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Short communication

Determination of amphetamine, methamphetamine and dimethamphetamine in Human Urine by Solid-Phase Microextraction (SPME)-Gas Chromatography/Mass Spectrometry

Seung-Woon Myung[a,*], Hye-Ki Min[a], Seungki Kim[a], Myungsoo Kim[a], Jin-Byom Cho[b], Taek-Jae Kim[b]

[a]*Bioanalysis and Biotransformatiom Research Center, Korea Institute of Science and Technology, P.O. Box 131, Cheongryang, Seoul 130-650, South Korea*

[b]*Department of Chemistry, Kyonggi University, Suwon, Kyonggi 440-760, South Korea*

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Abstract

A simple and rapid assay method for three stimulant drugs (amphetamine, methamphetamine, and dimethamphetamine) in human urine using solid-phase microextraction was developed. In solid-phase microextraction, the drugs were equilibrated between the adsorbent coated-fiber and aqueous sample matrix. After adsorption of the analytes, the fiber was directly transferred to the injector of a gas chromatograph, where the analytes were thermally desorbed and subsequently separated by the gas chromatograph and detected by mass spectrometer. The solid-phase microextraction method, which did not require solvents, was found to be a fast and simple analytical method. We optimized the solid-phase microextraction technique, for factors such as the NaCl salt effect (30%), pH effect (pH=12.4), equilibration time (30 min), desorption time (1 min) and coated-fiber type (100 μm poly(dimethylsiloxane)) and detected the stimulants in human urine, obtained from human subjects. The detection limits of each drug were below 1–10 ng/ml. The developed method can be applied to the abused drug test.

Keywords: Amphetamine; Methamphetamine; Dimethamphetamine

1. Introduction

Amphetamine, methamphetamine, and dimethamphetamine have powerful central nervous system (CNS) stimulant actions in addition to the peripheral α and β actions common to indirectly acting sympathomimetic drugs [1]. They increased self-confidence and alertness and improve physical performance and are abused as a tool of increase performance in competition, anorexic drug for the treatment of obesity, and mood enhancer. The IOC (International Olympic Committee) Medical Commission and other sports federations defined them as prohibited classes of pharmacological agents [2]. Therefore, their detection in biological fluids is important in toxicology, clinical, forensic, and sports fields. Traditionally, methods for the analysis of nitrogen-containing basic drugs have been based on liquid–liquid extraction [3] and have usually been designed for the extraction and detection of each

*Corresponding author

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group of substances separately [4]. Even if the liquid–liquid extraction is successful in designing a unified method, the high amounts of organic solvent residues generated in the liquid–liquid extraction procedure produces problems with regards to the workers' health and the environment safety.

Within the last few years, the development of solid-phase extraction procedures (SPE) [5–7] has allowed the generation of alternative methods to liquid–liquid procedures, obtaining cleaner extracts and optimum recoveries. The liquid–liquid extraction (LLE) and solid-phase extraction (SPE) methods are time-consuming, tedious and often require concentration of the extract prior to instrumental analysis. These methods also require large quantities of expensive and toxic solvents that can be harmful to the human and environment.

Recently a commercially available technique, solid-phase microextraction (SPME) [8], allows simultaneous extraction and pre-concentration of analytes from a sample matrix. This methodology has been used for the analysis of several compounds, such as organic acids [9], organophosphorous insecticides [10], tricyclic antidepressants [11], polychlorobutadienes [12], and phenols [13], amphetamines and related compounds [14,15], codeine and methadone [16]. This technique is significantly more rapid and simpler than both of LLE and SPE, and the requirement of solvents has been eliminated. The purpose of this study was to investigate use of the SPME for the GC/MS analysis for amphetamine, methamphetamine, and dimethamphetamine in human urine samples. The coated fiber was immersed directly into the urine sample until equilibration of the analytes adsorbed to the coating phase is attained. Analytes were then thermally desorbed into the injection port of gas chromatograph, and subsequently analyzed.

2. Experimental

2.1. Materials

All standards, which are free bases, were generous gifts from Prof. Dr. M. Donike in Deutsche Sporthochschule in Cologne, Germany. SPME devices and fiber assemblies were purchased from Supelco (Bellefonte, PA, USA), and a capillary column was purchased from Hewlett–Packard Co. Other common chemicals used were of analytical grade. The urine was obtained from human subjects who were administered the stimulants, and the International Olympic Committee (IOC) for accreditation for anti-doping analysis testing.

