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New method of derivatization and headspace solid-phase microextraction for gas chromatographic–mass spectrometric analysis of amphetamines in hair

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Abstract

A simple method for hair analysis of methamphetamine (MAMP) and amphetamine (AMP) by gas chromatography–mass spectrometry (GC–MS) was developed using simultaneous headspace solid-phase microextraction (HS-SPME) with derivatization. After alkaline-digestion of hair, the analytes derivatized with heptafluoro-*n*-butyryl chloride were adsorbed on a polydimethylsiloxane-coated fiber by HS-SPME and analyzed by GC–MS. Their mass spectra were, respectively, observable at 1 ng per sample. The standard curves in the range of 0.1–100 ng were linear. The intra-day coefficients of variation at each 0.5 ng were 12.5% for AMP and 3.8% for MAMP. The applicability of this method was demonstrated in some case studies. © 2001 Published by Elsevier Science B.V.

Keywords: Derivatization, GC; Amphetamines

1. Introduction

For examining long-term drug intake, hair analysis is very useful. In criminal or clinical investigations, it is often vital interest whether or not an amphetamine abuser has been taking the drug over a long period of time.

The method of extractive derivatization on an Extrelut column introduced in our previous report [1] is applicable to hair amphetamine analysis [2]. Utilizing this method, we have carried out many analyses of amphetamines in hair, and have been able to obtain reasonable results. However, the method itself needs certain pretreatment steps, e.g., washing the Extrelut filling, distilling solvents, adjusting the pH, etc. Over the last few years a new extraction method, solid-phase microextraction (SPME), has been developed and used for the analysis of amphetamines in several biological samples [3–9]. Since this method utilized the headspace manner (HS-SPME), no special pretreatment procedures were necessary [4–9]. Koide et al. introduced hair analysis of underivatized-amphetamine by HS-SPME and GC nitrogen–phosphorus detection [5]. For GC–MS investigation of amphetamines, pertinent derivatives are desired in order to acquire intact mass spectra as clear evidence. Heptafluoro-*n*-butyryl derivatives of amphetamines are commonly used for gas chromatography–mass spectrometry (GC–MS) analysis, and the derivatives can be

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prepared with heptafluoro-*n*-butyryl chloride in an aqueous solution.

Hair sample preparation for GC–MS analysis needs to be separated into three steps: alkaline-digestion, derivatization and extraction. However, a simple method or single step is always desirable in a practical laboratory. In this study, we attempted to unite the three steps into one simple performance step.

2. Experimental

2.1. Materials

2.1.1. Reagents

Methamphetamine (MAMP) hydrochloride was purchased from Dainippon Pharmaceutical (Osaka, Japan). Amphetamine (AMP) sulfate, 4-hydroxy-amphetamine (HAMP) hydrobromide, 4-hydroxy-methamphetamine (HMAMP) (free base), methamphetamine-d_5 (internal standard 1, I.S.-1) hydrochloride and 4-hydroxymethamphetamine-d_5 (I.S.-2) (free base) were prepared as described in our previous report [10]. Heptafluoro-*n*-butyryl (HFB) chloride was obtained from Tokyo Kasei Kogyo (Tokyo, Japan). The other common reagents used in this study were of analytical grade.

2.1.2. Tools

The SPME devices, 100-μm polydimethylsiloxane (PDMS) fiber assemblies, were purchased from Supelco (Bellefonte, PA, USA). The 12-ml vial used for headspace sampling could be sealed with a PTFE-coated silicon-rubber stopper and a plastic screw cap.

2.1.3. Specimens

Amphetamine-negative (blank) hair specimens provided by a volunteer were used for preparing blank and standard samples and amphetamine-positive hair specimens were collected at autopsies. All positive specimens contained MAMP more than 5 ng per 5 mg, at the level of which intact mass spectrum of MAMP could be observed by our previous report [2]. These were, respectively, stored in paper envelopes at room temperature until analysis, after being washed by Suzuki et al.'s method [11].

