitored in the selected ion monitoring (SIM) mode: amphetamine-TFA (*m/z* **140,** 91, 118), amphetamine-d_5-TFA (*m/z* **144,** 92, 123), methamphetamine-TFA (*m/z* **154,** 110, 118), methamphetamine-d_{11}-TFA (*m/z* **160,** 113, 126), MDA-TFA (*m/z* **135,** 162, 275), MDA-d_5-TFA (*m/z* **136,** 167, 280), MDMA-TFA (*m/z* **154,** 110, 135), MDMA-d_5-TFA (*m/z* **158,** 113, 136), MDEA-TFA (*m/z* **168,** 140, 303), MDEA-d_5-TFA (*m/z* **173,** 141, 308), MBDB-TFA (*m/z* **168,** 176, 303), MBDB-d_5-TFA (*m/z* **172,** 178, 308) and BDB-TFA (*m/z* **135,** 176, 289). Deuterated BDB was not available, so that MDA-d_5 was used as internal standard. For quantification, peak area ratios of the analytes to the internal standard were calculated as a function of the concentration of the substances.

2.3. Headspace SPDE procedure

The hair samples were washed for 5 min in deionised water, light petroleum and dichloromethane, respectively, using a Vortex Genie 2 mixer (Bender and Hobein, Zurich, Switzerland). After drying, the hair samples were cut into small pieces about 1 mm long. The washing solutions were analysed by conventional GC–MS procedures to exclude contamination.

Ten milligrams of hair were submitted to alkaline hydrolysis into a 10-ml headspace (HS) vial in the presence of 1 ml of NaOH (10 *M*) and 80 μl aqueous internal standard solution (250 ng deuterated analytes/ml). The vial was sealed using a silicone–PTFA septum and a magnetic cap and was shaken for 5 min at 50 °C in the agitator of the autosampler (650 rpm, agitator on time: 0:05 min, agitator off time: 0:02 min, Fig. 2a). The SPDE needle was inserted into the sample vial through the septum and the plunger was moved up and down at 200 μl/s for 50 times to extract the analytes dynamically (Fig. 2b). For on-coating derivatization, the syringe was positioned above a second vial containing 25 μl of MBTFA and the plunger was moved up and down six times (Fig. 2c). After the last filling cycle, the syringe was emptied, moved to the gas station and 2.5 ml of nitrogen were aspirated (Fig. 2d). For desorption of the analytes the needle was completely introduced into the hot injection port of the GC and was held there for 15 s for thermal equilibration (250 °C). The plunger was moved slowly down (10 μl/s) and the analytes were flushed into the GC system (Fig. 2e). Simultaneously with desorption, the GC run was started. After removing the SPDE needle from the injection port, syringe and

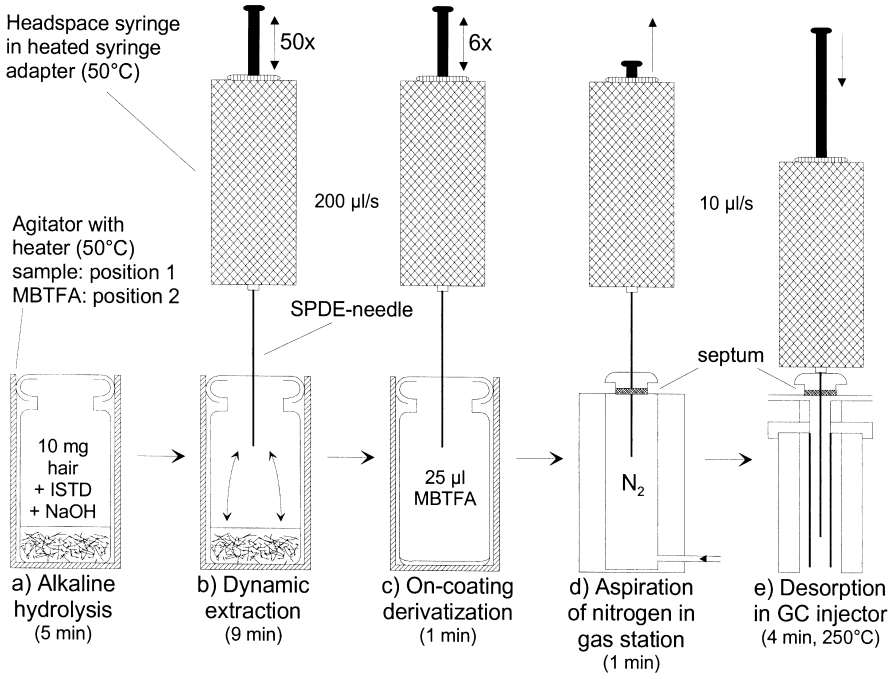

Fig. 2. Procedure for fully automated hair analysis with alkaline hydrolysis, solid-phase dynamic extraction (SPDE), derivatization and GC–MS.

needle were cleaned by flushing with nitrogen for 1 min, followed by a blank run to exclude carry-over.

