Fully Automated Determination of Amphetamines and Synthetic Designer Drugs in Hair Samples Using Headspace Solid-Phase Microextraction and Gas Chromatography–Mass Spectrometry

Frank Musshoff*, Heike P. Junker, Dirk W. Lachenmeier, Lars Kroener, and **Burkhard Madea**
Institute of Legal Medicine, University of Bonn, Stiftsplatz 12, D-53111 Bonn, Germany

Abstract

This study describes a fully automated procedure using alkaline hydrolysis and headspace (HS) solid-phase microextraction (SPME) followed by on-fiber derivatization and gas chromatographic (GC)–mass spectrometric (MS) detection of amphetamine, methamphetamine, methylendioxyamphetamine, methylendioxymethamphetamine, methylendioxyethylamphetamine, methylendioxyphenylbutanamine, and methylmethylendioxyphenylbutanamine in human hair samples. Ten milligrams of hair is washed with deionized water, petroleum ether, and dichloromethane. After the addition of deuterated internal standards the sample is hydrolyzed with sodium hydroxide and directly submitted to HS-SPME. After the absorption of analytes for an on-fiber derivatization procedure the fiber is directly placed into the HS of a second vial containing *N*-methyl-bis(trifluoroacetamide) before GC–MS analysis. The limits of detection are determined between 0.01 and 0.17 ng/mg. Absolute analyte recoveries are in the range between 0.3% and 7.5%. Linearity is proven over a range from 0.1 to 50 ng/mg with coefficients of correlation from 0.998 to 1. In comparison with conventional methods of hair analysis, this fully automated HS-SPME–GC–MS procedure is substantially faster and easier to perform without using solvents. It uses minimal sample amounts and has the same degree of sensitivity and reproducibility.

Introduction

Hair analysis for drug-of-abuse testing has been established as an important instrument in clinical and forensic toxicology (1–3). Various methods have been described for the determination of amphetamine and designer amphetamines in hair samples (4–12). Conventional procedures consist of several consecutive and time-consuming steps (e.g., digestion of the sample with alkaline, an acid extraction, an enzymatic treatment, or methanol sonication extraction of the hair matrix, followed by further clean-up by solid-phase extraction). Finally, the analytes are derivatized and analyzed, usually by gas chromatography (GC)–mass spectrometry (MS).

Solid-phase microextraction (SPME), discovered and developed by Pawliszyn et al. (13), has emerged in the past few years as a variable solvent-free alternative to conventional liquid–liquid extraction procedures. SPME in conjunction with analysis by GC–MS has been employed for a variety of classes of organic compounds, especially of volatile and semivolatile agents using the headspace (HS) technique. For the analysis of amphetamines and synthetic designer drugs, the direct extraction from an aqueous medium, direct immersion (DI)-SPME has been used for the analysis of urine samples (14–17) as well as serum samples (18). Compared with DI-SPME, the HS technique (HS-SPME) showed significant advantages because of the avoidance of organic solvents, simpler execution, and a lower chromatographic background signal. The methods have been described for the analysis of urine samples (19–24) as well as blood (25–27) and hair samples (10–12).

However, in hair analysis the SPME procedure was restricted because of the necessity of digestion prior to extraction. Sporkert and Pragst (11) described a procedure that combined alkaline hair hydrolysis, HS-SPME, derivatization, and GC–MS analysis for the determination of amphetamines. An "in-sample" derivatization was performed by the addition of methyl- or *n*-butyl-chloroformate to the hydrolyzed samples. The resulting carbamates were extracted by HS-SPME and analyzed by GC–MS. Recently, Liu et al. (12) developed a similar extractive derivatization method for the detection of amphetamines in hair samples using heptafluoro-*n*-butyryl chloride, and Namera et al. (24) used ethylchloroformate for the analysis of urine. However, for this procedure the sample vial had to be opened for the addition of agents for the derivatization after hydrolysis. For an "on-fiber" derivatization procedure, Jurado et al. (23) used trifluoroacetic anhydride (TFA), and Koster et al. (17) used pentafluorobenzoyl chloride for the analysis of urine samples. For the analysis of