2.1.4. Specimen for the comparative analysis exemplified

The hair of a 43-year-old male cadaver found in a forest was collected at an autopsy. According to the forensic medical findings and the police information, the corpse had been dumped after he was stabbed in the right chest. In the toxicological blood tests, methamphetamine at 1.2 μmol/100 ml and a trace level of aminopyrine were demonstrated. The blood MAMP concentration corresponded to intermediate-level in our temporary toxic evaluation [12]. By our previous method of hair analysis for amphetamine, MAMP was also estimated at 21.5 ng/mg (144 pmol/mg), and its distribution obtained from nine strands of hair is indicated in Fig. 4. The distribution of MAMP obtained by using five strands of specimen was compared with the previous result.

2.1.5. Internal standard (I.S.) solution and standard solutions

I.S.-1 (for AMP and MAMP) and I.S.-2 (for HAMP and HMAMP) as their hydrochlorides were together dissolved with 1 *M* HCl to obtain 10 ng/μl I.S. solution. Four standard compounds, AMP sulfate, MAMP hydrochloride, HAMP hydrobromide and HMAMP base were together dissolved in 1 *M* HCl to produce their standard solutions at serial concentrations of 0.1, 0.5, 1.0, 5.0, 10, 50 and 100 ng/μl. These solutions were kept in a refrigerator at 4°C.

2.1.6. Sample preparation

Five cm of hair were placed in a 12-ml vial containing 10 ng of each of the I.S.s (1 μl as the solution), and digested in 0.2 ml of 1 *M* NaOH at 70°C. Then, the cooled sample was diluted with 1.2 ml of 0.1 *M* phosphate buffer (pH 6.0), before being mixed with 10 μl of HFB-Cl for derivatization. The derivatives in the headspace of the vial were adsorbed onto the fiber at 60°C for 20 min after being stirred for a few seconds. The fiber was transferred into the injection port of GC and held for more than 3 min. The derivatives were diffused from the fiber in the liner (see Section 2.3) at 250°C.

2.1.7. Preparation for standard curves

Into a vial containing 5 cm of blank hair, 1 μl of I.S. solution and 1 μl of each of the standard solutions were added. Then, the sample preparation was performed in the same manner as described above.

2.2. Examination of applicability

To examine this method for practical application, some actual specimens obtained from stimulant abusers were analyzed utilizing this method. For our routine screening test, the mass spectral demonstration of MAMP was attempted using hair which was 5 cm long. The quantitative results were also compared with the results obtained by our previous method [2]. With the new method, five pieces of hair were used, while 10–15 pieces (more than 20 cm long or more than 1 mg) were used with previous method. The sample preparation for this examination was carried out in the same manner as the above. In order to measure MAMP distribution, hair specimens were cut into 1-cm lengths from the root, and five pieces of each of these lengths were, respectively, collected in a vial.

2.3. Instrumental

GC–MS analyses were performed on a QP-5000 operated in the positive electron impact (EI) mode (Shimadzu, Kyoto, Japan). The GC–MS conditions were as follows: an XTI®-5 capillary column (30 m× 0.25 mm I.D., 0.5-μm film thickness; Restek, Bellefonte); injection port temperature 250°C; column oven temperature, initially held at 70°C for 1 min, then increased at 25°C/min to 290°C; helium flow-rate, 2.1 ml/min (constant); interface temperature, 260°C. SPME sampling was performed in the splitless mode, the splitter being opened 1 min after the fiber was inserted. The ionizing energy was 70 eV. The inner diameter of the deactivated liner in the injection port was 2.0 mm. Quantitative data were obtained by selected ion monitoring at m/z 240 and 118 for AMP, at m/z 254 and 210 for MAMP, at m/z 240 and 330 for HAMP, at m/z 254 and 210 for HMAMP, and at m/z 258 for both I.S.s. The mass spectra for qualification were collected by the full ion scanning manner at every 0.5 s.