Spiked samples containing 2 ng of each analyte per mg hair, respectively, were prepared and analysed using the procedures described above. For the validation of the method peak purity and selectivity, intra- and inter-day precision at two different concentrations (2 and 20 ng/mg), absolute extraction recovery and sample stability were determined. The linearity of the calibration curve was evaluated between 0.05 and 20 ng/mg. For the determination of the limit of detection (LOD) and the limit of quantitation (LOQ) a separate calibration curve in the range of LOD (0.01–1 ng/mg) was established [18,19].

The method conditions that are independent of the extraction mode (SPDE or SPME) like alkaline hydrolysis, agitator speed, salt additions (not necessary for amphetamines), amount of derivatization reagent were set according to our previously developed SPME method [20] as given in the text above. The following SPDE specific parameters were optimised successively by testing three vials at each temperature and each point: temperature of agitator and headspace syringe (30–90 °C), number of filling cycles for extraction (10–100) and derivatization (1–8), speed of aspirating the syringe for extraction (50–250 µl/s) and desorption (10–200 µl/s), flush gas volume for desorption (250–2500 µl), predesorption time in the GC injection port (1–45 s) and desorption temperature (230–280 °C).

3. Results and discussion

3.1. Parameter optimisation for the SPDE method

3.1.1. Temperature

The incubation, extraction and derivatization of the samples at increased temperatures led to an improvement in analyte sensitivity, because thereby the transfer of the analytes from the aqueous phase to the gaseous phase and the film of PDMS inside the capillary is accelerated. The peak areas of amphet-

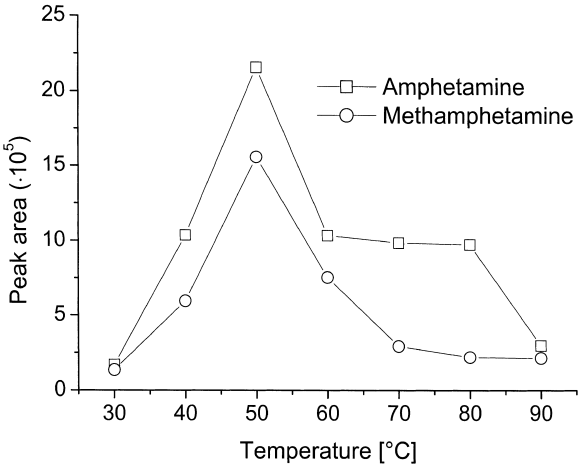

Fig. 3. Temperature profiles for the headspace SPDE of 2 ng/mg amphetamine and methamphetamine ($n=3$).

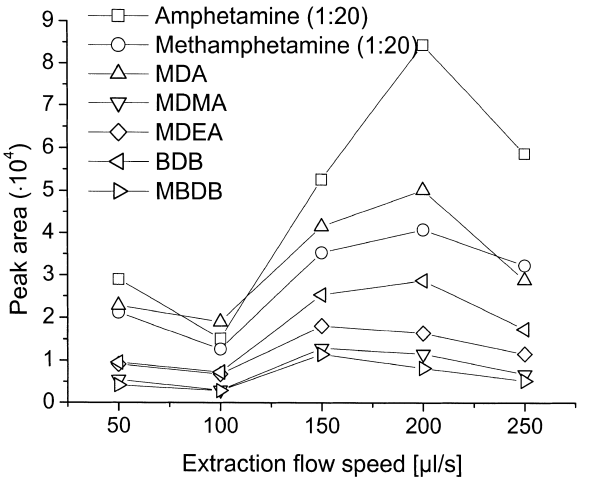

Fig. 5. Extraction profiles of amphetamines and synthetic designer drugs (2 ng/mg) ($n=3$).

amines and synthetic designer drugs showed a maximum at 50 °C (Fig. 3).