* Author to whom correspondence should be addressed: email f.musshoff@uni-bonn.de.

amphetamine and methamphetamine (MA) in serum samples, Lee et al. (18) used the vapor of an heptafluorobutyric anhydride–ethyl acetate solution for on-fiber derivatization in a laboratory-made HS device with an oil bath, but a comparatively high temperature of 270°C was necessary for this procedure. In a single case Sporkert and Pragst (11) used *N*-methyl-bis(trifluoroacetylamide) (MBTFA) for on-fiber derivatization of the analytes after hydrolysis of hair samples, and the trifluoroacetyl derivatives were analyzed by GC–MS. Using such an HS on-fiber derivatization, the method could be performed automatically with a multipurpose autosampler. In comparison with the HS-SPME method of Koide et al. (10), which did not employ derivatization of the analytes, the detection limits were markedly enhanced.

In this study, a fully automated method combining HS-SPME and GC–MS with prior alkaline hydrolysis of hair samples was validated using HS on-fiber derivatization with MBTFA for the analysis of amphetamine, MA, and various synthetic designer drugs.

Experimental

Reagents and materials

The following chemicals were purchased from Promochem (Wesel, Germany) as methanolic standard solutions: D,L-amphetamine, D,L-amphetamine-d_5, D,L-MA, D,L-MA-d_{11} (MA-d_{11}), D,L-3,4-methylendioxyamphetamine (MDA), D,L-3,4-methylendioxyamphetamine-d_5 (MDA-d_5), D,L-3,4-methylendioxyethylamphetamine (MDEA), D,L-3,4-methylendioxyethylamphetamine-d_5 (MDEA-d_5), D,L-3,4-methylendioxymethamphetamine (MDMA), D,L-3,4-methylendioxymethamphetamine-d_5 (MDMA-d_5), D,L-3,4-methylendio- xyphenyl-2-butanamine (BDB), D,L-*N*-methyl-1-(3,4-methylendioxyphenyl)-2-butanamine (MBDB), and D,L-1,2-dideutero-*N*-trideuteromethyl-1-(3,4-methylendioxyphenyl)-2-butanamine (MBDB-d_5). The solutions were stored at 8°C and used after dilution to the required concentrations. MBTFA was obtained from Macherey-Nagel (Düren, Germany). An SPME device for an autosampler with a replaceable 100-µm polydimethylsiloxane fiber was from Supelco (Deisenhofen, Germany). The fiber was conditioned at 250°C in the injection port of the GC for 1 h, according to the supplier's instructions.

GC–MS method

An Agilent Model 6890 Series + GC in combination with a Model 5973 Network MS and a CTC-Combi-PAL-Autosampler (Chromtech, Idstein, Germany) were used for analysis. Data acquisition and analysis were performed using standard software supplied by the manufacturer. Substances were separated on a fused-silica capillary column (HP-5MS, 30-m × 0.25-mm i.d., 0.25-µm film thickness) (J&W Scientific, Cologne, Germany). The GC temperature program was as follows: 110°C held for 1 min, 8°C/min up to 210°C, held for 2 min, 30°C/min up to 280°C, and held for 5 min. The temperatures for the injection port, ion source, quadrupole, and interface were set at 240°C, 230°C, 150°C, and 280°C, respectively. The splitless injection mode was used, and helium with a flow rate of 1.0 mL/min was used as the carrier gas. An SPME 0.75-mm-i.d. insert liner and a Merlin Microseal septum (Supelco) were also used.

Electron impact mass spectra of the analytes were recorded by total ion monitoring. Retention times and characteristic mass fragments were recorded, and the chosen diagnostic mass fragments were monitored in the selected ion monitoring (SIM) mode. The characteristic ions used for quantitation were as follows: amphetamine–TFA (*m/z* 140, 91, 118), amphetamine-d_5–TFA (*m/z* 144, 92, 123), MA–TFA (*m/z* 154, 110, 118), MA-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, and MDA-d_5 was used as the internal standard. For quantitation, peak area ratios of the analytes to the internal standard were calculated as a function of the concentration of the substances.