2.2. Instrumental

A Hewlett–Packard Model 5988A mass spectrometer (Hewlett–Packard, CA, USA) coupled to a HP5890A gas chromatograph (Hewlett–Packard, CA, USA) controlled by a HP 59970A MS ChemStation was used. The mass spectrometer was operated with a filament current of 300 μA and an electron energy of 70 eV in the EI mode. Quantification of the drugs was done by the selected ion monitoring (SIM) method and selected characteristic ions for amphetamine, methamphetamine and dimethamphetamine were $m/z=44$, 58, and 72, respectively and $m/z=91$ was a common ion for the three drugs. A cross-linked (5%)-Diphenyl-(95%)-dimethylsiloxane copolymer capillary column Ultra-2 (20 m length, 0.2 mm I.D. with a film thickness of 0.33 μm) was installed in the gas chromatograph and inserted directly into the ion source of the mass spectrometer. Helium was used as the carrier gas at a flow-rate of 0.9 ml/min. The oven temperature was held at 100°C for 1 min, raised to 300°C at 20°C /min and finally held at 300°C for 5 min. The injection was done in the split modes. The injector temperature was at 250°C and the transfer line temperature was at 290°C.

2.3. Analytical procedure

To obtain optimized conditions, standard aqueous solutions (5 μg/ml of three stimulants) were prepared by spiking an appropriate amount of the working standard (1000 μg/ml in MeOH) into 4 ml vials filled with 3 ml of distilled water. The fiber was immersed in the sample for the designed time with stirring at room temperature. After extraction, the fiber was directly transferred into the hot injection port (250°C) and desorbed for 1 min. Triplicate analyses were performed for all experiments.

2.4. Optimization

The effects on extraction efficiency of ionic strength and pH levels of a sample were investigated. The ionic strength was modified by the addition of NaCl in varying amounts to attain a solution containing 10, 20, 30% (w/w). In order to investigate the effect of pH, 5 *M* KOH solution was used. The standards extracted under various ionic strengths and pH levels were compared with the standard aqueous solution. Precision was determined with three extractions from human urine sample administered stimulants at the concentration of ca 0.3–1 μg/ml. The linearity of the method was tested by extracting aqueous standards with increasing concentrations over a range typically between 0.01–1 ug/ml. The detection limits were calculated as the concentration of analytes in the sample, which gave a signal-to-noise ratio (S/N) of three.

2.5. Urine sample analysis

3 ml of urine were placed in a 4 ml vial and 15 μl of 5 *M* KOH and 0.9 g of NaCl were added. The sample was stirred using the magnetic stirring bar. 100 μm poly(dimethylsiloxane) (PDMS) SPME fiber was immersed directly into the stirred urine for 30 min. The drugs were equilibrated between the adsorbent coated-fiber and aqueous sample. After adsorption of the analytes, the fiber was directly transferred to the injector of a gas chromatograph, where the analytes were thermally desorbed and subsequently determined by the GC–MS.

3. Results and discussion

3.1. Optimization

SPME is an equilibrium process that involves the partitioning of analytes from a liquid or gaseous sample into a polymeric phase according to their partition coefficients, *K* [8,10]. The following equation may be used to describe the SPME process in a two-phase system;

$$n_s = KV_sV_{aq}C^0_{aq}/KV_s + V_{aq}$$

where n_s is the amount extracted by the fiber coating, V_{aq} and V_s are the volumes of the aqueous phase and stationary phase, respectively, and C^0_{aq} is the initial concentration of the analytes in the aqueous phase. To obtain an optimum condition for the analysis of three stimulants using the SPME method, the effects of several parameters were investigated. A comparison of the extracting efficiency using 100 μm PDMS fibers, 7 μm PDMS fiber for mid- to non-polar semi-volatiles and 85 μm polyacrylate (PA) fibers for polar semi-volatiles fiber for the spiked control sample was performed. The stimulants showed a higher affinity for the 100 μm PDMS coating fiber than 7 μm PDMS and 85 μm PA. When it was confirmed that the 100 μm PDMS fiber was suitable for the analysis of the volatile stimulants, the experiments to find the optimum extraction conditions for the 100 μm PDMS coating were performed. Fig. 1 shows the effects of contact (immersion) time on extraction of drugs from the distilled water at pH = 12.4 and 30% NaCl in magnetic stirring agitation with the use of a 100 μm PDMS coated SPME-fiber. All experiments were performed under stirring in order to optimize the transfer of analytes from the aqueous sample into the fiber coating. The equilibrium for three stimulants was reached over 30 min of immersion.

