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Determination of amphetamine and methamphetamine in serum via headspace derivatization solid-phase microextraction–gas chromatography–mass spectrometry

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Abstract

This study evaluates solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry (GC–MS) to determine trace levels of amphetamine and methamphetamine in serum. Headspace post-derivatization in a laboratory-made design with heptafluorobutyric anhydride vapor following SPME was compared with that without derivatization SPME. The SPME experimental procedures to extract amphetamine and methamphetamine in serum were optimized with a relatively non-polar poly(dimethylsiloxane) coated fiber at pH 9.5, extraction time for 40 min and desorption at 260°C for 2 min. Experimental results indicate that the concentration of the serum matrix diluted to a quarter of original (1:3) ratio by using one volume of buffer solution of boric acid mixed with sodium hydroxide and two volumes of water improves the extraction efficiency. Headspace derivatization following SPME was performed by using 6 μl 20% (v/v) heptafluorobutyric anhydride ethyl acetate solution at an oil bath temperature of 270°C for 10 s. The precision was below 7% for analysis for without derivatization and below 17% for headspace derivatization. Detection limits were obtained at the ng/l level, one order better obtained in headspace derivatization than those achieved without derivatization. The feasibility of applying the methods to determine amphetamine and methamphetamine in real samples was examined by analyzing serum samples from methamphetamine abused suspects. Concentrations of the amphetamine and methamphetamine ranged from 6.0 μg/l (amphetamine) to 77 μg/l (methamphetamine) in serum.

Keywords: Amphetamines; Methamphetamine

1. Introduction

Amphetamine and methamphetamine are synthetic drugs used to treat mild depression, obesity, narcolepsy and certain behavioral problems in children [1]. They are also powerful central nervous stimulants capable of producing an euphoric static similar to cocaine [2]. The most commonly available amphetamines are amphetamine (*R,S*-1-phenyl-2-propanamine) and methamphetamine (*R,S*-*N*-methyl-1-phenyl-2-propanamine). Clandestine laboratories manufacture large quantities of illegally synthesized methamphetamine. Methamphetamine is abused worldwide, leading to considerable interest in forensics and toxicology. In clinical treatment, methamphetamine has predominantly α-adrengic agonist effects, subsequently, increasing blood pressure and cardiac activity. Capable of decreasing dopamine degradation at the neuronal level, selegiline (*N*,α-dimethyl-*N*-2-propynylphenethylamine) is a selective monoamine oxidase type inhibitor effectively used to treat Parkinson's disease [3,4]. In vivo metabolic

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studies demonstrate that selegiline is efficiently metabolized to methamphetamine by oxidative depropynylation. Methamphetamine is then further metabolized to amphetamine by oxidative demethylation [5]. For patients receiving selegiline treatment, plasma concentrations of amphetamines at 5–15 μg/l are encountered [6–8]. Therefore, the concentration of amphetamines in blood must be determined to monitor selegiline treatment in Parkinson's disease.

The analysis of blood samples has acquired a considerably greater value than urine investigation, particularly in forensic toxicology. Pharmacokinetic and pharmodynamic studies of methamphetamine in humans, normally require a sensitive and accurate method to detect the level of methamphetamine and its metabolites in biological fluids. Several analytical methods have been developed to quantitatively measure the presence amphetamine and methamphetamine in biological fluids, including radioimmunoassay (RIA) [9], gas chromatography (GC) with electron-capture detection (ECD) [10–12], flame ionization detection (FID) [13], or nitrogen–phosphorous detection (NPD) [14], or GC combined with mass spectrometry (MS) [15,16], and high-performance liquid chromatography (HPLC)–chemiluminescence detection [17].

Proper sampling largely determines the validity of an analytical sample for trace analysis. In particular, appropriately preparing biological samples is a prerequisite for chromatographic analysis. Previous investigations have developed various types of extraction methods for amphetamines in biological fluids, including liquid–liquid extraction [18] and solid-phase extraction (SPE) [19]. The conventional extraction method for amphetamine and methamphetamine in biological fluids is employed by using solvent extraction, generally at an alkaline pH, at which the amphetamine and methamphetamine are non-ionized [20]. However, solvent extraction is relatively time consuming, hazardous to human health as it uses large amounts of toxic organic solvent and multi-step procedures, possibly incurring loss of analytes. The feasibility of replacing conventional solvent extraction to isolate trace analytes with SPE has received increasing interest. However, the use of SPE can be lengthy, with a series of stages including washing, conditioning, eluting, and slow during the process. Moreover, SPE can be expensive because the cartridges are normally disposed of after one extraction; and disposal costs for the solvent are also quite expensive. Therefore, a simple, fast and solvent-free extraction technique must be developed, particularly for biological fluids analysis. Solid-phase microextraction (SPME) is based on an equilibrium of analyte concentration between the sample and the solid-phase fiber coating. Because of its solvent-free methodology, simplicity, rapidity and relatively low cost, SPME has been widely applied to extract organic compounds from aqueous samples [21]. Zhang et al. [22] reviewed the SPME technique, theory, method development and related applications.