3. Results

For qualitative analysis, mass spectra were observable at 1 ng per sample of AMP and MAMP (Fig. 1C,D). With regard to HAMP and HMAMP, their detection was irregularly recognized. The identifiable sensitivity in this method was apparently 5 times higher than in our previous work [2]. Although some artificial peaks relating to silicon-rubber relating components were observed (Fig. 1A), no peaks interfering with target compounds appeared as shown in Fig. 1B.

For quantitative analysis by GC-SIM, respective characteristic fragment ions were selected at m/z 254 for MAMP, at m/z 118 for AMP and at m/z 258 for I.S.s. For confirmation of trace analytes, the respective supplemental ions were monitored at m/z 210 for MAMP, and at m/z 240 for AMP. As seen in Fig. 2, MAMA and AMP were, respectively, identifiable at concentrations of 0.1 ng per sample by observing a pair of characteristic ions. The concentration was estimated by obtaining the area ratio of analyte peak with I.S. peak. Respective standard curves were linear within the range of 0.1 to 100 ng per a sample; $y=0.0352x+0.0191$ ($r^2=0.9997$) for AMP and $y=0.0881x+0.0659$ ($r^2=0.9993$) for MAMP. The reliabilities of the quantitative values are indicated as coefficients of variation in Table 1.

The applicability of this method was studied by examining several hair specimens collected from supposed MAMP abusers. MAMP was, in every actual case studied in this report, identifiable as an intact mass spectrum, while AMP was also observable in SIM (see an example of detection file in Fig. 3). In every MAMP-positive case, namely, more than 1 ng of MAMP was present in a 5-cm long hair. For quantitative evaluation of this method, actual hair specimens were analyzed by this method with SIM and the results were compared with results obtained by our previous method [2]. Comparative results between two methods are exemplified in Fig. 4, which shows MAMP distribution on hair expressed as a concentration level for every 1-cm section. The results obtained suggest that this method is also

