Yuji Nakahara,[1] *Ph.D.; Kazunori Takahashi,*[1] *Ph.D.; Mochihiko Shimamine,*[1] *Ph.D.; and Yasushi Takeda,*[2] *Ph.D.*

Hair Analysis for Drug Abuse: I. Determination of Methamphetamine and Amphetamine in Hair by Stable Isotope Dilution Gas Chromatography/Mass Spectrometry Method

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ABSTRACT: Determination of methamphetamine and amphetamine in hair was performed by gas chromatography/mass spectrometry using stable isotope-labeled internal standards, 2-methylamino-1-phenylpropane-2,3,3,3-d_4 and 2-amino-1-phenylpropane-2,3,3,3-d_4. Extraction of hair with methanol/5*M* hydrochloric acid (20:1) using ultrasonication was chosen as the standard method. The calibration curves for amphetamines in the hair were linear from 1 to 100 ng/mg ($r > 0.99$). The detection limit was 0.5 ng/mg at the 95% confidence level. The coefficients of variation (CV) ($n = 8$) of analysis using the spiked hair with methamphetamine were from 0.7 to 6%. The CV ($n = 8$) of analysis of the methamphetamine abuser's hair was 17.5%. Sectional analysis of monkey and human hair after methamphetamine ingestion suggested a good correlation between the duration of drug use and drug distribution in the hair.

KEYWORDS: toxicology, hair, methamphetamine, amphetamine, stable isotope dilution, gas chromatography/mass spectrometry (GC/MS), hair analysis, drug abuse history

Drug use is at present confirmed mainly by urinalysis. However, difficulties can be encountered during urinalysis. For example, only short-term information of drug use can be obtained, subjects can easily evade proper sampling or refuse to provide urine, and there is a difficulty with long storage. It has been previously illustrated [*1*] that hair analysis may overcome these deficiencies characteristic of urinalysis. Therefore, the practical use of hair analysis has been anticipated for forensic science analysis, therapeutic drug monitoring, drug abuse, and dope testing.

In forensic toxicology, gas chromatography/mass spectrometry (GC/MS) has been evaluated as a confirmative method [*2*] for drug use testing. It is important to use an appropriate internal standard for accurate and precise determination of trace amounts of a drug in hair by GC/MS. The deuterium-labeled target drug is superior to other compounds

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[1]Section chief and senior forensic chemists, respectively, Narcotics Section, National Institute of Hygienic Sciences, Tokyo, Japan.

[2]Head, Division of Drugs, National Institute of Hygienic Sciences, Tokyo, Japan.

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as an internal standard (IS) for GC/MS from the standpoints of reproducibility and accuracy because it has the same reactivity for derivatization and the same recovery from biological samples as the target drugs.

This paper describes the analysis of methamphetamine (MA) and amphetamine (AP) in hair by isotope dilution GC/MS using deuterium-labeled MA and AP, and its application to the determination of MA and AP in monkey and human hair.

Experimental Materials

Reagents

All the chemicals were of reagent grade, available commercially. Acetaldehyde-d_4 (99.6 atomic percent D) was purchased from Merck Sharp and Dohme (Montreal, Canada) and methamphetamine hydrochloride was obtained from Dainippon Pharmaceutical Co. (Osaka, Japan). Amphetamine sulfate was synthesized by the Ramirez and Burger method [*3*], and its purity was checked by thin-layer chromatography (TLC), GC/MS, nuclear magnetic resonance (NMR), and melting point (MP) [280°C (decomposition)].

Hair Sample

Monkey Hair—Prior to the administration of MA, the monkey's back hair was cut with an electric shaver and it was used as a control. Three crab-eating monkeys (male, 2.6 to 2.7 kg) were subcutaneously administered, once a day, 3 mg/kg of MA hydrochloride dissolved in physiological saline for three weeks. Back hair growing at the position where the control hair was previously cut was collected at the eighth week after the last drug administration.

Human Hair—Hair samples were collected from the vertex of the scalp by cutting approximately 2 mm from the scalp. The root sides of the hair samples obtained were bundled with a rubber band and the samples were wrapped in aluminum foil, on which was drawn the orientation of the hair shafts.

We compared the correlation between the self-reported drug history of the human subject and the results of sectional analysis, assuming that scalp hair grows at the rate of about 1.1 cm/month.

Scalp hair of an MA abuser (female, 35 years old) was provided by Dr. Konuma of the National Shimofusa Sanatorium (Chiba, Japan). The abuser had used MA intravenously at 60 to 90 mg/day for 50 days from the 100th day before hair collection (Fig. 3). Hair samples (12 cm) at the vertex area were cut close to the scalp.