Derivatization is a convenient means of obtaining better chromatographic separations with increasing volatility and detector sensitivity as well as selectivity, particularly with ECD. Analyzing amphetamine and methamphetamine generally requires derivatization of the amino group prior to GC analysis to improve the detection sensitivity. Conventionally used derivatization methods include trifluoroacetyl [15], trichloroacetyl [6], acetyl [13], pentafluorobenzoyl [11], pentafluorobenzene sulfonyl [23], and heptafluorobutyryl [10]. In this study, amphetamine and methamphetamine adsorbed on the fiber coating of the SPME system are derivatized after extracting from serum with heptafluorobutyric anhydride vapor in a laboratory-made headspace device. The optimum conditions for determining amphetamine and methamphetamine in serum are also systematically studied. The feasibility of applying the proposed methods is demonstrated as well. To do so, the SPME behavior, detection limits, linear dynamic detection ranges and repeatability are elucidated by determining the amount of amphetamine and methamphetamine. To confirm the proposed method's effectiveness, the optimized procedure was combined with GC–MS to determine the amount of methamphetamine and metabolites in serum of samples taken from individuals who underwent a physical examination while suspected of abusing methamphetamine.

2. Experimental

2.1. Reagents

All chemicals were of research grade and used

without further purification. Amphetamine, methamphetamine and [$^2H_5$]methamphetamine (MA-d_5) were purchased from Sigma (St. Louis, MO, USA). MA-d_5 was used as an internal standard. Stock standard solutions of amphetamine and methamphetamine were prepared in methanol and maintained at 4°C in a refrigerator. A standard solution of heptafluorobutyric anhydride (HFBA) was obtained from Supelco (Bellefonte, PA, USA), and 20% (v/v) in ethyl acetate solution was used to produce HFB derivatives of amphetamine and methamphetamine. The buffers for various pH values were then prepared by mixing potassium dihydrogenphosphate or disodium hydrogenphosphate with sodium hydroxide or boric acid with sodium hydroxide. Next, silanization of glassware was performed, prior to use, by soaking the glassware overnight in toluene solution at a concentration of 10% dichlorodimethylsilane. The glassware was rinsed with toluene and methanol and then thoroughly dried for 4 h. Sera were obtained from a local hospital in Taichung, mid-Taiwan. Serum samples were prepared by spiking with stock standard amphetamine and methamphetamine solution and the MA-d_5 internal standard into serum that was pooled from many individuals. The serum samples of suspected methamphetamine abusers collected were kept frozen at −20°C until further treatment.

2.2. Sampling

The SPME fibers used were coated with poly(dimethylsiloxane) (PDMS) at 100 μm thickness (supplied by Supelco). The fibers were conditioned under helium at a flow-rate of 1.0 ml/min in the hot injection port of a gas chromatograph at 250°C for 1 h prior to use. All analyses were performed with 4.6-ml vials containing 3 ml of solution. The stir bar (3/8 in.×3/16 in.; 1 in.=2.54 cm) was utilized to agitate the sample during extraction. Optimization of the SPME was then studied with respect to extraction efficiency for amphetamine and methamphetamine used to spike a 3-ml serum sample. After extraction, the needle on the SPME manual holder was set at its maximum length 4.5 cm in the GC injector and, then, the fiber was directly exposed to the hot injector for analysis. In derivatization studies, a PDMS-coated fiber was placed into a sample vial to extract amphetamine and methamphetamine from serum. After extraction equilibrium was reached, and the fiber was transferred into the headspace derivatization system that was made in our laboratory [24]. In the headspace derivatization experiments, amphetamine and methamphetamine were derivatized with HFBA vapor produced from 6 μl 20% HFBA ethyl acetate solution which was heated at 270°C using silcone oil.

