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Determination of amphetamine and methamphetamine in human hair by headspace solid-phase microextraction and gas chromatography with nitrogen–phosphorus detection

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Abstract

A simple and rapid method for the determination of amphetamine (AP) and methamphetamine (MA) in human hair was developed by headspace solid-phase microextraction (SPME) and gas chromatography with nitrogen–phosphorus detection (GC–NPD). The hair (1 mg) was dissolved in 0.2 ml of a 5 *M* sodium hydroxide solution in a tightly sealed vial by shaking at 75°C for about 5 min. In order to adsorb AP and MA on the SPME fiber, 100 μm of polydimethylsiloxane fiber was exposed to the headspace of the vial, and the vial was heated at 55°C for 20 min. Then the fiber was removed from the vial and inserted into the injection port of the GC–NPD system using a CBJ-17 capillary column. The compounds adsorbed on the fiber were analyzed by exposing the fiber at 220°C for 30 s in the GC injection port. By using this method, AP and MA in human hair could be analyzed simply and rapidly without any interference from coexisting substances. The percentages of AP and MA extracted from human hair by the SPME method were 48 and 62%, respectively, and relative standard deviations were below 10% ($n=5$). The calibration curves for AP and MA were linear in the ranges of 0.4–15 and 4–160 ng/mg hair, respectively. The detection limits of AP and MA at a signal-to-noise ratio of three were 0.1 and 0.4 ng/mg hair, respectively. This method could be applied to the analysis of an abuser's hair sample.

Keywords: Amphetamine; Methamphetamine

1. Introduction

Amphetamine (AP) and methamphetamine (MA) are a major class of central nervous system stimulants. Most drug abuse cases in Japan involved the abuse of MA, which damages ones personality and also causes an increase in the number of criminals. Recently, stimulant abuse has spread from adults to juveniles and is one of the most serious problems in Japan. Ordinarily, stimulant abuse is verified by examining urine from an abuser. However, using urine analysis, stimulant abuse could not be detected in cases where the abuse had occurred more than one week before the analysis took place, as drugs could no longer be detected in urine. In contrast, using examination of hair, we can obtain verification of

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longer term abuse and also a history of abuse [1], because the drugs are incorporated into hair and remain for several months or even years. Thus, hair analysis of drugs has become of interest in the field of forensic science [2].

Various methods for hair analysis of AP and MA have been developed using gas chromatography (GC) [3], GC–mass spectrometry (GC–MS) [4–8], GC–MS–MS [9], high-performance liquid chromatography [10,11] and ion mobility spectrometry [12]. Although some of these methods are sensitive and selective, most of these methods require complicated and time-consuming pretreatment of hair samples. For example, solvent extraction following alkaline digestion of hair sample requires considerable effort to remove interfering substances in the hair digest. On the other hand, direct extraction using methanol–hydrochloric acid is an easier procedure than the alkaline digestion method, but the hair must be cut finely with a scissors and an overnight extraction step is required. Recently, a solid-phase microextraction (SPME) method was developed as a new solventless extraction technique by Arthur and Pawliszyn [13]. Using this technique, organic compounds in aqueous samples are adsorbed directly from the sample onto a fused-silica fiber coated with an appropriate stationary phase, and then the fiber needle can be directly injected into a GC injection port for analysis. The SPME method has been used for extraction of various compounds of forensic interest [14–19], because of its simplicity and rapidity. Recently, Yashiki et al. [14] reported the analysis of AP and MA in urine using the SPME method combined with headspace (HS). In the present work, we investigated a simple and rapid method for the determination of AP and MA in human hair by HS–SPME and GC with nitrogen–phosphorus detection (NPD).

2. Experimental

2.1. Reagents

AP sulfate was provided by Dr. Takako Inoue of the National Research Institute of Police Science (Tokyo, Japan). MA hydrochloride was purchased from Dainippon Pharmaceuticals (Osaka, Japan). α-Phenethylamine (α-PEA) hydrochloride was prepared from α-PEA (Aldrich, Milwaukee, WI, USA) by the addition of 12 *M* hydrochloric acid and was used as an internal standard for AP. *N*-Propyl-β-phenethylamine (pro-β-PEA) hydrochloride was synthesized from β-PEA hydrochloride (Wako, Osaka, Japan) and propionaldehyde (Wako) by the method of Jacob et al. [20] and was used as an internal standard for MA. The purity of these synthesized compounds was confirmed by thin-layer chromatography and GC–MS. The chemical structures of AP, MA and the internal standards are shown in Table 1. Each compound was dissolved in distilled water to make a stock solution with a concentration of 0.1–1 mg/ml and these were used after dilution with 0.4 *M* hydrochloric acid to the required concentration. All other chemicals were of analytical-reagent grade.

