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Journal of Chromatography B, 842 (2006) 106–110
JOURNAL OF CHROMATOGRAPHY B
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Single hair analysis of methamphetamine and amphetamine by solid phase microextraction coupled with in matrix derivatization ☆

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Received 19 December 2005; accepted 15 July 2006
Available online 12 September 2006

Abstract

A sensitive method for detection of methamphetamine (MA) and amphetamine (AP) in human hair was developed using solid phase microextraction (SPME) and one-pot derivatization. MA and AP were directly derivatized to *N*-propoxycarbonyl derivatives in an aqueous solution by propylchloroformate in a one-pot reaction before extraction by SPME. The derivatives were extracted to a coating of SPME from a headspace of the vial. The adsorbed derivatives were thermally desorbed in the injection port of a gas chromatograph. Pentadeuterated MA was used as an internal standard. The absolute recoveries of MA and AP from the spiked hair were 2.80–17.5%, respectively. The calibration curves showed linearity in the range of 0.05–20 ng/0.08 mg/vial for MA and 0.1–20 ng/0.08 mg/vial for AP in hair. Detection limits (S/N = 3) of MA and AP were 0.02 and 0.05 ng/0.08 mg/vial. The coefficients of variation of intraday were 1.04–26.4%. Additionally, this proposed method was applied to segmental analysis in clinical and medico-legal cases of MA intoxication.

Keywords: Amphetamine; Methamphetamine; Hair; Segmental analysis; Solid phase microextraction

1. Introduction

Methamphetamine (MA) and its metabolite amphetamine (AP) are powerful stimulants of the central nervous system and are abused in many countries. Acute poisoning and death due to intoxication or overdose have been reported after intake of MA and related drugs. In forensic and clinical laboratories, abuse of these drugs is usually verified by a detection of the parent drugs and/or their metabolites in urine and blood obtained from abusers. However, urine and blood analysis have the following disadvantages: (1) the period of detectability (half-life time) after intake of the drugs is short and (2) the concentration of drugs in urine is affected by the pH of urine. Thus, a more suitable biological material is needed to confirm long-term use of drugs. For these reasons, attention has recently been focused on hair analysis to determine the usage periods for many drugs [1–5]. The slow growth of hair and its absorption of any drugs and their metabolites allow investigation over a much longer period, compared with other biological materials. Many chromatographic methods have been reviewed to detect amphetamines in hair [6–8], where extraction of the targets from hair matrix prior to chromatography was necessary. The analysis of free amphetamines by gas chromatograph (GC) or gas chromatograph–mass spectrometer (GC–MS) is hindered by difficulties in sensitivity and reproducibility because of adsorption on and interaction with the column. Derivatization is usually needed for the GC analysis of these compounds. However, sample preparation procedures become laborious, costly, and time-consuming. A simple and accurate method is needed for routine analysis and screening of amphetamines in hair for forensic, judicial, and clinical purposes.

Our final goal was to develop a simple and accurate method for determination of amphetamines in biological materials, which could be used for routine works. Acylation, such as trifluoroacetylation is usually used for derivatization of amphetamines in many laboratories. However, there are some serious problems in the trifluoroacetylation of amphetamines; for example, a loss of the derivatives during sample preparation and stability of the derivatives [9]. Recently, alkylchloroformates have been reported for derivatization of amines in urine [10]. The

☆ This paper was presented at the 43rd International Meeting of the International Association of Forensic Toxicologists, Seoul, Korea, 29 August–2 September 2005.

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doi:10.1016/j.jchromb.2006.07.039

alkylchloroformates quickly react with primary and secondary amines in an alkali aqueous solution. Therefore, these reagents are suitable for our aim to derivatize amphetamines. In our previous study, we examined ways to extract and simultaneously derivatize MA and AP in urine and blood on the Extrelut column [11–13]. It was difficult to apply a segmental hair analysis using those methods, because the sensitivity was insufficient. In this study, we developed a more sensitive method for determination of MA and AP in hair using SPME and applied this to reveal the history of MA use in medico-legal and clinical cases of MA intoxication.