Fig. 1 depicts the derivatization apparatus made in our laboratory. The derivatization system consists of a modified 10-ml test tube with an inner tube (5 cm×0.3 cm) and a heating mantle. Briefly, 6 μl of 20% HFBA in ethyl acetate was placed in a test tube partially submerged in 6 ml of silicone oil. The lower portion of the system was immersed in an oil bath maintained at 270°C with a heating mantle. The SPME fiber after extraction was pierced through the rubber septum into the inner tube of the system, whereas the amphetamine and methamphetamine adsorbed on the fiber were immediately derivatized with the HFBA vapor from the bottom of the tube. The HFBA vapor was produced by heating the inner tube in oil bath. The derivatization procedures must be performed in a hood with safety equipment, adding as little derivatization agent as possible to

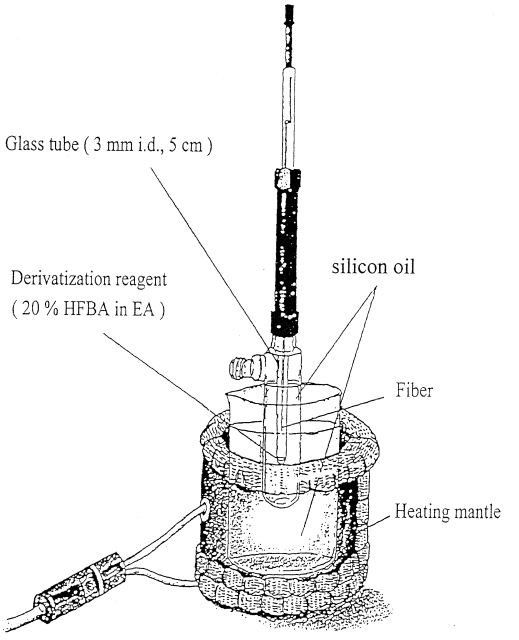

Fig. 1. Headspace derivatization kit for amphetamine and methamphetamine using SPME.

Table 1
Analytical conditions of amphetamines, as determined by GC–MS with various ionization modes

Compound	M_r	t_R (min)	Quantitated ion (m/z)		Confirmed ion (m/z)	
			EI	PCI	EI	PCI
Amphetamine	135	3.97	44	136	91	119
Methamphetamine	149	4.33	58	150	91	119
MA-d_5	154	4.33	62		92	
Amphetamine-HFB[a]	331	5.27	240		118	
Methamphetamine-HFB	345	5.97	254		210, 118	
MA-d_5-HFB	350	5.97	258		213	

[a] Heptafluoro-*n*-butyryl.

avoid explosion of the glass apparatus at a too high vaporization pressure of HFBA.

2.3. Gas chromatography–mass spectrometry

Analysis by GC–MS was performed with a Hewlett-Packard (HP) MS Engine mass spectrometer (Palo Alto, CA, USA) with a HP 5890 Series II gas chromatograph. A split/splitless injector was used in the splitless mode. GC separations were performed with a 30 m×0.25 mm I.D., 0.25 μm stationary phase thickness fused-silica capillary column DB-5 MS (J&W Scientific, Folsom, CA, USA). Helium was used as a carrier gas and maintained at a rate of 1 ml/min by using the electronic pressure control. The transfer line was maintained at 250°C. For fiber injection, the injector was held isothermally at 260°C. The ion source of mass spectrometer was maintained at 230°C and 150°C for electron impact ionization (EI) and chemical ionization (CI), respectively. The GC oven was initially set at 60°C, programmed to 120°C at a rate of 30°C/min and from 120°C to 180°C at a rate of 20°C/min, then finally increased at 30°C/min to 250°C (held for 0.5 min). The total analysis time of a single run is 8.3 min. Table 1 lists the analytical selected ion monitoring (SIM) conditions for the amphetamine, methamphetamine and derivatives studied, as performed by MS.

3. Results and discussion

3.1. GC–MS determination

For monitoring trace amphetamine and methamphetamine in serum, the highest sensitivity of the GC–MS technique must be developed. The SIM mode of MS is usually chosen in quantitative analyses. In general, the most abundant ion was used to monitor and quantify, the specific ion was used as the confirmed ion. Various ionization modes of MS, including EI with an electron energy of 70 eV, and CI with methane as reagent gas, were used to trace the optimum ionization mode for analysis of amphetamine and methamphetamine. Negative ion CI could not work in our instrument, therefore, the positive ion mode was performed in this study. The mass-to-charge ratio ranging from 60 to 350 u was used to determine appropriate masses for SIM. To evaluate the optimum ionization technique for trace analysis of amphetamine and methamphetamine in serum, amphetamine and methamphetamine analyzed in various ionization modes of MS were compared in a standard solution with respect to the responses obtained. According our results, the sensitivity of EI is higher than that obtained by positive chemical ionization (PCI). Therefore, in this study, EI is chosen to investigate the optimum conditions of SPME.