Table 1 shows the factor increase obtained for all conditions using a 30 min extraction time, which was also used for control samples of the same concentration, a neutral pH and with no salt added. Firstly, the extraction efficiency according to varying pH was investigated. The peak areas of the drugs extracted with pH = 12.4 were compared with that of control sample (pH = 7, no salt). The amount extracted to the coating fiber with pH = 12.4 was increased by 30–50 times as compared to the one with pH = 7. When the pH was increased, their acid–base equilibrium shifted toward the neutral form, which had a greater affinity for the coating fiber, thereby the sample was moved to the coating fiber. Secondly, the addition of base and salt in combination was investigated as a means of enhancing the amount extracted by the fiber. In general, the extracted amounts of the drugs are increased with the addition of salt depending on the polarity and solubility of the drugs. This effect was observed for high pK_a compounds which are amphetamine (pK_a =

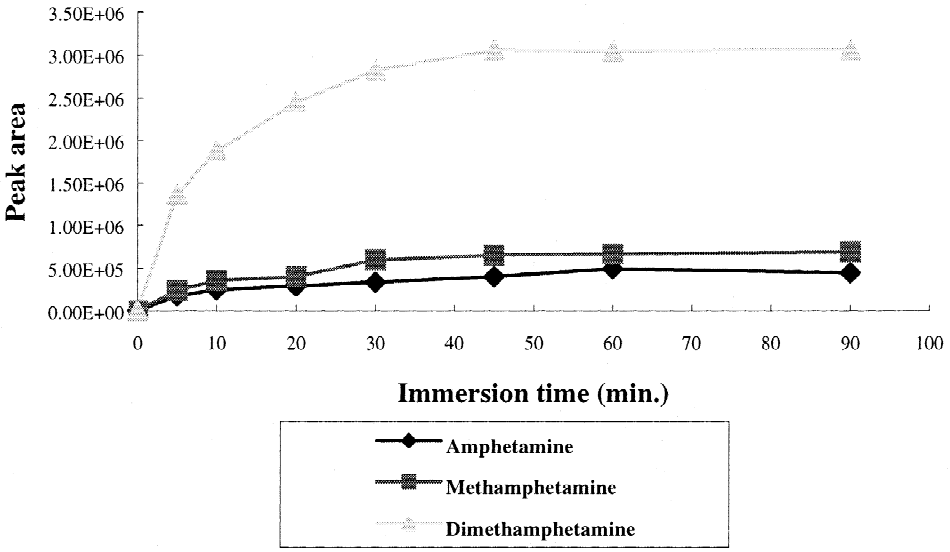

Fig. 1. The effects of immersion time on extraction of stimulants with the use of 100 μm PDMS coated SPME fiber.

9.9), methamphetamine ($pK_a = 10.1$) and dimethamphetamine ($pK_a = 9.8$). The addition of salt to the water solution generally causes a decrease in solubility of the analytes in the aqueous phase which favors movement of the analytes into the fiber coating, thereby improving the extraction efficiency of the method [16]. The extracted amount of methamphetamine which is higher dissociation constant increased significantly with the addition of salt (180 times increasing at 30% salt compared to the control condition), but extraction of amphetamine and dimethamphetamine, which are lower pK_a, showed less increase than methamphetamine. The result of the extraction amount according to the polarity of the compound was reported by Pawliszyn et al. [10]. As a result, with pH = 10 and 30% salt conditions, the amount extracted for every analyte in the human urine got the highest sensitivity. The extraction amounts of amphetamine, methamphetamine, and dimethamphetamine increased 94, 179, 61 times respectively compared to the control sample. When the base of pH = 12.4, salt of 30% NaCl were combined, they were found to be the optimum conditions for analyzing stimulants using SPME method.