HS-SPME procedure

The washing of the hair samples was performed according to a modified procedure of Kauert et al. (28); the samples were subsequently washed for 5 min in 5 mL of deionized water, petroleum ether, and finally dichloromethane using a Vortex Genie 2 mixer (Bender & Hobein AG, Zurich, Switzerland). After drying, the hair samples were cut into small pieces approximately 1 mm long. The washing solutions were analyzed by conventional GC–MS procedures to exclude external contamination.

Ten milligrams of hair was submitted to alkaline hydrolysis into a 10-mL HS vial in the presence of 1 mL of NaOH (10M) and an aqueous internal standard solution (250 ng deuterated analytes/mL, 80 µL). 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 was 0:05 min, and agitator off-time was 0:02 min). For absorption, the needle of the SPME device containing the extraction fiber was inserted through the septum of the vial, and the fiber was exposed into the HS of the vial for 10 min. For analyte derivatization, the fiber was exposed to a second vial containing 25 µL of MBTFA for 2 min at 50°C. The compounds absorbed on the fiber were des-

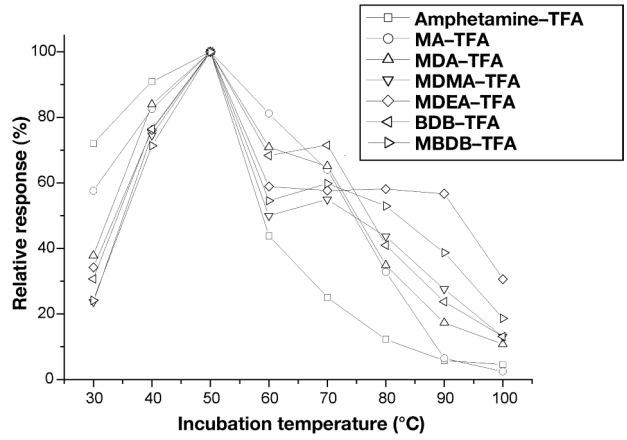

Figure 1. Influence of the incubation temperature on the extraction yield ($n = 3$).

orbed by exposing the fiber in the injection port for 4 min followed by analysis.

For validation, spiked hair samples containing 2 ng/mg of the analytes were analyzed using the procedure described previously. Before validation, the following parameters were optimized successively: conditions of hydrolysis, the addition of various salts, incubation time and temperature, agitator speed, extraction time and temperature, derivatization time and amount of derivatization reagent, desorption time and temperature, and depth-of-fiber insertion into the vial and injection port.

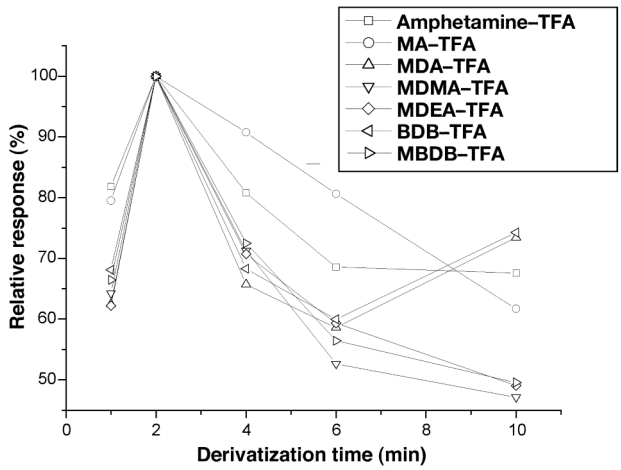

Figure 2. Influence of the derivatization time on the extraction yield (n = 3).