3.2. Selection of optimum condition for SPME

For the analyte extraction during SPME experiments, the amount extracted depends on the mass transfer of an analyte through the aqueous phase, the time of extraction and the stationary phase coated on the fiber of SPME. In this study, a relatively nonpolar PDMS was chosen as a fiber coating to extract amphetamine and methamphetamine in the serum. Many factors affect the mass transfer of amphetamine and methamphetamine from serum to the fiber

in SPME, including matrix effects, ionic strength and pH value. The mechanism of SPME is based on an equilibrium between the analytes concentration in the sample and that in the solid-phase fiber coating. The equilibrium time affects the optimum extraction time which in turn determines the amount extracted. Stirring a solution is normally used to increase the rate of mass transfer and decrease the equilibrium time. Herein, all extractions performed in this study were under conditions of stirring around 1000 rpm. According to variance analysis of the experimental results, the peak area counts of amphetamine and methamphetamine increased with the extraction time from 10 to 60 min. Methamphetamine and amphetamine reached equilibrium in 40 min. Therefore, the extraction time was chosen as 40 min to allow simultaneous extraction of the amphetamine and methamphetamine in serum. Notably, the properties of the analytes, injector temperature or desorption temperature, position of the fiber in the injector port, initial oven temperature, and desorption time heavily affected the thermal desorption of amphetamine and methamphetamine from the fiber into the GC system. Although a higher desorption temperature can reduce the desorption time, a higher desorption temperature easily causes thermal degradation of some thermally labile analytes. Too high a desorption temperature may also cause the bleeding of the stationary phase of the fiber coating and shorten the lifetime of the fiber. The desorption temperature monitored ranged from 200 to 280°C. According to our results, the peak areas of amphetamine and methamphetamine increase with an increasing temperature and reach a steady maximum in over 260°C. Carryover or memory effect could be problematic for analytes with relatively high boiling points frequently encountered when using the SPME technique to analyze organic compounds. A second desorption of the fiber performed at the maximum temperature of 280°C, after the initial desorption run was used to determine whether or not the analytes remain on the fiber. The lack of carryover is found for amphetamine and methamphetamine at a desorption temperature exceeding 260°C. Therefore, the desorption temperature of 260°C was chosen to extract the studied amphetamine in serum.

The amount of amphetamine and methamphetamine desorbed from the fiber depends not only on the desorption temperature, but also on the desorption time and the depth of the fiber in the injector. The long desorption time also produces a broadening analyte peak and memory effect. Desorption time was also investigated within a range of 0.5 to 5 min, by leaving the fiber in the injector for a progressively longer period of time and maintaining the injector temperature at 260°C. According to those results, the amount of amphetamine and methamphetamine desorbed increased with an increasing time and reached an equilibrium of over 2 min. The fiber's depth in the injector was measured from 1.5 to 4.5 cm. The amphetamine and methamphetamine desorbed increased with increasing depth of the fiber. Notably, for all subsequent experiments, the amphetamine and methamphetamine were desorbed at 4.5 cm, the maximum length of the syringe carriage in the injector for 2 min.

3.3. Matrix effects

Changes in the sample matrix yield significant differences in the signal intensity of analytes of a varying structure in the SPME method. Varying the pH from 6 to 10 was monitored to examine how pH affects the extraction of amphetamine and methamphetamine in serum. The extraction increased with increasing pH values to reach maximum at pH 9.5 and, then, decreased by increasing the basicity of serum (Fig. 2). Amphetamine and methamphetamine are weak basic compounds with secondary or primary amines. At a high pH, the acid–base equilibrium of basic amphetamine and methamphetamine significantly shift towards the neutral form, which

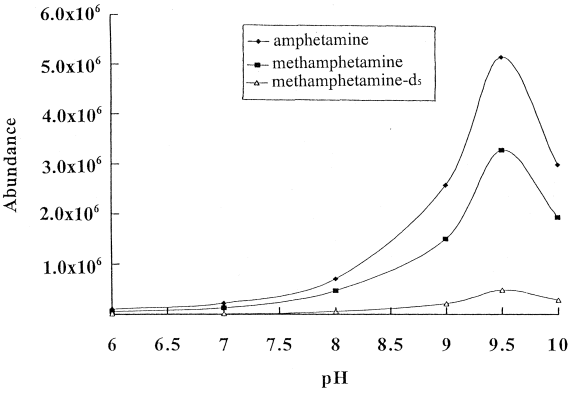

Fig. 2. Dependence of peak areas of amphetamine and methamphetamine on pH of extraction by SPME.

has a higher affinity for the fiber, thereby increasing the amount extracted. At a higher pH, the extraction efficiency of amphetamine and methamphetamine decreased, possibly due to the more neutral components formed in the serum competition of the active sites of the fiber.