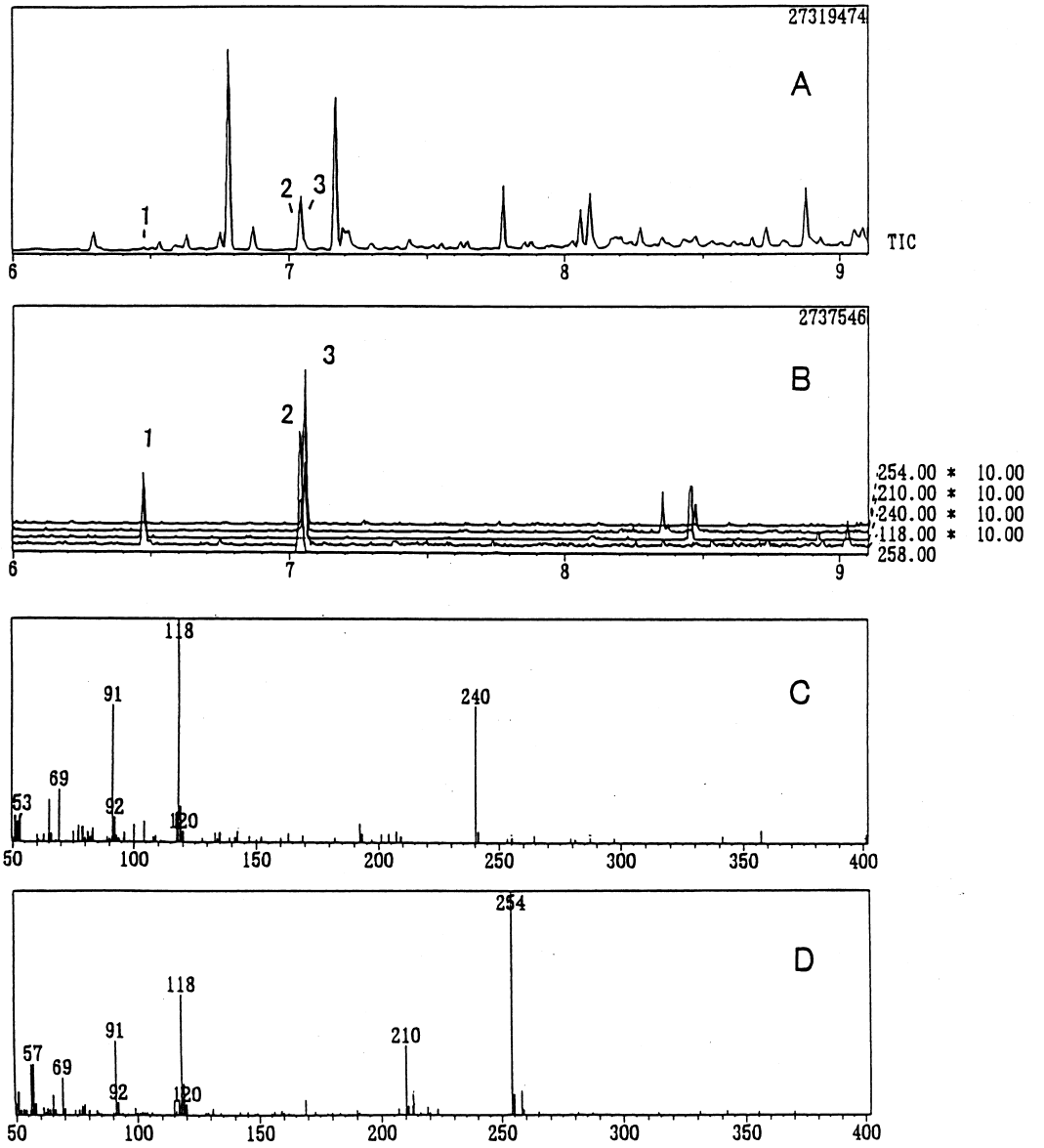

Fig. 1. Detection of amphetamines in a standard hair sample. The sample was prepared from a 5-cm long hair spiked with 1 ng each of authentic standards and 10 ng each of internal standards. In this example, phenolic groups are unobservable. (A) Total ion chromatogram; (B) mass chromatograms reconstructed with their characteristic fragment ions; (C,D) mass spectra identified with methamphetamine and amphetamine. Retention times (min): (1) amphetamine 6.47; (2) methamphetamine-d_5 (I.S.-1) 7.04; (3) methamphetamine 7.06.

applicable to practical analysis, as was our previous method. The findings obtained from actual case studies clearly indicate that this method is of use for practical forensic investigations.

4. Discussion

For obtaining higher sensitive and characteristic mass spectra, amphetamines are often per-

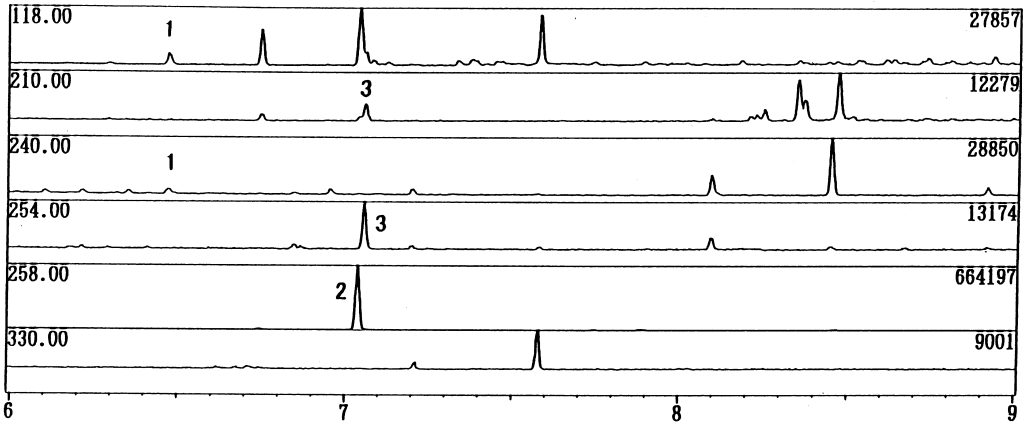

Fig. 2. SIM chromatograms obtained from 5-cm long hair spiked with 0.1 ng of each of the authentic standards and 10 ng of each of the internal standards. The numbers indicated above the peaks correspond to the respective numbers in Fig. 1.