Control human hair was obtained from healthy volunteers at our Institute.

Apparatus

GC/MS analyses were performed using a Hewlett-Packard 5890 gas chromatograph equipped with 7673A autosampler and an MSD 5970 (Avondale, Pennsylvania). The gas chromatography (GC) was carried out with a 10 m by 0.25-mm inside-diameter, 0.3-μm cross-linked methyl silicone fused-silica WCOT (Gaskuro Kogyo Inc., Tokyo, Japan). The injection port temperature was 150°C (splitless mode) and helium was the carrier gas (25 kPa head pressure). The oven temperature was held at 60°C for 0.5 min following injection and then programmed to 250°C at a rate of 20°C/min.

High-resolution mass measurements were carried out by VG Analytical Zab-2SEQ high-resolution mass spectrometer using the chemical ionization mode (isobutane).

2-Methylamino-1-Phenylpropane-2,3,3,3-d_4 (MA-d_4)

After 60 mL of benzyl magnesium chloride in ether [obtained by refluxing benzyl chloride (12.7 g) and magnesium (2.75 g) for 2 h in anhydrous ether] was added to 20 mL of *N*-methylethyleneimide-d_4 in ether [obtained by introducing methylamine gas into acetaldehyde-d_4 in ether], the reaction mixture was refluxed for 1 h. After cooling, 200 mL of 0.75*M* sulfuric acid (H_2SO_4) was added to the reaction mixture to decompose excess reagent. The acid layer was separated, basified with 6*M* sodium hydroxide (NaOH), and extracted three times with 200 mL of chloroform ($CHCl_3$). The combined $CHCl_3$ extract was treated with a few drops of concentrated hydrochloric acid (HCl)/ethanol (1:9) and evaporated to dryness. The solid residues were recrystallized from ethanol under the following conditions: MA-d_4/HCl; mp 133 to 135°C; infrared spectrum (IR) [potassium bromide (KBr)] cm^{-1}, 3420, 1598; high-resolution mass measurement [isobutane/chemical ionization (CI)]; MH^+ at *m/z* 154, 146 ($C_{10}H_{12}ND_4$ = 154, 153).

2-Amino-1-Phenylpropane-2,3,3,3-d_4 (AP-d_4)

AP-d_4 was prepared in the same manner as for MA-d_4 except that ethyleneimine-d_4 and concentrated H_2SO_4/ethanol (1:20) were used in place of *N*-methylethyleneimine-d_4 and concentrated HCl/ethanol (1:9), respectively. The solid obtained was recrystallized from methanol/ethanol (1:1) under the following conditions: AP-d_4/½ H_2SO_4; m.p., 270 to 275°C (dec.); IR(KBr) cm^{-1}, 3420, 1610, 1556; high-resolution mass measurement (isobutane/Cl); MH^+ at *m/z* 140, 137 ($C_9H_{10}ND_4$ = 140, 138).

Trifluoroacetylated (TFA) MA-d_4 and AP-d_4 were measured by GC/electron ionization mass spectrometry (EIMS) (GC/EIMS). These structures were confirmed by comparison with each cluster ion pair as follows: TFA-methamphetamine: M^+ [249(TFA-MA-d_4):245(TFA-MA)], M^+-69 (M^+-CF_3)[180(TFA-MA-d_4):176(TFA-MA)], M^+-91 (base peak) [158(TFA-CH_3N=$CDCD_3$):154(TFA-CH_3N=$CHCH_3$)], M^+-127[122(ArCH=$CDCD_3$):118(ArCH=$CHCH_3$)]. TFA-amphetamine: M^+[235(TFA-AP-d_4):231(TFA-AP)], M^+-69 (M^+-CF_3)[166(TFA-AP-d_4):162(TFA-AP)], M^+-91 (base peak) [144(TFA-HN=$CDCD_3$):140(TFA-HN=$CHCH_3$)], M^+-127[122(ArCH=$CDCD_3$):118(ArCH=$CHCH_3$)].