Adding a salt solution to the sample matrix has varying effects on the equilibrium process, depending on the analyte structure and properties. Saturated sodium chloride (5 *M*) or potassium chloride (5 *M*) was added to the serum samples to examine the effect of salting out affects. Three extractions were performed for every condition. Table 2 summarizes the magnitude of enhancement in extraction, as attributed to the addition of salt, pH value and combined pH and salt. The slight improvement in extraction is attributed to decreases in the solubility of the amphetamine and methamphetamine caused by the salt, ultimately forcing these analytes into the fiber. Under the combined base and salt conditions, the improvement of the extraction is not better than that obtained with pH alone. The reason is at a basic condition, the saturated salt hinders the shift of the ionized form of amphetamine and methamphetamine towards the neutral form, which has a higher affinity for the fiber. Therefore, the serum is adjusted only at pH 9.5 to extract amphetamine and methamphetamine in serum by SPME.

In the serum matrix effect study, the serum samples were spiked with 0.25 mg/l methamphetamine, 1.25 mg/l amphetamine and 50 μg/l MA-d_5 standard solution, a mixed solution of 1 ml serum with 1 ml pH 10 buffer solution and then was diluted with water to form ratios of 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7 of the original composition to trace the matrices effect during the extraction of amphetamine and methamphetamine of serum by using SPME method. For this test, 3 ml of diluted serum was investigated under the studied optimum conditions. Triplicate analysis was performed for different dilution conditions. Fig. 3 summarizes those results. The greatest extraction was obtained at a 1:3 ratio of the diluted serum solution. According to those results, interferents in the serum interfere with the extraction of amphetamine and methamphetamine with the SPME method. At a higher ratio of dilution serum, a lower extraction efficiency produced came from the amphetamine and methamphetamine concentration diluted.

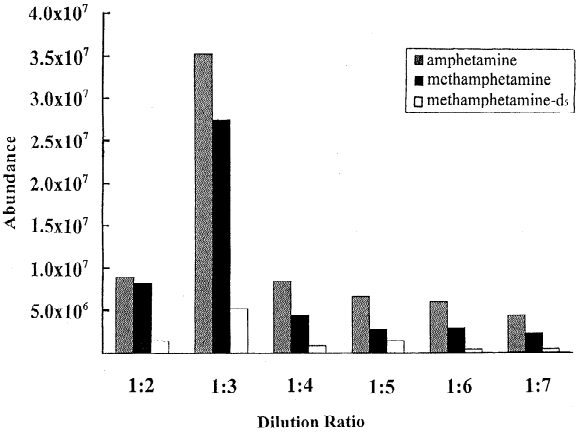

Fig. 3. Effect of dilution ratio of serum on the extraction efficiency.

3.4. Post-derivatization of following SPME

After finishing the studies of the optimum conditions of SPME for amphetamine and methamphetamine in serum, post-derivatization following SPME via headspace was investigated. Derivatiza-

Table 2
Matrix effect enhancement of extraction of amphetamine and methamphetamine in serum with SPME

Compound[a]	Recovery (%)						
	Original serum	pH 9.5	With NaCl	With KCl	With pH 9.5 and NaCl	With pH 9.5 and KCl	With pH 9.5 and NaCl+KCl
Amphetamine	0.6	4.2	1.4	0.7	3.7	2.7	3.1
Methamphetamine	1.2	7.2	1.7	1.1	5.5	4.1	4.9
MA-d_5	1.1	6.8	1.7	0.9	5.2	3.8	4.5

[a] Amphetamine 200 μg/l, methamphetamine 200 μg/l, MA-d_5 50 μg/l.

tion is a feasible means of obtaining better chromatographic separations by increasing the volatility and detector sensitivity. In this study, we performed the post-derivatization of amphetamine and methamphetamine with HFBA on the fiber of SPME following extraction by using the vapor of HFBA. According to the results in Fig. 4, the peak shapes and signal-to-noise ratios of amphetamine and methamphetamine are better than those obtained without derivatization. Extraction efficiency was compared by using post-derivatization following SPME and SPME without derivatization as well as SPME following the addition of HFBA ethyl acetate derivatization solution in serum. The highest extraction efficiency was achieved by using post-derivatization following SPME. The sensitivities for methamphetamine and amphetamine with post-derivatization following SPME are better than those obtained in without derivatization by a factor of 17 and 4, respectively.