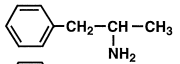
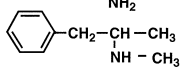
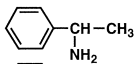
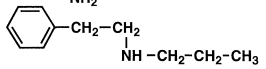
2.2. Hair sample

Hair samples were obtained from abusers and non-abusers.

Each hair sample was cut into lengths of about 5 mm. A 1 mg amount of hair was washed with 2 ml of 0.1% sodium *n*-dodecylbenzenesulfonate, 2 ml of distilled water and 2 ml of methanol, in turn.

A control hair solution was used to establish the optimal analytical method and for construction of the calibration curve. The control hair solution was prepared as follows: A 0.3 g amount of hair from a non-abuser was dissolved in 3 ml of 1 *M* sodium hydroxide by shaking at 75°C, then 4.5 ml of distilled water were added to give a solution con-

Table 1
Chemical structures of amphetamine, methamphetamine and the internal standards used in this study

Compound	Structure
Amphetamines	
Amphetamine (AP)	
Methamphetamine (MA)	
Internal standards	
α-Phenethylamine (α-PEA)	
N-Propyl-β-phenethylamine (Pro-β-PEA)	

taining 1 mg of hair in 25 μl of 0.4 *M* sodium hydroxide.

2.3. Analytical procedure

Hair sample (ca. 1 mg), 50 μl of the internal standards solution and 150 μl of 6.7 *M* sodium hydroxide were placed in a 2-ml vial with a silicone rubber–PTFE septum and screw-cap top. The vial was sealed tightly and shaken at intervals by hand while heating in a water bath at 75°C until the hair was dissolved (about 5 min). When establishing the optimal conditions and constructing the calibration curve, 25 μl of control hair solution, 25 μl of an AP–MA standard mixture, 50 μl of the internal standards solution and 100 μl of 10 *M* sodium hydroxide were placed in a 2-ml vial. The vial was sealed tightly and shaken.

After cooling, the needle of the SPME device was passed through the septum. The extraction fiber in the needle was exposed to the headspace of the vial for 20 min at 55°C. After the fiber was retracted into the needle, the needle was removed from the vial and immediately inserted into the GC injection port for desorption of AP and MA at 220°C for 30 s in the injection port. The procedure is shown schematically in Fig. 1.

2.4. Apparatus

The SPME device was purchased from Supelco (Bellefonte, PA, USA). The fiber assembly, coated with 100 μm of polydimethylsiloxane, was conditioned in the GC injection port for 30 min at 250°C to remove fiber contaminants. The 2-ml grass vials with silicone rubber–PTFE septa were obtained from Hewlett-Packard (Wilmington, DE, USA).

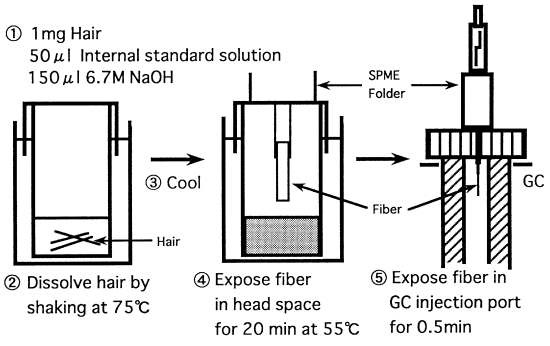


Fig. 1. Illustration of the analytical procedure used for analyzing amphetamine and methamphetamine in human hair by headspace solid-phase microextraction and gas chromatography.

GC analysis was carried out using a Hewlett-Packard 5890 Series II Plus equipped with a nitrogen–phosphorus detector. The column used for the separations was a CBJ-17 capillary column (30 m× 0.53 mm I.D., with a film thickness of 1 μm; Shimadzu, Kyoto, Japan). The operating conditions for the split injection system were as follows: column temperature, held initially at 100°C for 5 min then raised to 220°C at 10°C/min before being held at 220°C for 3 min. The injection and detector temperatures were 220°C. The flow-rate of the carrier gas (helium) was 4 ml/min and the split ratio was 2:1. The response of the recorder was set to one fourth after 9.7 min.