Methods

The hair samples were washed three times with 10 mL of 0.1% sodium dodecylsulfate (SDS) and with 10 mL of water, under ultrasonication for 1 min. After the hair was dried, it was cut into sections about 2 cm long and then cut up as fine as possible with scissors. Approximately 10 mg of each section of finely cut hair was accurately weighed, extracted with 2 mL of MeOH/5*M* HCl(20:1) for 1 h under ultrasonication, and the solution stored at room temperature overnight. After 100 μL of the IS methanol solution containing MA-d_4 and AP-d_4 at 3 μg/mL each was mixed with the hair extract and the hair filtered off, the filtrate was evaporated with a nitrogen stream. The residue was dissolved in 200 μL of trifluoroacetic anhydride/ethylacetate (1:1) and warmed at 55°C for 20 min. The reaction solution was evaporated with a nitrogen stream and the residue was redissolved in 100 μL of ethylacetate. One μL of the ethylacetate solution was automatically injected into a gas chromatograph. The selected ion monitoring of TFA-AP, TFA-AP-d_4, TFA-MA, and TFA-MA-d_4 at each base peak is shown in Fig. 1.

Results and Discussion

Calibration Curves, Linearity, and Reproducibility

The calibration curves for the measurements of MA and AP were constructed by the analysis of extracted and derivatized samples of 10 mg of control hair to which the standard

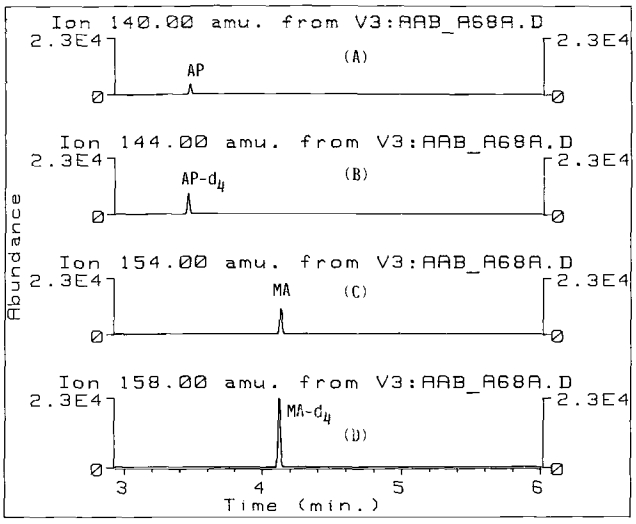

FIG. 1—*Selected ion monitoring of TFA-amphetamine* (A), *TFA-amphetamine-d_4* (B), *TFA-methamphetamine* (C), *and TFA-methamphetamine-d_4* (D).

solutions of MA and AP had been added at 1 to 100 ng/mg of hair, together with IS as mentioned about. The calibration curves for MA ($y = 1.02x + 0.0086$) and AP ($y = 1.12x - 0.0015$) in the hair were linear over the concentration range 1 to 100 ng/mg with correlation coefficients $r = 0.999\ 97$ for MA and $r = 0.999\ 96$ for AP. Both detection limits were 0.5 ng/mg at the 95% confidence level.

Reproducibility of the method was determined by processing spiked hair of healthy volunteers at two concentrations with respect to a calibration curve run each day. Eight samples were analyzed for each concentration. The coefficients of variations (CV) for MA and AP were 1.73 and 4.38% at 4 ng/mg and 0.70 and 6.08% at 8 ng/mg, respectively (Table 1).

Washing

External contamination on the surface of the hair should be removed before analysis. Baumgartner et al. [*1*] have reported that the contamination on the surface of the hair of mice and humans administered morphine could be removed thoroughly by three 0.1% SDS washings and three water rinses. We used this washing method for our study. It

TABLE 1—*Reproducibility of hair analysis using hair spiked with drugs and drug abuser's hair* (n = 8).

	MA		AP	
	Avg. ± SD	CV, %	Avg. ± SD	CV, %
Known concentration,				
4	3.97 ± 0.069	1.73	4.31 ± 0.189	4.38
8	8.03 ± 0.056	0.70	8.08 ± 0.491	6.08
Abuser's hair[a]	5.07 ± 0.888	17.5	. . . . .	. . .

[a]This abuser had been abusing MA daily at 40 to 80 mg/day for four months. The hair length was 8 cm.

was confirmed that the second and third SDS washing solutions contained no methamphetamine.

Extraction Method

There are generally two methods for the extraction of basic drugs from hair: extraction with organic solvent, after the solubilization of hair with concentrated NaOH, [*4,5*] and direct extraction with acid solution or acidic methanol [*6–8*].