Derivatization time, oil bath temperature and amount of reagent are the major parameters that determine the efficiency in derivatization. In an oil bath temperature study from 190 to 290°C, the maximum derivatization was obtained at 270°C. When the oil bath exceeded 270°C, the desorption of the adsorbed fiber reduced the overall extraction efficiency. Those studies reveal that the amount of HFBA and derivatization time is 6 μl and 10 s, respectively, which produces the optimum results. Herein, post-derivatization of amphetamine and methamphetamine following SPME was performed by adding 6 μl HFBA (20% in ethyl acetate solution) at 270°C for 10 s. After finishing the study of the optimum desorption temperature, in the GC injector as for without derivatization analysis, the fiber was set at 260°C for 2 min to avert the carryover effect.

3.5. Precision and detection limits

Eight consecutive fiber extractions with the same concentration under the optimum conditions studied herein were used to examine the precision of SPME method. A 3 ml serum solution containing 150 μg/l amphetamine and 30 μg/l methamphetamine was also investigated. The repeatability was expressed as relative standard deviation (RSD) of the fiber below 7%. In post-derivatization following SPME, a 3 ml

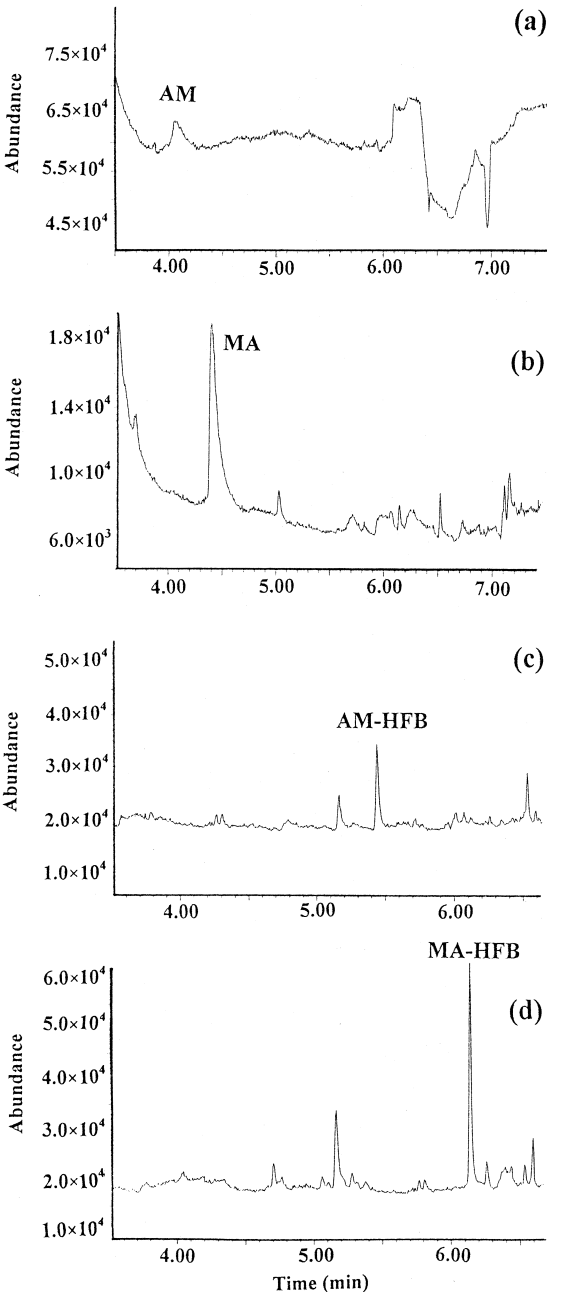

Fig. 4. Mass ion chromatograms of 5 μg/l (a) amphetamine, (b) methamphetamine, (c) amphetamine derivative, (d) methamphetamine derivative, produced by SPME–GC–MS. AM: Amphetamine; MA: methamphetamine; AM-HFB: amphetamine derivatives; MA-HFB: methamphetamine derivatives.