2. Experimental

2.1. Materials

Methamphetamine hydrochloride was purchased from Dainippon Pharmaceutical Co. (Osaka, Japan). Amphetamine hydrosulfate and pentadeuterated methamphetamine (MA-d_5, internal standard) hydrochloride were supplied from Dr. Hara of Fukuoka University (Fukuoka, Japan). Propylchloroformate (purity 95%) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Other reagents and solvents used were purchased at the highest commercial quality from Wako Pure Chemical Inc. (Osaka, Japan). Stock standard solutions of amphetamines (1.0 mg/ml) were dissolved in 0.01 M HCl and stored at 4 °C in a refrigerator. Working solutions were diluted from the standard solutions with 0.01 M HCl.

A manual assembly of SPME with a replaceable extraction fiber coated with polydimethylsiloxane (PDMS, 100 μm) was purchased from Supelco (Supelco Japan, Tokyo). The fibers were conditioned in a GC injection port at 250 °C for 1 h prior to use. Fibers were changed after 50 uses.

2.2. Hair samples

Drug free hairs collected from healthy adult males and females who had not taken any drugs were used to make blank or spiked hair with amphetamines. Drug free hair and abuser's hair collected from clinical and medico-legal cases were kept at room temperature until analyzed. Each hair was successfully washed with sodium dodecylsulfate (0.1%, once), distilled water (3 times), ethanol (once), and then dried at room temperature. Each hair was cut into lengths of 1 cm each prior to extraction and the average weight of a piece of hair (1 cm) was 0.08 ± 0.02 mg. Spiked hair was prepared as follows: a drug free hair was put in an extraction vial, and then the known amounts (solution) of AP and MA was carefully dropped onto the hair.

2.3. Gas chromatography–mass spectrometry

The gas chromatograph–mass spectrometer (GC–MS) used was a Hewlett-Packard 6890 GC and 5973A MSD, equipped with a 30 m × 0.25 mm (I.D.) fused silica capillary column (Hewlett-Packard, HP-5MS, film thickness 0.25 μm). The oven temperature was set at 100 °C for 1 min, and then programmed from 100 °C to 300 °C at 10 °C/min, and held at 300 °C for 3 min. The temperatures of the injection port and the detector were set at 250 °C and 280 °C, respectively. Splitless injection mode was used. The mass selective detector was operated in the electron impact (EI) mode at 70 eV of electron energy. Helium with a flow rate of 1.0 ml/min was used as a carrier gas. To confirm the mass fragment of the derivatives, data were obtained in a full scan mode with a scan range from *m/z* 50 to 450. Ions used for quantitation were *m/z* 130 for AP derivative, and *m/z* 144 for MA derivative, *m/z* 148 for MA-d_5 (IS) derivative.

2.4. Extraction and derivatization by SPME

A piece of washed hair (1 cm) was placed into a 10 ml-volume extraction vial and weighed to calculate the concentration of AP and MA in hair. Sodium hydroxide (1 M, 0.5 ml) and IS (1.0 μg/ml, 5 μl) were placed into the vial and sealed rapidly with a silicon septum and a vial cap. The vial was heated at 70 °C for 20 min to digest the hair. After cooling down, propylchloroformate (10 μl) was added in the vial. The SPME needle was then inserted into the vial and the extraction fiber was exposed at 70 °C for 20 min in the headspace. After extraction, the fiber was pulled back into the needle and the needle was then inserted into the injection port of the GC–MS. The fiber was exposed for 3 min in the injection port for desorption of the analytes from the fiber.

2.5. Method validation

To determine calibration curves, hair samples spiked with MA and AP at concentrations ranging from 0.01 to 20 ng/0.08 mg/vial were prepared and analyzed using the above procedure. The calibration curves were obtained by plotting the peak area ratio between the derivatives of MA (AP) and that of MA-d_5 (IS). The criteria for the limits of detection and quantitation were S/N = 3 and 10. Repeatability was evaluated by analyzing spiked hair containing three different concentrations (0.5, 5.0 and 15 ng/0.08 mg/vial) of MA and AP on the same day in five replicates.