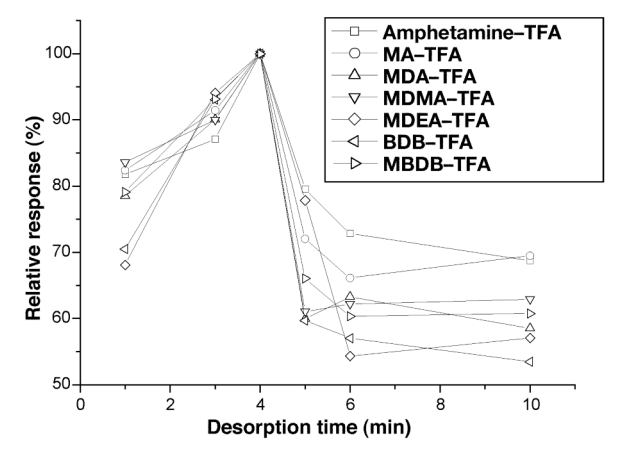

Figure 3. Influence of the desorption time on the extraction yield (n = 3).

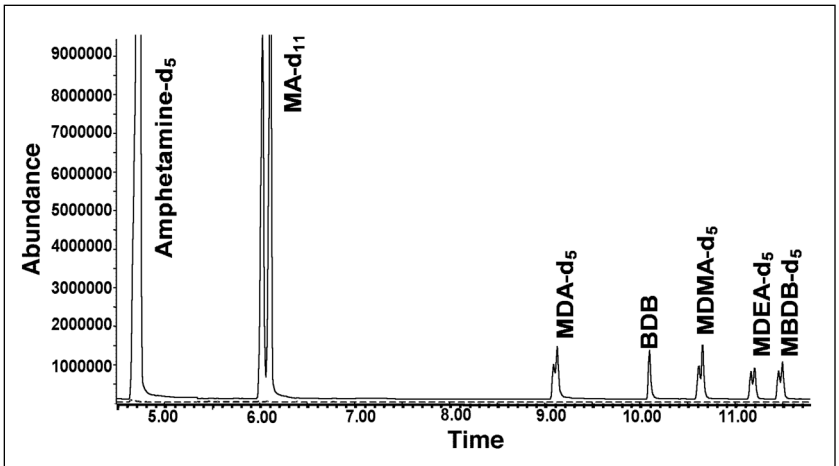

Figure 4. Total ion chromatogram of a spiked hair sample (5 ng/mg) compared with a blank hair sample (dotted line).

Results and Discussion

Parameter optimization for the HS-SPME method

Additions

The fully automated extraction of hair samples either in buffer solutions (phosphate buffer pH 2–10) or by acidic hydrolysis gave inadequate results. Alkaline hydrolysis in the presence of sodium hydroxide (10M) was found to be optimal. The addition of salts (ammonium sulfate, sodium sulfate, sodium carbonate, sodium bicarbonate, sodium chloride, sodium fluoride, or sodium iodide) did not increase the extraction yield, contrary to the salting-out effect observed with other substance classes.

Heating temperature

The incubation of the samples at increased temperatures before the absorption process led to an improvement of sensitivity, because the crossing of the analytes was thereby facilitated from the aqueous into the gaseous phase. The analytes showed a maximum response at 50°C, though at higher temperatures a reduction of the extraction yield could be observed (Figure 1).

Incubation time

The duration of the incubation of the samples in the agitator before absorption also had a substantial influence on the extraction yield. A duration of 5 min was found to be optimal.

Agitator speed

The optimum was achieved at 600 rpms.

Extraction

For the HS-SPME it is necessary that a 3-phase equilibrium adjusts between the liquid phase of the sample, the gaseous phase, and the solid phase of the fiber. The equilibrium of the semivolatile analytes was reached after 10 min.

Derivatization

The derivatization was finished after 2 min. A longer derivatization time led to a decrease of the extraction yield (Figure 2). It is important to note that for each sample, a separate vial with a derivatization reagent has to be used, because using only one vial in an analysis sequence can lead to sample carry-over. The use of 25 µL MBTFA was sufficient for derivatization.