Table 3
Estimated limits of detection for SPME coupled with GC–MS for amphetamines and heptafluorobutyric anhydride derivatives

Compound	LOD (μg/l)	RSD (%, n=8)
Amphetamine	0.6	7
Methamphetamine	0.4	6
Amphetamine-HFB	0.08	17
Methamphetamine-HFB	0.05	19

serum solution containing 25 μg/l of each compound was used. The precision was found to be below 17% by using MS-SIM. Therefore, the precision of the SPME method was acceptable.

In SPME without derivatization, the linearity was studied at a concentration ranging from 1 μg/l to 200 μg/l for amphetamine and methamphetamine with MA-d_5 used as the internal standard. The correlation coefficients are 0.9993 and 0.9947 for amphetamine and methamphetamine, respectively. In post-derivatization with HFBA vapor following SPME, the linear ranges were examined by extracting the spiked serum samples ranging from 0.5 μg/l to 200 μg/l for amphetamine and methamphetamine with 50 μg/l MA-d_5 used as the internal standard under the optimum conditions. The studied amphetamine and methamphetamine in serum were analyzed, in which SPME is linear with linear correlation coefficients exceeding 0.998 in all cases.

The linear range experiments provide the necessary information to estimate the detection limits, based on the lowest detectable peak that has signal-to-noise ratio of 3. Table 3 compares the limits of detection (LODs) obtained using post-derivatization with HFBA vapor following SPME and without derivatization. According to this table, the obtained LODs for amphetamine and methamphetamine in derivatization are better than those achieved without derivatization. The LODs to determine amphetamine and methamphetamine in serum can be reduced to 80 ng/l and 50 ng/l, respectively.

4. Application

This study also examined the effectiveness of the proposed method in determining amphetamine and methamphetamine in real samples by analyzing the serum samples obtained from methamphetamine abused suspects that underwent a physical examination in a hospital. SPME was operated at the optimum conditions studied herein. The serum samples were analyzed by spiking with 50 μg/l of MA-d_5 used as an internal standard. Fig. 5 illustrates the chromatograms of a real serum sample and a serum blank sample. Table 4 indicates amphetamine and methamphetamine as determined in real serum samples by using post-derivatization following SPME. According to our results, amphetamine was

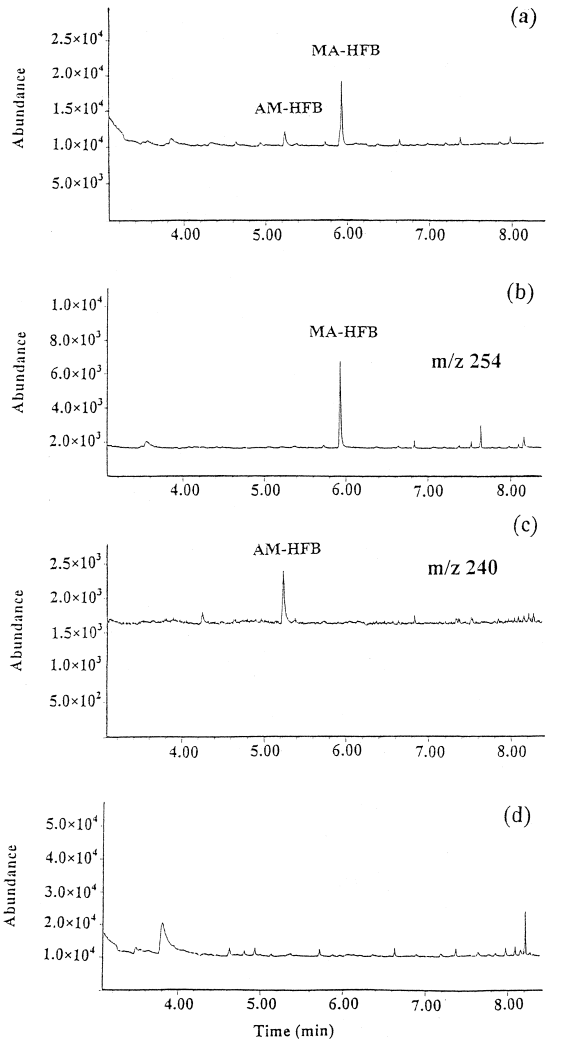

Fig. 5. (a) Mass ion chromatogram of the serum sample of a drug abused suspect (S_4), (b) extracted ion m/z 240, (c) extracted ion m/z 254, (d) serum blank, produced by headspace derivatization following SPME–GC–MS.