3. Results and discussion

3.1. Optimum extraction conditions

Although several methods, such as alkaline digestion [8], enzymatic digestion [21], methanol sonication [22], etc. were reported for hair pretreatment, we used the alkaline digestion method for this purpose. By using this method, AP and MA were easily eluted from hair into alkaline solution and vaporized into the headspace of the vial. Among the commercially available SPME fibers tested, polydimethylsiloxane proved to be the most satisfactory stationary phase for this purpose. In order to establish the optimum extraction conditions with this fiber, a test sample containing AP (22 ng/mg hair) and MA (24 ng/mg hair) was prepared in the control hair solution and these compounds were extracted into the SPME fiber under various conditions. As shown in Fig. 2, exposure of the SPME fiber to the headspace in 0.2 ml of 5 *M* sodium hydroxide at 55°C proved to be adequate for maximal extraction of AP and MA. Although diffusion of these compounds in the alkaline solution increases as the temperature rises, the increasing temperature has a negative effect on adsorption to the fiber. Therefore, the extraction of AP and MA was higher at 55°C as a consequence of these opposing parameters. In contrast, the adsorp-

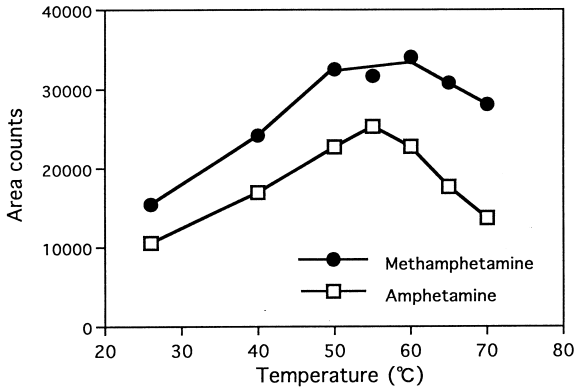


Fig. 2. Effect of temperature on the headspace solid-phase microextraction of amphetamine and methamphetamine in hair.

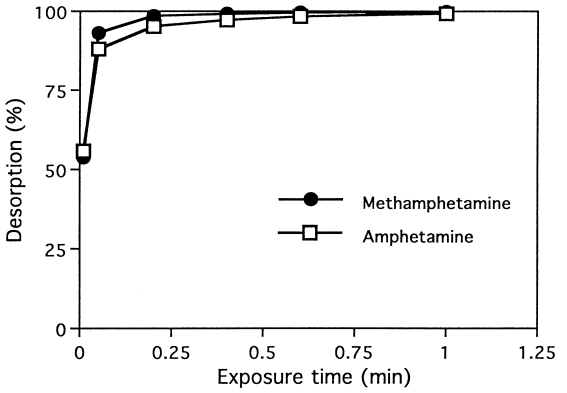


Fig. 4. Effect of exposure time of a 100-μm polydimethylsiloxane fiber on the thermal desorption of amphetamine and methamphetamine.

tion of AP and MA on the fiber gradually increased with time (Fig. 3), however, the adsorption ratios of these compounds against their internal standards remained constant after 15 min. Therefore, an adsorption time of 20 min was used. The percentages of AP and MA extracted from hair samples by the HS–SPME method were 48 and 62%, respectively, and the relative standard deviations were below 10% ($n=5$).

3.2. Optimum desorption condition

In order to establish the optimum desorption time, the exposure time of the fiber in the GC injection port was studied. As shown in Fig. 4, AP and MA adsorbed onto the SPME fiber from the solution containing hair (74 ng of AP and 80 ng of MA had been added) were desorbed rapidly at 220°C. Within 0.5 min, over 97 and 99% of AP and MA were desorbed from the fiber, respectively.

The remaining AP and MA in the fiber could be removed by reexposing the fiber in the GC injection port at 250°C for 3 min after each analysis. However, it was impossible to remove AP and MA completely from the fiber using this method when the fiber had been used for samples containing high concentrations of these compounds.

Therefore, the fiber was changed frequently when carryover could not be removed. (Carryover can be avoided performing blank test verification before each analysis).

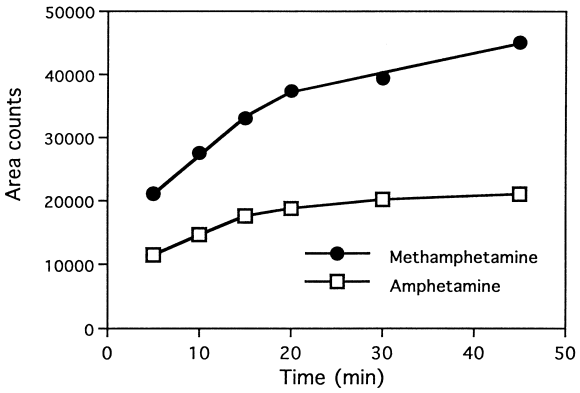


Fig. 3. Effect of adsorption time on the headspace solid-phase microextraction of amphetamine and methamphetamine in hair.