3. Results and discussion

3.1. Sample preparation

First of all, hair and an alkaline solution (1 M NaOH) were put in a test tube to digest hair. Though the hair was dissolved clearly, the relative recovery of AP and MA was 30% (Fig. 1). There was a concern that amphetamines would escape from the test tube because of their volatility. Therefore, the vial was capped with butyl rubber in order to avoid low recovery when a hair sample was digested in a vial. Unfortunately, the recovery of amphetamines was not satisfactory with butyl rubber (relative recovery: 45–70%). Three kinds of septum (silicon, butyl rubber covered with/without aluminum foil) were examined to improve the recovery. When butyl rubber was used as a septum, the derivatives were absorbed by the rubber and the recovery of amphetamines was low. The recovery was slightly improved

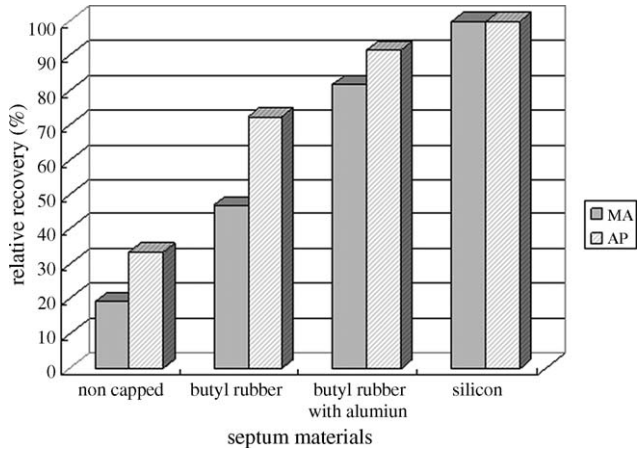

Fig. 1. The effect of the septum material on the recovery of amphetamines.

by covering butyl rubber with aluminum foil. The recovery rate of AP was lower than that of MA because the water solubility of AP ($\log P_{OW}$: 1.83) was higher than that of MA ($\log P_{OW}$: 2.22). The best recovery rates and the highest peak area were ultimately achieved by using silicon septum.

Four main methods [(1) alkaline, (2) acid, (3) methanol and (4) enzyme hydrolysis] were reported to digest a hair [14]. Alkaline hydrolysis has the potential to simultaneously digest the hair matrix and derivatize amphetamines in hair to the derivatives in a one-pot reaction. In this study, alkaline hydrolysis was adopted to digest a hair. For the effect on the recovery of amphetamines in hair, a concentration of NaOH (1, 3 and 5 M), digestion temperature (70 and 80 °C) and time (10 and 20 min) were examined. The recovery of MA was decreased when the digestion time was shorter. The recovery of MA when digesting for 10 min was ca. 60% comparing with digesting for 20 min. It