Table 4
Amphetamine concentrations in serum of drug abused suspects

Subject	Amphetamine (μg/l)	Methamphetamine (μg/l)
S_1	18	28
S_2	20	ND[a]
S_3	21	ND
S_4	6	77

[a] ND: Below limit of detection.

found in all serum samples. Amphetamine was detected ranging from 6.0 μg/l to 21 μg/l, and methamphetamine was detected at 28 μg/l and 77 μg/l. The serum samples of those two suspects revealed amphetamine but no methamphetamine, which is deduced from the degradation of methamphetamine to amphetamine in serum for a long time, over 1 year.

5. Conclusion

This study evaluated SPME combined with GC–MS to determine trace amounts of amphetamine and methamphetamine in serum samples. Better chromatographic shape and sensitivity were obtained to derivatize amphetamine and methamphetamine with gas post-derivatization with HFBA heated in an oil bath. The proposed method is precise and detection limits at the ng/l level of amphetamine in serum are obtained. Headspace post-derivatization following SPME is one-order better than that obtained in without derivatization. In addition, the feasibility of applying SPME–GC–MS to determine the amount of amphetamine and methamphetamine in methamphetamine abused suspects' serum was tested. Amphetamine and methamphetamine were detected ranging from 6.0 μg/l (amphetamine) to 77 μg/l (methamphetamine). The proposed method offers a low level sensitivity to trace amphetamine and methamphetamine in serum containing a high degree of interference.

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References

[1] J.D. Cook, S.M. Schanberd, Biochem. Pharmacol. 19 (1970) 1165.
[2] M. Sato, Psychopharmcol. Bull. 22 (1986) 751.
[3] G.P. Reynolds, J.D. Elsworth, K. Blau, M. Sandler, A. Lees, G.M.Br. Stern, J. Clin. Pharmacol. 6 (1978) 524.
[4] J.J. Knoll, Neural Transm. 25 (1987) 45.
[5] S.R. Philips, J. Pharm. Pharmacol. 33 (1981) 739.
[6] J.S. Salonen, J. Chromatogr. 527 (1990) 163.
[7] C.L. Hornbeck, R.J. Crarny, J. Anal. Toxicol. 13 (1989) 144.
[8] R.W. Taylor, S.D. Le, S. Philip, N.C. Jain, J. Anal. Toxicol. 13 (1989) 293.
[9] C. Ward, A.J. McNally, D. Rusyniak, S.J. Salamone, J. Forensic Sci. 39 (1994) 1486.
[10] R.B. Bruce, W.R. Maynard Jr., Anal. Chem. 41 (1969) 977.
[11] M. Terada, T. Yamamoto, T. Yoshida, Y. Kuroiwa, S. Yoshimura, J. Chromatogr. 237 (1982) 285.
[12] T. Kraemer, H.H. Maurer, J. Chromatogr. B 713 (1998) 163.
[13] P. Lebish, B.S. Finkle, J.W. Brackett Jr., Clin. Chem. 16 (1970) 195.
[14] S. Cheung, H. Nolte, S.V. Otton, R.F. Tyndale, P.H. Wu, E.M.J. Sellers, J. Chromatogr. B 690 (1997) 77.
[15] S. Suzuki, T. Inoue, H. Hori, S. Inayama, J. Anal. Toxicol. 13 (1989) 176.
[16] N. Nagasawa, M. Yashiki, Y. Iwasaki, K. Hara, T. Kojima, Forensic Sci. Int. 78 (1996) 95.
[17] K. Nakashima, K. Suetsugu, K. Yoshida, S. Akiyama, S. Uzu, K. Imai, Biomed. Chromatogr. 6 (1992) 149.
[18] H. Shin, M. Donike, Anal. Chem. 68 (1996) 3015.
[19] K. Hara, Y. Kashimura, M. Hieda, M. Kageura, J. Anal. Toxicol. 21 (1997) 54.
[20] R. Kronstrand, J. Anal. Toxicol. 20 (1996) 512.
[21] K.J. Hageman, L. Mazeas, C.B. Grabanski, D.J. Miller, S.B. Hawthorne, Anal. Chem. 68 (1996) 3892.
[22] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[23] P.R. Paetsch, G.B. Baker, L.E. Caffaro, A.J. Greenshaw, G.A. Rauw, R.T. Coutts, J. Chromatogr. 573 (1992) 313.
[24] M.R. Lee, R.J. Lee, Y.W. Lin, C.M. Chen, B.H. Hwang, Anal. Chem. 70 (1998) 1963.