3.1.2. Extraction

The extraction time and extraction recovery depend on the number of filling cycles, the plunger speed and the volume aspirated through the syringe. However, even if the equilibrium was not completely reached, 50 cycles were used as a good compromise concerning time of analysis and sensitivity (Fig. 4). The optimal extraction flow speed was 200 µl/s (Fig. 5). The best results were achieved using a volume of 1000 µl for aspirating and dispensing.

3.1.3. Derivatization

The derivatization reaction started slowly (1–4 cycles) considering the time needed for MBTFA to diffuse into the needle coating. The peak areas increased at five cycles, the reaction was finished after six cycles. More derivatization cycles led to a decrease in the extraction recovery which may be caused by desorption processes (Fig. 6). The rela-

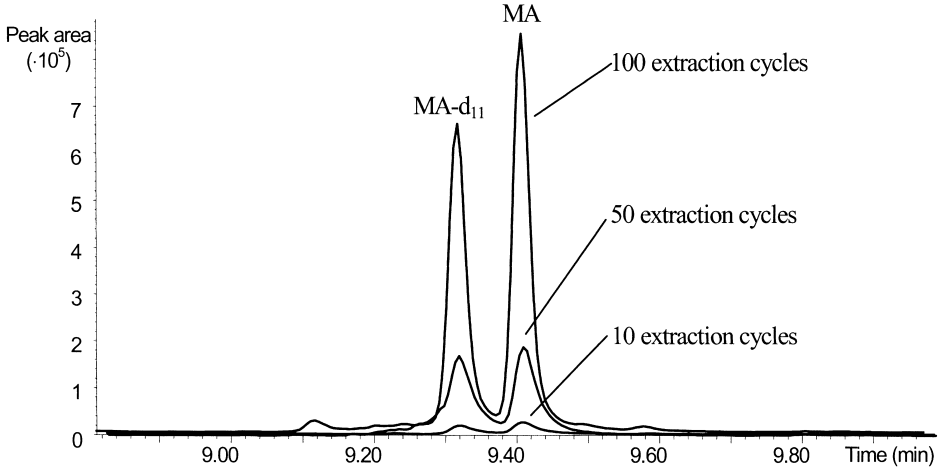

Fig. 4. GC–MS total ion chromatograms of 2 ng/mg methamphetamine (MA) measured by different numbers of extraction cycles.

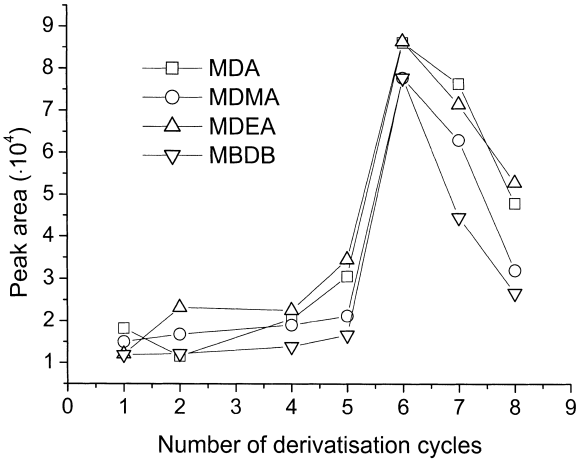

Fig. 6. Derivatization-time profiles with MBTFA (2 ng/mg of each analyte) ($n=3$).

tively small time window with maximum recovery can reproducibly be adjusted with the autosampler and had no negative influence on the results. It is important to note that for each sample, a separate vial with derivatization reagent has to be used, because otherwise a carry-over was noticed. The use of 25 μl MBTFA is sufficient for derivatization.

3.1.4. Desorption

The predesorption time in the injection port for thermal equilibration should not be longer than 15 s, at longer times a peak tailing was observed resulting in decreased sensitivity (Fig. 7). In this period of time, the thermal equilibration of the needle is achieved, so that the analytes are completely desorbed. The possibility of a condensation of the analytes into the syringe body is excluded by the nitrogen pressure. In the blank runs, no carry-over was observed. A reaction of the analytes with the metal surface of the needle leading to a loss of analytes or spurious peaks in the chromatogram were likewise not observed.

The volume and plunger speed have a significant influence on the desorption process. The response increased with incrementing desorption volume, being highest at the full syringe volume of 2.5 ml. Above a plunger speed of 50 μl/s the pressure in the injection port was too high, so that the GC system showed an error message. Additionally at these faster desorption speeds the analytes had no time to diffuse from the PDMS film into the nitrogen stream, so that a decrease in the chromatographic response and a peak tailing was observed. The best response was reached with the slowest adjustable speed of 10 μl/s and a nitrogen volume of 2.5 ml (Fig. 8).