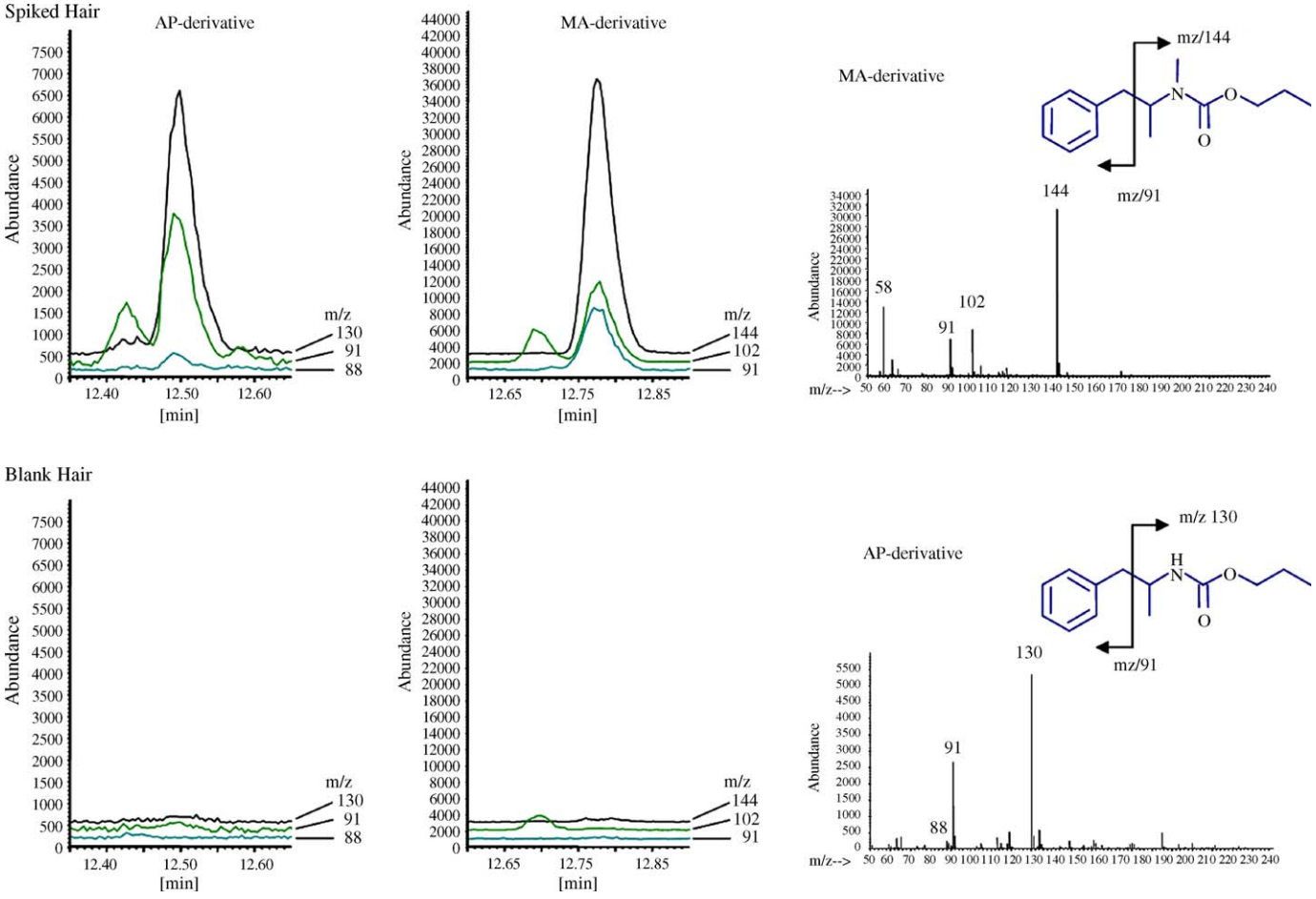

Fig. 2. Typical extracted ion chromatograms and full mass spectra of the derivatives extracted from spiked and blank hair: left: *N*-propoxycarbonyl AP, right: *N*-propoxycarbonyl MA.

Table 1
Quantitation limit and linearity of this method

Compound	Limit of detection (ng/0.08 mg/vial)	Range of linearity (ng/0.08 mg/vial)	Linearity[a]	Correlation coefficient
AP	0.05	0.10–20	$y = 0.029x + 0.018$	0.999
MA	0.02	0.05–20	$y = 0.160x + 0.068$	0.999

[a] x are amounts of analytes (ng/0.08 mg/vial) and y are peak area ratios.

Table 2
Intra-day precision for analysis of MA and AP

Compound (ng/0.08 mg/vial)	Intra-day ($n = 5$)	
	Recovery(%)	CV(%)
AP		
0.5	5.08	26.4
5.0	2.86	9.49
15.0	2.80	6.40
MA		
0.5	17.5	5.75
5.0	12.0	1.04
15.0	11.2	1.14

was considered that the digestion for 10 min was insufficient for the extraction of amphetamines from hair. The recovery of AP was slightly increased with the rise of NaOH concentration, but was not influenced by digestion temperature and time. Our strategy was decided the digestion time was longer by using of lower concentrated alkali solution because a high concentration alkali usually affects the fiber. When 1 M NaOH was used, the hair was completely digested and the solution was clear. However, the hair was not completely digested with high concentration of NaOH, and clots of hair matrix were observed. Therefore, hair was digested with 1 M NaOH and heated at 70 °C for 20 min. This extraction time (20 min) was the best for routine analysis, considering the operating time of GC–MS. The next extraction can be performed while previous GC–MS analysis, and the following analysis is possible at the same time as the finish of previous analysis.