3.2. Validation and applications

Table 2 summarized the detection limits, precision and the linear range of the stimulant drugs with GC–MS by SPME. The experiments were performed under the condition of pH = 12.4, 30% salt concentration, and 30 min of equilibrium time. The detection limit was evaluated using a S/N of 3. The detection limit of amphetamine, methamphetamine, and dimethamphetamine was below 10, 10, 1 ng/ml, respectively and stimulants analysis by the SPME method represented the good sensitivity than liquid–

Table 1
The extracted amount ratio according to the pH and salt

Analytes	pH = 7 ± NaCl				pH = 12.4 + NaCl			
	0%	10%	20%	30%	0%	10%	20%	30%
Amphetamine	1	0.31	0.26	0.29	33.83	45.77	68.76	94.73
Methamphetamine	1	0.36	0.45	0.29	53.05	82.73	134.05	179.71
Dimethamphetamine	1	0.37	0.29	0.33	49.57	58.11	68.94	61.39

Table 2
The precision and detection limits of the drugs by the SPME method

Analyte	Limit of detection (ng/ml)	Linear range (μg/ml)	Correlation coefficient (r^2)	Precision	
				Concentration (μg/ml)	% R.S.D.
Methamphetamine	10	0.05–1	0.9849	0.37, 0.33, 0.36	4.3
Methamphetamine	10	0.05–1	0.9930	1.05, 1.01,1.18	6.6
Dimethamphetamine	1	0.05–1	0.9945	0.50, 0.57, 0.52	1.4

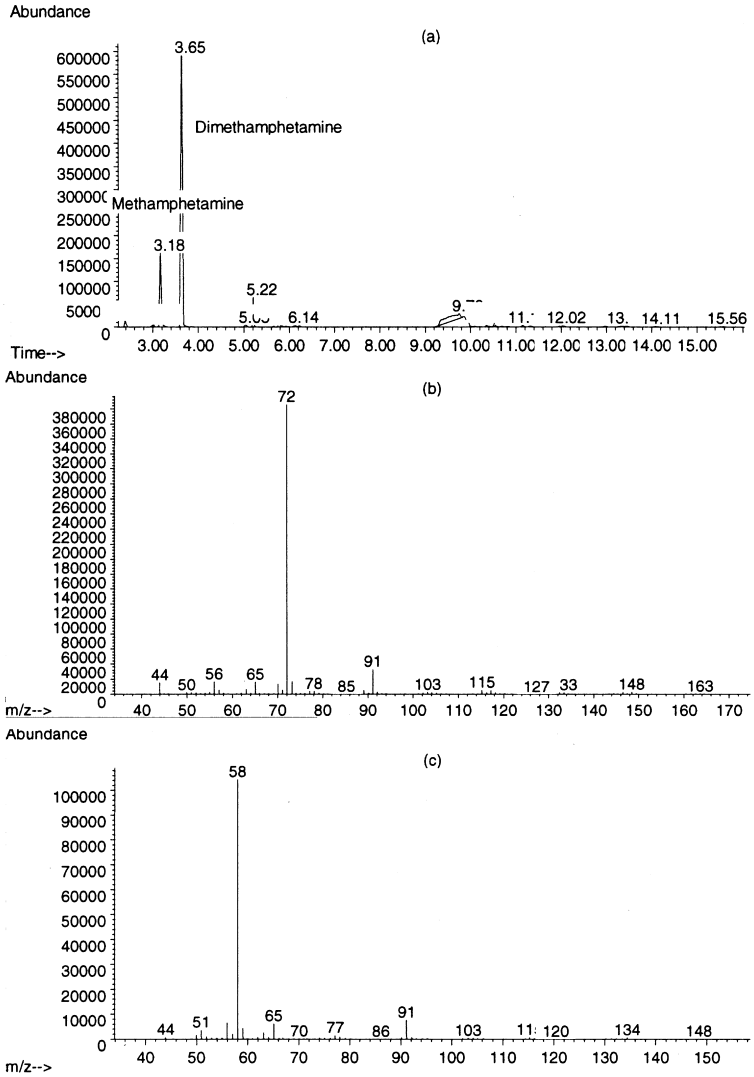

Fig. 2. (a): Total ion chromatogram of a SPME extract of a urine from a human subject administered dimethamphetamine, (b) and (c): EI mass spectrum of the dimethamphetamine and the metabolite (methamphetamine).

