TECHNICAL NOTE

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Detection of Methamphetamine and Amphetamine in a Single Human Hair by Gas Chromatography/ Chemical Ionization Mass Spectrometry

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ABSTRACT: A detailed procedure of an extremely sensitive method for quantitation of methamphetamine and amphetamine in human hair by gas chromatography (GC)/chemical ionization (CI) mass spectrometry (MS) is presented. *N*-methylbenzylamine was used as an internal standard. The samples, after extraction with an organic solvent, were derivatized with trifluoroacetic anhydride before the GC/MS analysis. Quantitation was made with quasi-molecular ions of the derivatives by selected ion monitoring in the CI mode. The detection limit was about 10 pg in an injected volume. The high sensitivity enabled us to measure both stimulants in a single human hair in actual cases.

KEYWORDS: toxicology, methamphetamine, amphetamine, hair, gas chromatography/chemical ionization mass spectrometry

There is a great need for analysis of methamphetamine and amphetamine in human samples because they are widely abused and cause serious social problems; urine and blood have been generally used as forensic science materials. The use of hair is advantageous because it is easy to obtain and drugs are not broken down in it for a long time. Detection of drugs from human hair was tried for phencyclidine [*1*], phenobarbital [*2*], and opiate [*3*].

In the present study, we have established a detailed procedure for sensitive quantitation of methamphetamine and amphetamine in a single human hair by gas chromatography (GC)/ chemical ionization (CI) mass spectrometry (MS).

Materials and Methods

Chemicals

Methamphetamine-hydrogen chloride was purchased from Dainippon Pharmaceutical Co., Ltd., Osaka; *N*-methylbenzylamine from Tokyo Kasei Kogyo Co., Ltd., Tokyo; and

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trifluoroacetic anhydride (TFA) and 2% Thermon-3000 on Chromosorb W acid-washed dimethyldichlorosilane (AW-DMCS), 80–100 mesh from Wako Pure Chemical Industries, Ltd., Osaka. *d*-amphetamine sulfate was kindly donated by Dr. Yoshiko Yamamoto, Department of Legal Medicine, Kyoto University School of Medicine, Kyoto. *N*-methylbenzylamine was crystallized as a hydrogen chloride salt from a mixture of hydrochloric acid and ethanol by adding acetone.

Human Hair

Hair, offered by suspects of methamphetamine abuse, was kindly donated by a local police; hair from nonusers was also used.

GC/MS Conditions

A JMS-D300 GC/MS instrument with a JMA-2000E computer-controlled data analysis system was used. GC separation was made on a 2.0-m by 2.0-mm (internal diameter) glass column packed with 2% Thermon-3000 on Chromosorb W 80–100 mesh.

GC conditions were: injection temperature 180°C, column temperature 140°C, and helium flow rate 30 mL/min.

CI MS conditions were: reagent gas methane, electron energy 200 eV, ionization current 300 μA, separator temperature 150°C, ion source temperature 180°C, and chamber pressure 133 Pa (1.0 torr).

Electron impact (EI) spectra were recorded at 70 eV.

Procedure

A single hair was weighed and washed in a mixture of equal volumes of methanol and water by an ultrasonic washing machine. After drying it in air, it was completely dissolved in 2.0 mL of 2.5*N* sodium hydroxide to which 2.0 ng of *N*-methylbenzylamine had been added as an internal standard, by heating it at 80°C for 10 min. The mixture was extracted twice with 2.0 mL of *n*-pentane. The combined extracts were evaporated to dryness in vacuo and 100 μL of TFA was added to the residue. After heating it at 80°C for 30 min, it was dried under the stream of nitrogen. The residue was dissolved in 50 to 200 μL of ethyl acetate and 2 μL of it was subjected to the GC/MS analysis as described before.