Table 1
Reliability of intra-day and day-to-day assays (each $n=5$)

Compound	Intra-day (C.V.%)[a]		Day-to-day (C.V.%)	
	0.5 ng	50 ng	0.5 ng	50 ng
Amphetamine	12.5	13.4	12.2	15.1
Methamphetamine	3.8	2.5	9.3	4.0

[a] C.V., coefficient of variation.

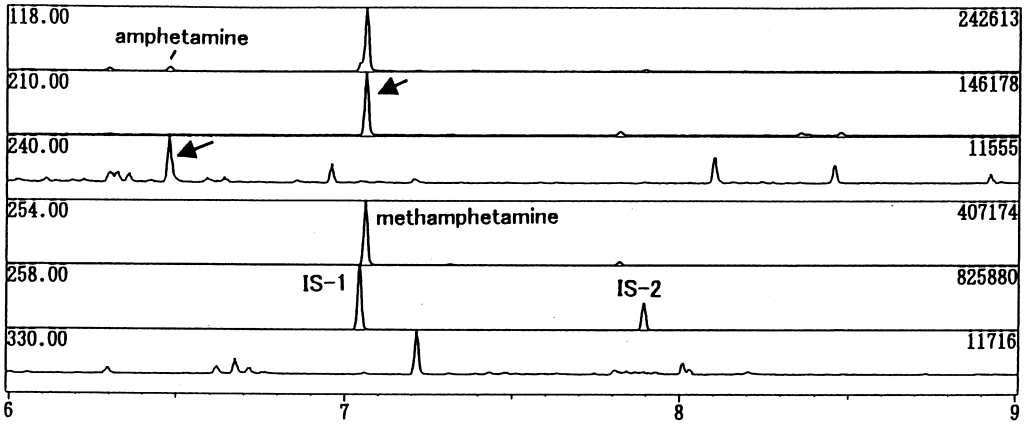

Fig. 3. SIM chromatograms of a 5-cm long hair in an actual case (46-year-old female). Estimated contents are less than 0.02 ng for amphetamine and 4.48 ng for methamphetamine. The intact mass spectrum of methamphetamine was observed. I.S.-1, methamphetamine-d_5; I.S.-2, 4-hydroxymethamphetamine-d_5.

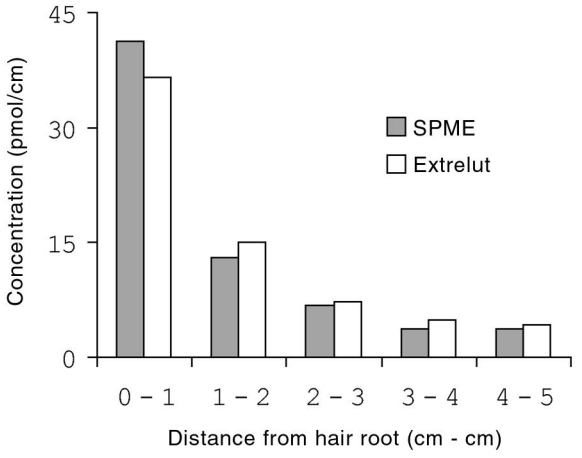

Fig. 4. Comparison of this method with our previous method. Hair specimens obtained from a 43-year-old male cadaver were measured by both methods. In the latest method, extractive derivatization was used. Both results coincide regarding MAMP distribution on hair.

fluoroacylated for GC–MS analysis. Perfluorocarbonic anhydrides are commonly used as the perfluoroacylating reagents [7–11,13]. However, when using their reagents, anhydrous conditions are required. Accordingly, the derivatizations are usually performed after extraction. Even in HS-SPME, the derivatizations were performed at a dry place such as an injection port of the instrument [7,8]. Meanwhile, *n*-propyl chloroformate [4], pentafluorobenzyl bromide [14] and heptafluoro-*n*-butyryl chloride can be used for derivatization in aqueous solution. In this study, the heptafluoro-*n*-butyrylation in aqueous solution was demonstrated with HS-SPME. The sample introduction with HS-SPME in which the use of organic solvents is unnecessary consequently provided very clear mass spectra at a lower level.