liquid extraction method [17]. The relative standard deviation (R.S.D.) within a day measured by the SPME method for three stimulants in human urine samples was 1.4–6.6%. For the quantification of the stimulants in the human urine sample, the equations for the curves were: $y=140427x+12152$ for amphetamine, $y=307295x+7291$ for methamphetamine, and $y=100000x+1483$ for dimethamphetamine. The correlation coefficient (r^2) of each calibration curve was 0.9848 for amphetamine, 0.9930 for methamphetamine, and 0.9945 for dimethamphetamine. Two human urine samples, known to be positive for stimulants drug, were analyzed by the developed method. Although sensitivities were different, the three stimulants could be determined simultaneously with minimal interference. The GC–MS chromatograms for each of the two samples were presented in Figs. 2 and 3 and show parent and its metabolite.

Fig. 2 shows the dimethamphetamine (3.65 min) and methamphetamine (3.18 min) which is a metabolite of the dimethamphetamine, and their EI mass spectra (Fig. 2). For the quantification, SIM (selected ion monitoring) mode was used and the selected ions were $m/z=72$ and $m/z=91$ for dimethamphetamine, $m/z=58$ and $m/z=91$ for methamphetamine. The extracted amounts of the dimethamphetamine and methamphetamine were 0.51 ± 0.01 μg/ml and 0.15 ± 0.03 μg/ml, respectively. Fig. 3 shows the total ion chromatogram and its EI mass spectra obtained from the SPME extract of human urine administered methamphetamine. This chromatogram contained the methamphetamine and amphetamine which is one of methamphetamine's metabolites, and they were detected at a retention time of 3.18 min and 2.79 min, respectively and the amounts of amphetamine and methamphetamine extracted from human urine were 0.35 ± 0.02 and 1.08 ± 0.07 μg/ml, respectively. Selected ions of amphetamine for the quantitative analysis were $m/z=44$ and $m/z=91$.

4. Conclusion

The SPME method appears very useful for the analysis of the volatile stimulant drugs in urine. Using the 100 μm PDMS fiber, very good sensitivity and precision could be reached. This developed method is simple, has low background noise and does not require the use of organic solvent. These factors make it attractive for rapid screening in anti-doping test in sports and for clinical and forensic use.

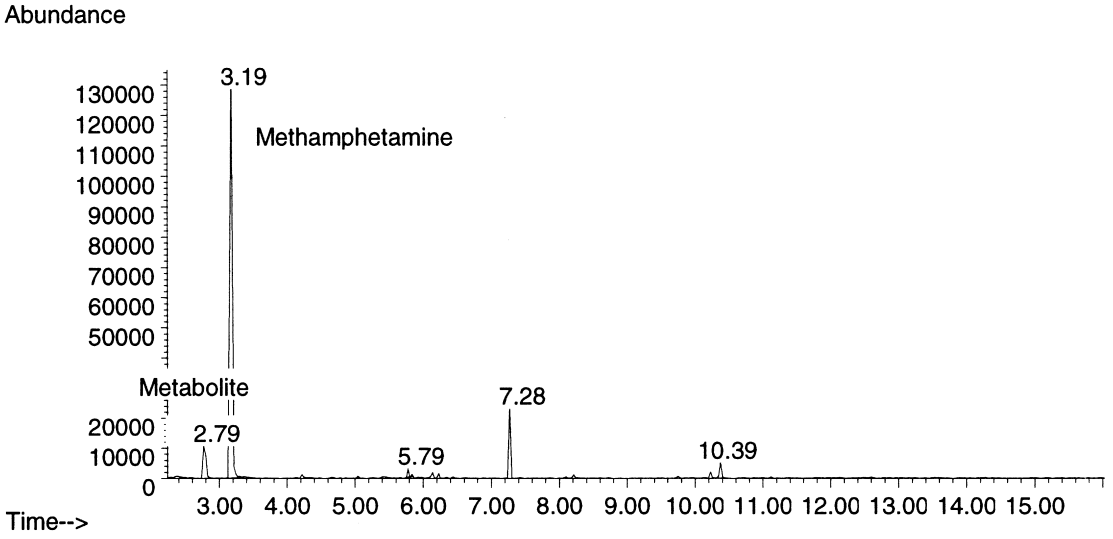

Fig. 3. Total ion chromatogram of SPME extract of a human urine from a human subject administered methamphetamine.

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