Results

Figures 1 to 3 show CI and EI mass spectra of the TFA derivatives of the authentic methamphetamine, amphetamine, and *N*-methylbenzylamine (internal standard). The EI spectra of methamphetamine and amphetamine agreed well with the data published [*4–6*]; neither molecular nor quasi-molecular peaks were observed in the spectra. In the CI spectra, strong quasi-molecular peaks were detected at *m/z* 246, 232, and 218 for methamphetamine, amphetamine, and *N*-methylbenzylamine (internal standard), respectively. Their percentages of total abundance were as high as 44.3% for methamphetamine, 55.3% for amphetamine, and 60.4% for *N*-methylbenzylamine. Thus the quasi-molecular peaks, produced in the CI mode, were used for quantitation.

Selected ion monitoring (SIM) was performed with the quasi-molecular ions for the authentic compounds and hair of the nonusers and users of methamphetamine as shown in Fig. 4. The peaks corresponding to the TFA derivatives of methamphetamine, amphetamine, and *N*-methylbenzylamine appeared at 3.8, 4.6, and 2.4 min of retention time, respectively (Fig. 4*a*). There were no interfering peaks of impurities around the peak of each compound (Fig. 4*b* and *c*), which warrants the specificity of the present assay.

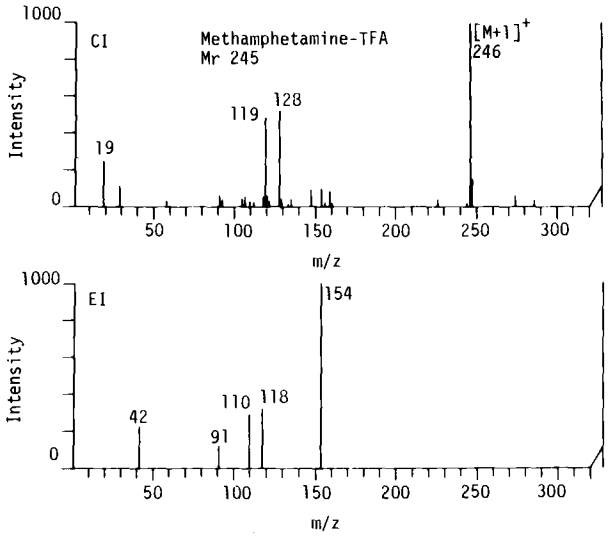

FIG. 1—*CI and EI mass spectra of methamphetamine-TFA.*

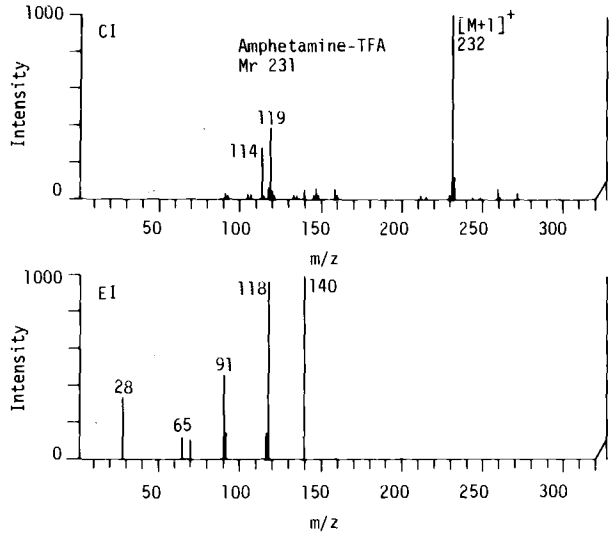

FIG. 2—*CI and EI mass spectra of amphetamine-TFA.*

For the SIM quantitation of methamphetamine, the ion intensity ratios (area) at *m/z* 246 to 218 (internal standard) were plotted against the concentrations of methamphetamine; generally satisfactory linearity was obtained up to 100 pg per 2 μL (the volume injected to the GC port) (Fig. 5). Figure 6 shows the same calibration plots for amphetamine. The sensitivity limit was 10 pg/2 μL for both compounds.