3.2. Validation data

To optimize the extraction and derivatization of MA and AP in hair, our previous method was modified [15]. Typical extracted

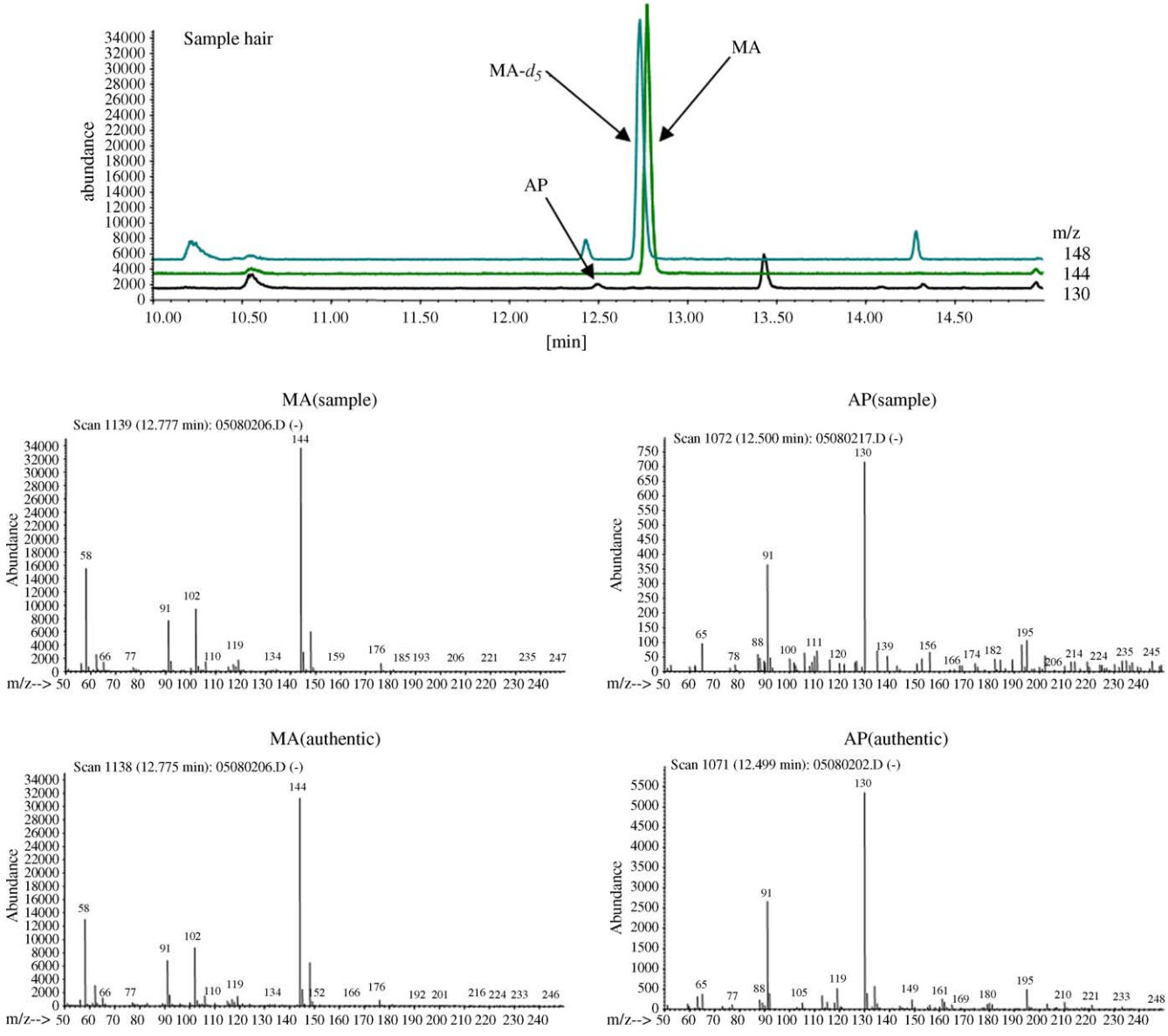

Fig. 3. Extracted ion chromatogram and mass spectrum of the derivatives from a clinical case.

ion chromatograms of the derivatives extracted from spiked and blank hair are shown in Fig. 2.