The results of extraction experiments investigated by the acidic extraction and ether extraction following alkaline solubilization are given shown in Table 2. There were not large differences between acidic extraction and alkaline-ether extraction, in the extraction efficiency of MA and AP from monkey hair, but the standard deviations (SD) of acidic methods were smaller than those of alkaline methods. In addition, less of the hair components were extracted by acidic extractions than by alkaline methods. Moreover, the former is simpler and easier than the latter. The best method for extraction of MA and AP from hair was the ultrasonic extraction of 10 mg of hair sample in 2 mL of MeOH/5*M* HCl (20:1), followed by standing overnight.

External Contamination of Hair Samples

In order to investigate the external contamination of hair by MA/HCl, the effects of standing time and drug concentration on drug adsorption by the hair were studied. As shown in Table 3, control hair soaked in an aqueous solution of 10 μg/mL of MA/HCl adsorbed no MA until 24 h, even though the surface of the hair was contaminated with 2.6 ng/mg of MA. However, in the case of hair soaked in 20 and 50 μg/mL of MA solution, 0.5 and 3 ng/mg of MA were adsorbed on or in the hair, respectively, even after washing. This result agreed with the report by Harrison et al. [*9*] on the strong affinity between amphetamine and melanin in the hair.

However, it is open to question whether MA is adsorbed on the surface or in the hair shaft. Because MA adsorbed on the surface might be eluted with washing more easily than MA incorporated in hair shaft, it is assumed that a considerable amount of MA would permeate the hair.

Sectional Analysis of Hair Sample

The monkey hair (2.2 cm) was cut into three sections from the root side to approximately 0.7 cm each, and each section was analyzed. As shown in Fig. 2, 12.8 ng/mg of MA and 10 ng/mg of AP were found in the distal section grown during drug administration. In the middle section, small amounts of MA and AP were found, but there was no measurable MA in the root section.

A human hair specimen (12 cm, female) was cut into six sections from the root side approximately 2 cm each and analyzed. No MA was found in the third to the distal section. However, approximately 4 and 10 ng/mg of MA were found in the first and second sections, respectively. These results corresponded to the duration of methamphetamine use as shown in Fig. 3.

Conclusions

The method described in this paper provides an accurate and sensitive analysis method for the determination of methamphetamine and amphetamine in hair. Methods using alkaline solubilization and acid methanol extraction were tried. As a result, the extraction of amphetamines in hair with methanol/5*M* HCl (20:1) under ultrasonication was chosen

TABLE 2—*Recovery of methamphetamine and amphetamine from hair by acidic extraction and ether extraction following alkaline solubilization.*

Solvent	Volume, mL	Procedure[a]	Results, ng/mg ± SD[b] MA	AP
MeOH/5*M* HCl (20:1)	2	sonication (1 h) + ON	15.5 ± 0.7	8.2 ± 1.0
	2	sonication (1 h)	10.8 ± 0.6	5.1 ± 0.6
	2	sonication (2 h)	12.8 ± 1.1	8.2 ± 1.2
DMSO/2.5*M* NaOH (4:1)[c]	1	sonication (0.5 h) + Et_2O extraction	11.8 ± 2.4	7.3 ± 2.8
2.5*M* NaOH	1	4°C (ON) + Et_2O extraction	10.8 ± 1.8	7.1 ± 2.1

[a]ON = overnight; Et_2O = diethyl ether.
[b]Sample: monkey hair described in the text. The number of measurements was three.
[c]DMSO = dimethylsulfoxide.

TABLE 3—*Effect of soaking time and concentration of methamphetamine on external contamination of hair.*

Concentration of MA in Water, μg/mL	Soaking Time, h	Washing with 0.1% SDS, MA(ng/mg) ± SD	
		Before	After
5	24	0.87 ± 0.81	ND[a]
10	24	2.47 ± 1.53	ND
20	24	2.73 ± 0.23	0.57 ± 0.07
50	24	8.57 ± 0.31	2.93 ± 0.16
10	4	0.05 ± 0.03	ND
10	8	0.17 ± 0.06	ND
10	16	0.33 ± 0.07	ND
10	24	2.47 ± 1.53	ND

[a]ND = not detected.