We selected the most highly intensive ion of the respective compounds for quantification. Although other characteristic ions were also monitored in order to peak-qualification, some interfering ions with their trace level detection sometime were frequently observed as described in our previous reports [1,2]. Accordingly, the quantitative analysis was performed using respective single ion after the intact mass spectra were positively confirmed.

For preparing the standard curves, we used 5 cm of hair, the accurate weight of which could not be measured by a regular chemical balance. In our actual hair test, we are permitted to obtain only about 50 strands of hair from a crime-suspected person. Accordingly, we use a few strands of hair especially for SPME performance, because of the difficulty of recollection of specimens. Some specimens should be stored for reconfirmation testing. Although the quantitative data must be reported in terms of ng/mg, we used ng/cm (or pmol/cm) for performing the actual case study.

Based on its relatively high sensitivity, this method is applicable for actual cases of hair testing. In practice, MAMP and AMP were more clearly identifiable on mass spectra and/or SIM chromatograms. For quantitative analysis, the estimated concentration of MAMP in this method was almost the same as that in the previous method [2].

5. Conclusion

For GC–MS analysis of amphetamines in hair, a sensitive, accurate and rapid sample introducing system is proposed which integrates all the steps of alkaline-digestion, derivatization and headspace-SPME into one simple step.

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References

[1] K. Hara, S. Kashinura, Y. Hieda, M. Kageura, J. Anal. Toxicol. 21 (1997) 54.
[2] K. Hara, S. Kashimura, S. Tomojiri, N. Tanada, M. Kageura, K. Tanaka, Jpn. J. Forensic Toxicol. 16 (1998) 190.
[3] A. Ishii, H. Seno, T. Kumazawa, M. Nishikawa, K. Watanabe, H. Hattori, O. Suzuki, Jpn. J. Forensic Toxicol. 14 (1996) 228.
[4] H.G. Ugland, M. Krogh, K.E. Rasmussen, J. Pharm. Biomed. Anal. 19 (1999) 463.
[5] I. Koide, O. Noguchi, K. Okada, A. Yokoyama, H. Oda, S. Yamamoto, H. Kataoka, J. Chromatogr. B 707 (1998) 99.
[6] C. Jurado, M.P. Gimenez, T. Soriano, M. Repetto, J. Anal, Toxicology 24 (2000) 11.

[7] A. Namera, M. Yashiki, J. Liu, K. Okajima, K. Hara, T. Imamura, T. Kojima, Forensic Sci. Int. 109 (2000) 215.
[8] N. Nagasawa, M. Yashiki, Y. Iwasaaki, K. Hara, T. Kojima, Forensic Sci. Int. 78 (1996) 95.
[9] F. Centini, A. Masti, C.I. Barni, Forensic Sci. Int. 83 (1996) 161.
[10] K. Hara, M. Kageura, Y. Hieda, S. Kashimura, Z. Rechtsmed. 100 (1988) 321.
[11] O. Suzuki, H. Hattori, M. Asano, J. Forensic Sci. 29 (1984) 611.
[12] K. Hara, T. Nagata, K. Kimura, Z. Rechtsmed. 96 (1986) 93.
[13] H. Tsuchihashi, K. Nakajima, M. Nishikawa, K. Shiomi, J. Chromatogr. B 731 (1989) 217.
[14] M. Sato, T. Mitsui, J. Pharm. Biomed. Anal. 16 (1997) 139.