To test recoveries of methamphetamine, amphetamine, and *N*-methylbenzylamine, 6 ng of each compound was added to 2.0 mL of the 2.5*N* sodium hydroxide solution in the pres-

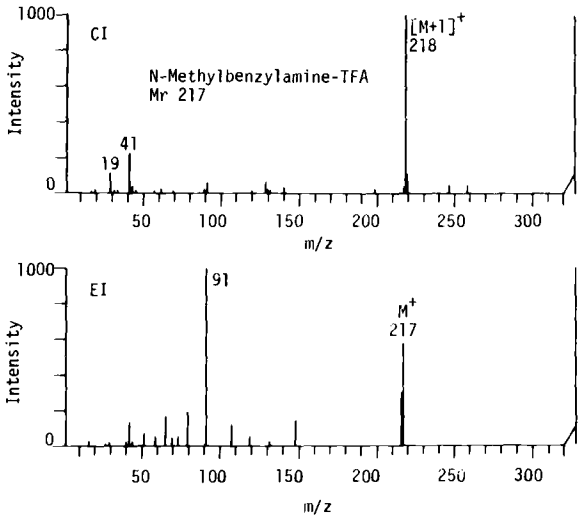

FIG. 3—*CI and EI mass spectra of* N-*methylbenzylamine-TFA.*

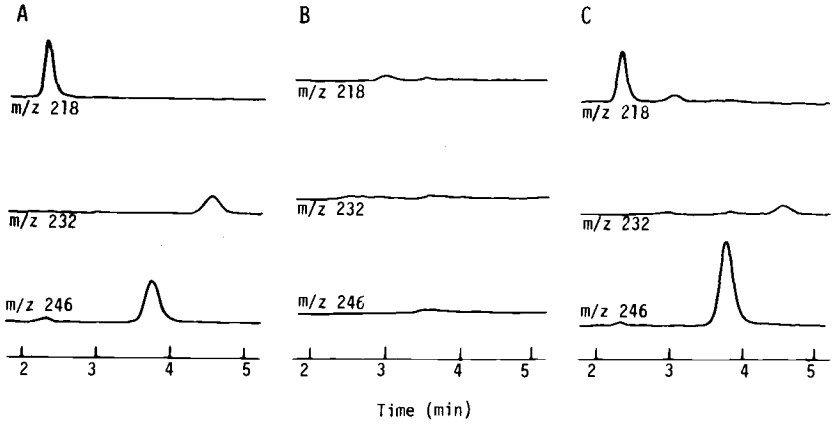

FIG. 4—*SIM of the mixture of the authentic methamphetamine—100 pg/2 μL, amphetamine—100 pg/2 μL, and* N-*methylbenzylamine-TFA—20 pg/2 μL* (a); *an extract of hair of a nonuser* (b); *and an extract of hair of a user of methamphetamine* (c).

ence of hair of a nonuser and processed as described before. The recoveries for methamphetamine, amphetamine, and *N*-methylbenzylamine were 81.5, 81.3, and 78.9%, respectively.

Using this method, methamphetamine and amphetamine in hair of suspects of methamphetamine abuse were carefully quantitated as listed in Table 1. The value of amphetamine, a metabolite of methamphetamine [*7*], was about five times less than the methamphetamine value.

Discussion

To our knowledge, this is the first report to discover methamphetamine or amphetamine in human hair by GC/MS. Very recently, Niwaguchi et al [*6*] have also reported experimen-

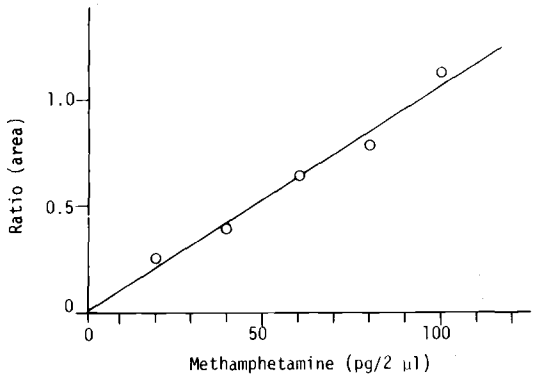

FIG. 5—*Calibration curve for methamphetamine-TFA by SIM. The vertical axis shows the peak area ratio calculated as* m/z *246/*m/z *218. Two nanograms of* N*-methylbenzylamine were added to each vial (20 pg/2 μL of the injected volume). The amount of methamphetamine was expressed as the form of its free base.*