Desorption

The thermal desorption of the analytes took place in the injector of the GC. A desorption time of 4 min at 240°C appeared to be optimal (Figure 3).

Depth of fiber insertion in vial and injection port

The depth of the fiber insertion into the injector of the GC affected the extraction yield. A depth of 52 mm was found to be optimal.

Validation

Figure 4 presents chromatograms of spiked and blank hair samples. During routine analyses of authentic samples, no interferences were observed. Validation data are demonstrated in Tables I and II. For the semivolatile analytes the extraction yields were between 8.0% and 12.6%, which are in the typical range for an HS-SPME procedure. The linearity of the SPME method was investigated by varying the concentration of the analytes in spiked hair samples over a 0.1–50-ng/mg range. The calibration curves were constructed from peak areas using the SIM mode and showed a linear relationship for each drug in the given concentration range. Precision resulted in ranges between 0.9% and 13.2% (intraday) and 1.7% to 17.0% (interday), the bias was between 0.3% and 17.5% (intraday) and 2.0% to 22.4% (interday). The presented method for the determination of amphetamine, MA, MDMA, MDA, MDEA, BDB, and MBDB by means of fully automated HS-SPME and GC–MS with on-fiber derivatization showed limits of detection (e.g., 0.01 ng/mg for amphetamine and MA) that were comparable or lower than the values indicated in literature, which were obtained by conventional extraction (0.01 ng/mg (6), 0.1 ng/mg (7), or 0.25 ng/mg (8) requiring at least 50–100 mg of hair) or SPME (0.04 ng/mg for amphetamine and 0.02 ng/mg for MA determined by Sporkert and Pragst (11)).

Table I. Validation Results

	Spiked concentration (ng/mg hair)	Intraday (n = 6) Precision* (%)	Intraday (n = 6) Bias† (%)	Interday (n = 18) Precision* (%)	Interday (n = 18) Bias† (%)
Amphetamine	0.5	2.1	9.2	3.2	9.9
	2	1.6	3.1	2.1	4.7
	40	0.9	0.9	1.7	2.4
MA	0.5	6.8	2.3	8.2	3.7
	2	5.7	0.3	8.0	3.3
	40	2.6	0.8	3.6	2.0
MDA	0.5	13.2	1.8	15.1	7.0
	2	6.8	0.8	9.9	2.7
	40	6.0	6.5	5.9	7.6
MDMA	0.5	2.3	1.6	8.2	5.1
	2	3.5	2.9	7.4	3.1
	40	1.8	6.9	4.2	7.0
MDEA	0.5	3.3	7.8	8.0	9.6
	2	1.7	2.7	4.1	1.5
	40	1.8	7.5	2.1	4.3
BDB	0.5	8.1	17.5	12.1	22.4
	2	6.2	6.6	9.6	8.1
	40	4.1	3.3	4.2	6.7
MBDB	0.5	9.8	3.8	17.0	11.1
	2	4.5	11.7	7.1	14.0
	40	2.9	7.8	4.8	8.2

* Precision, standard deviation/mean value x 100 (%)
† Bias, (measured concentration—spiked concentration)/spiked concentration × 100 (%)

Table II. Extraction Yield, Limit of Detection and Quantitation (LOD/LOQ), Linear Range, and Correlation Coefficient of the Calibration Curves

	Extraction yield* (%)	LOD† (ng/mg)	LOQ† (ng/mg)	Linear range (ng/mg)	Correlation coefficient
Amphetamine	10.2	0.01	0.03	0.1–50	0.9991
MA	8.0	0.01	0.05	0.1–50	0.9998
MDA	11.4	0.10	0.33	0.1–10	0.9968
MDMA	12.6	0.06	0.20	0.1–10	0.9992
MDEA	11.1	0.04	0.14	0.1–50	1.0000
BDB	10.3	0.17	0.54	0.1–50	0.9988
MBDB	9.2	0.10	0.31	0.1–50	0.9999

* Percentages of SPME-extracted amount per total amount (20 ng) as determined by injection of a methanolic solution (n = 3).
† LOD and LOQ were determined by a calibration curve established from samples containing the analytes in the range of LOQ according to German norm DIN 32645.