Because of the relatively long desorption time, the GC column was held at 90 °C to trap the analytes. At higher oven temperatures, peak tailing appeared, lower temperatures (30, 50 or 70 °C) did not improve the chromatographic separation.

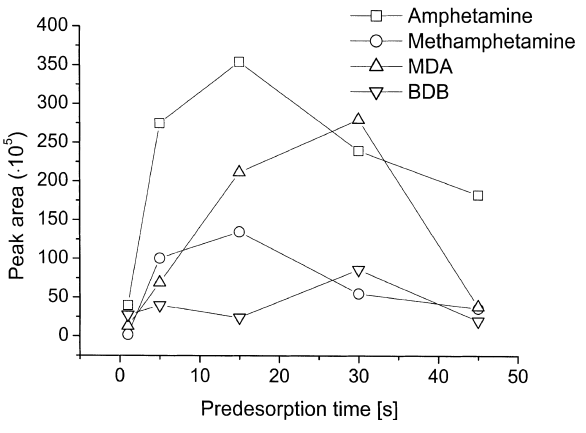

Fig. 7. Influence of the predesorption time in the hot injection port on the extraction recovery ($n=3$).

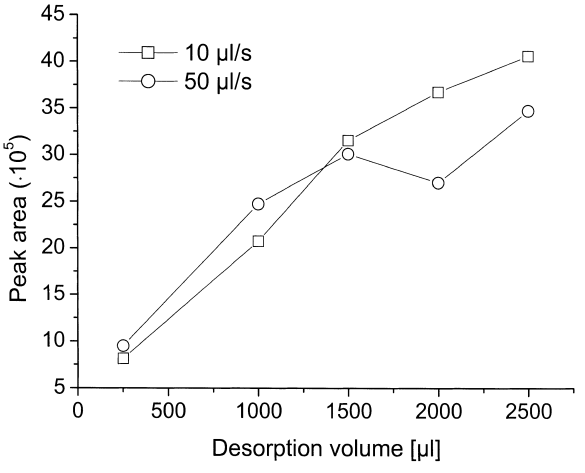

Fig. 8. Effect of the desorption volume and desorption flow speed on the extraction recovery (amphetamine, 2 ng/mg hair) ($n=3$).

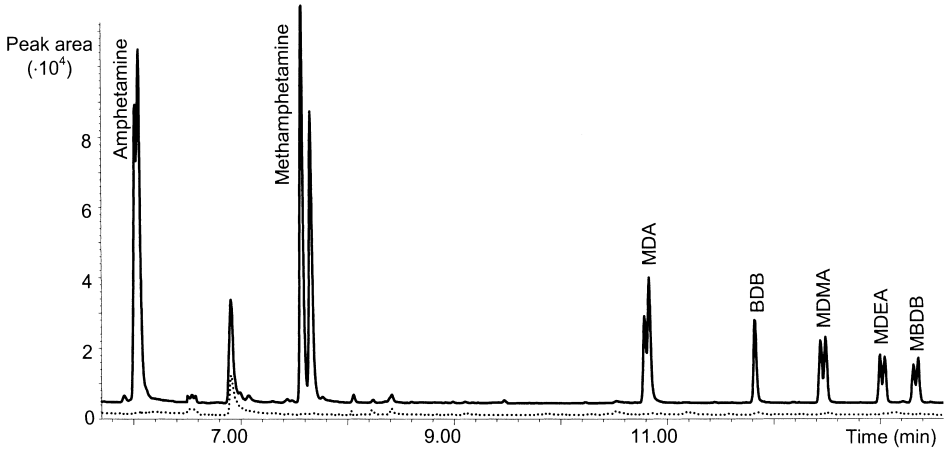

Fig. 9. Total ion chromatogram of a spiked hair sample containing the analytes and their deuterated analogs (amphetamine and methamphetamine: 1 ng/mg, MDA, BDB, MDMA, MDEA and MBDB: 2.5 ng/mg) in comparison to a blank hair sample (dotted line).