The calibration curves showed linearity up to 20 ng/0.08 mg/vail for MA and AP in hair. Detection limits (S/N = 3) of MA and AP were 0.02 and 0.05 ng/0.08 mg/vial, respectively (Table 1). The coefficients of variation (0.5, 5.0, 15 ng/0.08 mg/vial) were 1.05–26.4% (Table 2). The absolute recovery was 2.80–17.5%, respectively. Though the above recovery rate seemed poor, the whole amount extracted by SPME could be introduced into the GC–MS. As a result, the sensitivity of this method was higher than that of a conventional liquid–liquid or solid phase extraction.

The applications have been also reported for detection of amphetamines in hair [16–20]. Koide et al. extracted amphetamine and methamphetamine from the headspace and conducted analysis with a nitrogen–phosphorus detector [16]. This method did not employ a derivatization; therefore, the detection limits were markedly increased. Liu et al. extracted amphetamine and methamphetamine from headspace after derivatization using heptafluoro-*n*-butyryl chloride [18]. Musshoff et al. has reported on-fiber derivatization of amphetamines using the vapor of *N*-methyl-bis-(trifluoroacetamide) (MBTFA) [19]. To our knowledge, MBTFA affected subsequent analyses and damaged the coating of the fiber. Although this method has an interesting derivatization technique, we decided this was not suitable for routine analysis. The dynamic range of this method was narrow and this could not be applied to an automated procedure, because the vial was opened and the addition of the derivatizing reagent was needed during the procedure.

3.3. Application of segmental analysis

A 37-year-old male was arrested on April 22nd. He reported having injected MA or its salt between April 1st and 21st. He did not have a stimulant intake for about 1 month after his arrest (the period that he was detained). Thus, his hair was collected on May 17th, and the history of his drug use was investigated. When his hair was collected, the hair was cut at the surface of the scalp. Extracted ion chromatogram extracted MA and AP in hair collected from him is shown in Fig. 3. Sharp and symmetrical peaks of MA and AP were obtained without disturbance of endogenous interferences. Mass spectrum of MA was also obtained. Concentrations of MA and AP in 1 cm long hair segments are shown in Fig. 4. His testimony was proven from the analysis result. Although very little MA was detected in a segment of 1 cm from the scalp, this result was expected. Generally, 2 weeks are required for hair to grow beyond the scalp, though the hair extends by 10–14 mm a month. Thus, results will not accurately reflect the history of drug abuse when hair cut at the surface of the scalp is used. In order to improve this problem and to reflect the history of drug abuse, it is necessary to pull out the hair. There were various problems because the amount of the hair was large when the reported methods were used. However, the burden for patients decreases with the proposed method because a single piece of hair is sufficient for testing.

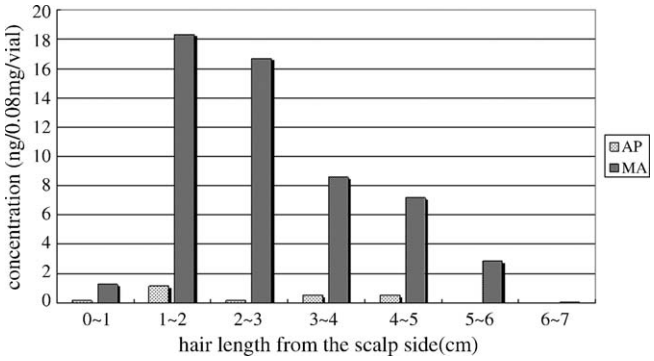

Fig. 4. Concentrations of MA and AP in 1 cm long hair segments of a 37-year-old MA abuser. One-centimeter segments were numbered from the scalp.

Acknowledgment

The authors would like to thank Dr. Hara (Fukuoka University), for a supply of standard compounds and a suggestion for digestion of hair.

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