For all applications of SPME the CTC-Combi-PAL-Autosampler offers extensive advantages. All steps that were necessary for the HS-SPME (such as heating and shaking of the sample, extraction in the HS at increased temperature, and desorption in the injector of the GC) were programmable and automatically executed, whereby the number of sources of error was reduced distinctly concerning the reproducibility of the individual working steps. A large advantage of the HS technique in relation to direct immersion is the protection of the SPME fiber and the exclusion of matrix effects, which affects the system and chromatography. According to our own experience, approximately 90–100 samplings are possible using the HS technique compared with 20–30 samplings using DI.

Compared with the detection of underivatized analytes the determination of TFA derivatives after an on-fiber derivatization resulted in sharper peaks as well as an improved chromatographic resolution and larger analyte sensitivity. In comparison with this procedure, the addition of the derivatization agents directly to the sample after hydrolysis of the hair matrix is comparatively time-consuming (12,16,24,27). Another alternative is on-column derivatization, which has been described by Nagasawa et al. (25) and Namera et al. (26); they injected heptafluorobutyric acid or anhydride in the injection port of the GC before the fiber was inserted. Desorption and derivatization were achieved by exposing the fiber in the injection port. Such a procedure, however, cannot be performed automatically.

The potential contamination of the hair by external sources (e.g., pulverized drugs) could probably generate false-positive results. Therefore, in those cases with positive results for one of the analytes, we analyzed the washing solutions using conventional GC–MS methods. In hair samples from drug abusers we found low amounts only in the petroleum ether solution. The third wash with dichloromethane was negative in all cases, which demonstrated that no analytes were extracted from the interior of the hair by using this washing procedure.

In order to demonstrate the applicability of the developed method on real samples, hair samples of drug abusers were analyzed. An HS-SPME chromatogram of an authentic hair sample is shown in Figure 5.

Amphetamines and synthetic designer drugs were found in

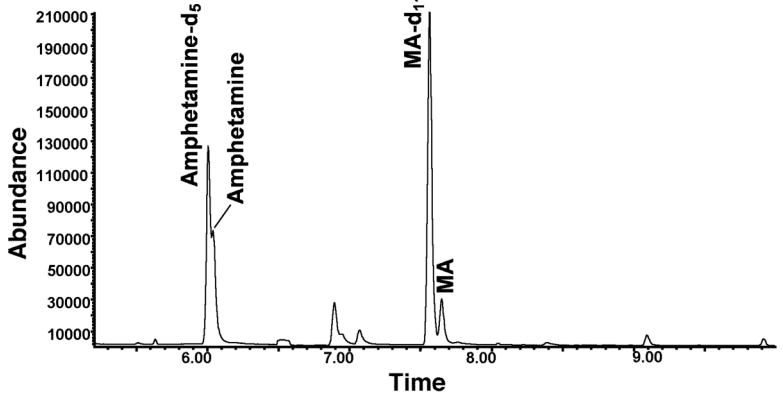

Figure 5. Total ion chromatogram of an authentic hair sample containing 0.55 ng/mg amphetamine and 0.22 ng/mg MA.

20 hair samples from drug abusers. The following concentrations were determined: 0.2–21.8 ng/mg of amphetamine, 0.1–9.6 ng/mg of MA, 1.3–26.4 ng/mg of MDA, 0.5–12.4 ng/mg of MDMA, 0.1–9.9 ng/mg of MDEA, and 0.2–0.9 ng/mg of MBDB.

Conclusion

The application of fully automated HS-SPME followed by GC–MS analysis for the determination of amphetamine, MA, MDMA, MDA, MDEA, BDB, and MBDB in hair was tested and successfully applied to the analysis of hair samples from drug abusers. The SPME turned out to be a substantially simpler and faster procedure than the conventional sample processing. The method meets the sensitivity and selectivity requirements of clinical and forensic toxicology.

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