3.1.5. Validation

In Fig. 9, chromatograms of spiked and blank hair samples are presented. During routine analyses of 10 authentic samples from non-drug users no interfering peaks from the hair matrix were observed. Peak purity and selectivity are ensured. The stability of the analytes in simultaneously prepared samples after alkaline hydrolysis in 10 *M* NaOH was tested by comparing the results of reference samples at the start and end of a sequence in the autosampler. Additionally, the stability under storage conditions (8 °C) was evaluated. No significant loss of analytes was detected. Further validation data are demonstrated in Table 1. For the semivolatile analytes, the extraction recoveries were in the range between 10.2 and 16.7%. The detection limits using the SPDE conditions described above were 0.03–0.19 ng/mg, which are similar or situated below the values obtained with the corresponding SPME method [20]. Precision resulted in ranges of 1.4–4.1% (intra-day)

Table 1
Validation results: extraction recovery, limits of detection (LOD) and quantitation (LOQ), intra- and inter-day precision and calibration curves

	Extraction recovery[a] (%)	LOD[b] (ng/mg)	LOQ[b] (ng/mg)	Precision[c]				Regression line			
				Intra-day		Inter-day		Linear range (ng/mg)	Slope	Intercept	Corr. coeff.
				2 ng/mg (%)	20 ng/mg (%)	2 ng/mg (%)	20 ng/mg (%)				
Amphetamine	12.9	0.04	0.14	1.6	1.4	4.6	4.2	0.05–20	0.603	0.016	0.999
Methamphetamine	10.2	0.05	0.21	3.4	2.6	5.9	4.3	0.05–20	0.594	0.013	0.998
MDA	15.0	0.03	0.11	2.4	2.3	7.8	4.2	0.05–20	0.604	0.005	0.998
MDMA	16.7	0.13	0.70	3.0	2.4	7.3	4.5	0.1–20	0.528	0.026	0.995
MDEA	14.7	0.19	1.94	4.1	3.5	9.5	4.4	0.2–20	1.227	−0.017	0.992
BDB	12.7	0.07	0.40	3.7	3.1	14.6	14.4	0.1–20	0.231	0.016	0.992
MBDB	11.6	0.18	1.37	3.6	2.6	10.2	8.8	0.2–20	0.455	0.019	0.999

[a] Extraction recovery: The absolute amount of analytes extracted by SPDE was calculated by comparison with the corresponding direct injection of a methanolic sample solution onto the GC column (initial amount: 20 ng, $n=3$): recovery = peak area SPDE/peak area liquid injection × 100.

[b] Limits of detection and quantitation were determined by establishing a specific calibration curve from samples containing the analyte in the range of LOQ. The limits were calculated from the residual standard deviation of the regression line [18,19].

[c] Precision is expressed as RSD (%), intra-day ($n=6$), inter-day ($n=18$).

and 4.2–14.6% (inter-day). The calibration curves were constructed from peak areas using the SIM mode and show a linear relationship for each drug. Regarding the validating data, the procedure is sensitive, selective and reproducible. The applicability of the developed method was demonstrated by analysing hair samples from drug abusers.

All in all the new HS-SPDE procedure using a multipurpose autosampler seems to be suitable for the determination of amphetamines and designer drugs in hair samples in a convenient automated method. All single steps like heating and shaking of the sample, alkaline hydrolysis, absorption, derivatization and desorption in the injector of the GC are programmable and are executed automatically, whereby the number of sources of error is reduced distinctly which is a main factor concerning the reproducibility. A large advantage of the SPDE technique in relation to SPME is the robustness of the capillary. It is nearly impossible to damage the SPDE device mechanically in contrast to the fragile SPME fibres.

The advantage of the headspace technique in contrast to the direct sampling of an aqueous solution through the SPDE needle is the protection of the stationary phase coating and the exclusion of matrix effects, which affect the system and chromatography. The headspace analysis of hair digests performed by SPDE has demonstrated to allow up to 200 samplings with the same capillary, which is more than twice the samplings possible with SPME. The absolute extraction recovery with SPDE was 50% higher compared to a SPME fibre.

4. Conclusions

The research shows that SPDE can be very successfully used for the determination of amphetamines and synthetic designer drugs in hair after on-coating derivatization with MBTFA.

The SPDE as a further development of SPME turned out to be equally suitable for the requirements of clinical and forensic toxicology regarding sensitivity and selectivity. In general, SPDE is an excellent sample preparation technique because of its robustness, greater capacity, excellent reproducibility, low detection limits and simple automation.

In the future, the extension of the application range of automated SPDE is possible by the growing number of available needle coatings.

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