3.3. GC analysis

NPD–GC chromatograms obtained from normal hair samples are shown in Fig. 5A,B. Sharp peaks and good chromatographic resolution for each compound were obtained. AP and MA in hair could be analyzed within 20 min without any interference from coexisting substances. AP and MA provided excellent NPD responses and the detection limits to give a signal-to-noise ratio of three under our GC conditions were 0.1 and 0.4 ng/mg hair, respectively (Table 2). Although the recoveries of AP and MA using the HS–SPME method were 48–62%, as shown in Section 3.1., these compounds could be analyzed sufficiently in hair samples by using struc-

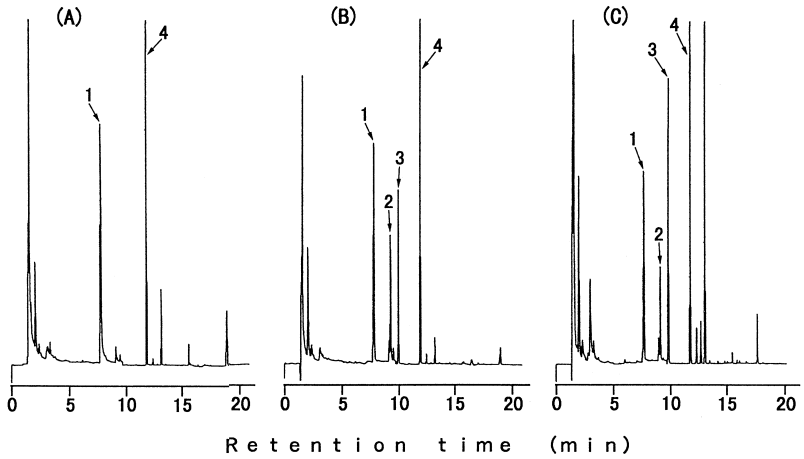


Fig. 5. GC–NPD chromatograms obtained from hair samples. (A) Normal hair, (B) normal hair to which standard amphetamine (1.5 ng) and methamphetamine (16.1 ng) had been added, (C) hair from an abuser. The GC conditions are given in Section 2.4. The recorder's responses were reduced to one fourth after 9.7 min. Peaks: 1=α-phenethylamine (internal standard); 2=amphetamine; 3=methamphetamine; 4=*N*-propyl-β-phenethylamine (internal standard).

tural homologues as the internal standard, as these showed similar behavior to AP and MA during the SPME step and were well separated from AP and MA on chromatograms. To obtain the actual concentrations of AP and MA in the hair of an abuser [23], the calibration curves for each compound were constructed from the peak area ratios against each internal standard. As shown in Table 2, a linear relationship was obtained, with correlation coefficients that were above 0.998, in the ranges 0.4–15 ng/mg hair for AP and 4–160 ng/mg hair for MA.

3.4. Analysis of hair from an abuser

In order to demonstrate the applicability of the method to a real sample, the contents of AP and MA were determined in hair taken from an abuser of MA (AP is a main metabolite of MA). As shown in Fig. 5C, AP and MA in the abuser's hair could be analyzed by this method without any interferences, although some unknown peaks were observed on the chromatogram. The contents of AP and MA in the abuser's hair were 1.14±0.19 and 34.8±3.5 ng/mg (n=5), respectively. The concentration of MA was determined to be about 30 times higher than that of AP.

4. Conclusion

These experiments have conclusively demonstrated that AP and MA can be accurately and precisely determined in samples of human hair using HS–SPME followed by GC–NPD analysis. This method is simple, rapid and sensitive, and can be used to determine AP and MA in the hair of abusers. We

Table 2
Linear regression data and detection limits for amphetamine and methamphetamine

Compound	Range (ng/mg hair)	Regression line[a]	Correlation coefficient[b] (r)	Detection limit (ng/mg hair)
Amphetamine	0.4–15	$y=0.2053x+0.0405$	0.9983	0.1
Methamphetamine	4–160	$y=0.0173x-0.0047$	0.9994	0.4

[a] y=peak-area ratio; x=concentration of compound (ng/mg hair).
[b] n=12.

believe that this method provides a practical and effective method for the analysis of stimulants in hair.

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