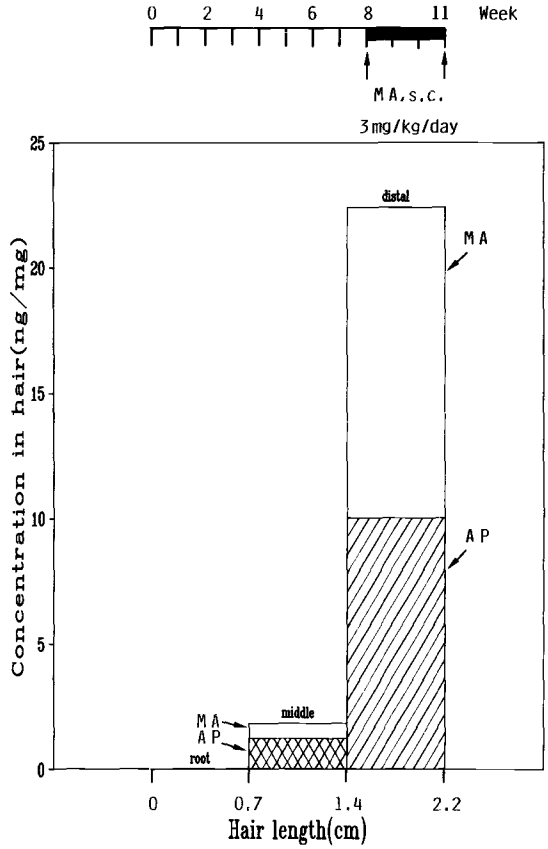

FIG. 2—*Sectional analysis of hair of monkeys administered methamphetamine for three weeks at 3 mg/kg/day. Open columns, MA; crosshatched columns, AP (hair = 2.2 cm, three sections).*

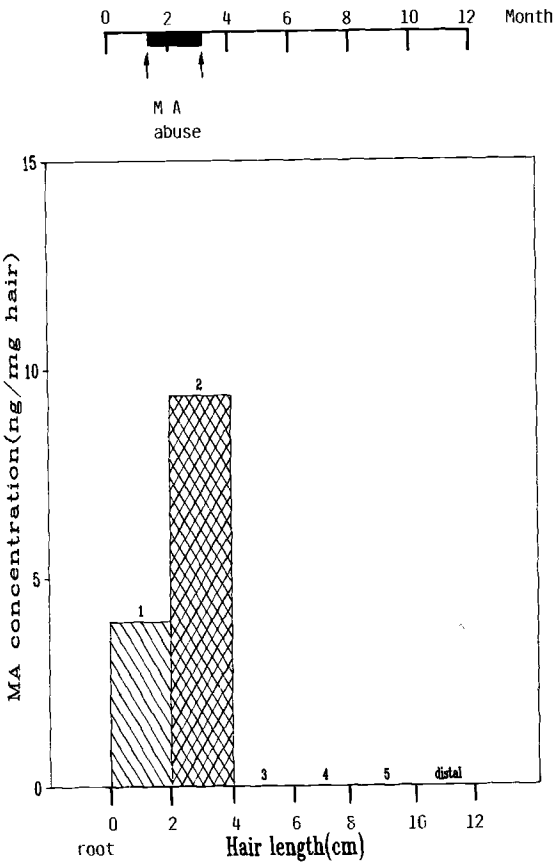

FIG. 3—*Sectional analysis of methamphetamine abuser's hair (female). She had been abusing methamphetamine intravenously at 60 to 90 mg/day for about 50 days (hair = 12 cm, six sections).*

because of the better extraction efficiency, less variable results, and the less extraction of hair components. The reproducibility of the method and linearity of the calibration curves were fairly good because of the use of stable isotope internal standards. However, there were considerable differences of variation between extraction of MA-spiked hair and the MA abuser's hair.

Soaking hair in a solution of MA suggested that methamphetamine could permeate the hair or was strongly adsorbed on the surface of the hair only when the hair was soaked in a highly concentrated solution of methamphetamine for a long period of time. It is still open to question whether drugs in hair would come from the bloodstream or from sweat containing drugs. However, the fact that amphetamine was detected in the hair of the monkey administered methamphetamine strongly suggests that the drugs detected in hair were secreted from the systemic circulation. Moreover, sweat containing more than 20 μg/mL of MA is unlikely, since MA concentrations in the sweat of monkeys chronically administered MA at 3 mg/kg/day for five days were, at most, 4 μg/mL and, on the average, 1 μg/mL [*10*]. When an oral dose of 20 to 25 mg dimethylamphetamine was given to healthy subjects, the maximum drug concentration in sweat was 4.27 μg/mL [*11*].

As a result of sectional analyses of a MA abuser's hair and monkey hair, a good

correlation between the duration of drug use and sectional drug distributions was observed.

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Address requests for reprints or additional information to
Dr. Y. Nakahara
National Institute of Hygienic Sciences
1-18-1, Kamiyoga, Setagaya-ku,
Tokyo, Japan 158