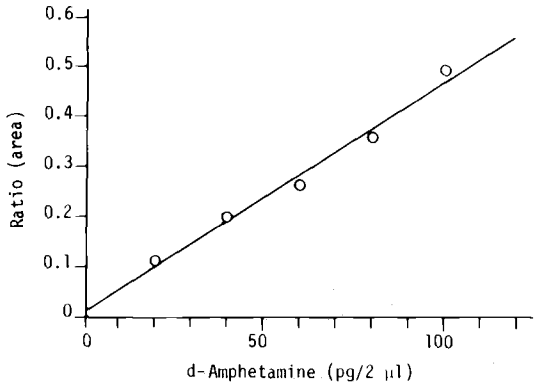

FIG. 6—*Calibration curve for amphetamine-TFA by SIM. The vertical axis shows the peak area ratio calculated as* m/z *232/*m/z *218. Two nanograms of* N*-methylbenzylamine were added to each vial (20 pg/2 μL of the injected volume). The amount of amphetamine was expressed as the form of its free base.*

tal data on determination of methamphetamine in rat hair after its administration and suggested the usefulness of human hair as a forensic science material.

The EI mode has been used in most reports dealing with GC/MS analyses of the stimulants in biological samples [*4–6*]. However, we have adopted GC/CI MS in the present study, because the CI mass spectra of the TFA derivatives of methamphetamine and amphetamine showed strong quasi-molecular peaks, which were useful for more sensitive detection of the compounds, while the EI spectra showed neither molecular nor quasi-molecular peaks of them (Figs. 1 and 2). When the fragment peaks in the EI mode were used for quantitation, they were remarkably interfered with by impurities contained in hair [*6*]. Our method in the CI mode was several times more sensitive than the conventional EI methods [*4–6*] and completely free of interference by impurities (Fig. 4).

We have employed 2% Thermon-3000 for gas chromatographic separation, which was used by Terada et al [*8*] for determination of methamphetamine in human urine by electron

TABLE 1—*Methamphetamine and amphetamine concentrations in hair obtained from suspects of methamphetamine abuse.* [a]

Sample	Age/Sex	Concentration, ng/mg in Hair[b]	
		Methamphetamine	Amphetamine
1	36/M	14.5	5.1
2	34/M	32.4	8.0
3	27/M	47.2	12.0
4	33/F	41.6	2.6
5	40/M	8.3	2.5
6	23/M	0	0
7	22/F	0	0
8	21/F	10.2	1.0
9	29/F	3.1	0.8
Mean ± S.D.		17.5 ± 15.0	3.6 ± 3.5

[a] The doses of methamphetamine and the frequency of its abuse are obscure, but it seems likely that several 10 mg of the amine are usually injected intravenously for each abuse.
[b] The amount of each compound was expressed as the form of the free base.

capture (EC) GC. We tried this EC method for human hair, but is was unsuccessful owing to interfering impurities present in it (unpublished observation). However, the 2% Thermon-3000 was satisfactory in its excellent separatory ability and very low adsorption of test compounds to the column in SIM.

Since the use of deuterated methamphetamine or amphetamine [*4*] seems undesirable in actual forensic science practice because of their cost or labor to synthesize them, we have tested *N*-methylbenzylamine, *N*-methylphenylethylamine, and phenylpropylamine as candidates for the internal standard (unpublished observation). Phenylpropylamine gave the same mass number and also the same retention time as amphetamine. *N*-methylphenylethylamine was good as an internal standard, but it is a biogenic monoamine, which may be present in human tissues [*9*]. *N*-methylbenzylamine is not biogenic and was satisfactory as an internal standard in its retention time (Fig. 4), extractability to organic solvents, and appearance of a quasi-molecular peak in the CI mass spectrum (Fig. 3).

The recoveries of methamphetamine, amphetamine, and *N*-methylbenzylamine added to hair of nonusers were equally around 80%. The minor loss of the amines may be mainly due to the loss of the organic solvent (*n*-pentane) during its transfer. This is fully compensated by taking the internal standard.

The concentration of methamphetamine in hair of the users was higher than we had expected (Table 1). Thus, only a single hair was sufficient to be subjected to measurements by the present assay method. Nagata [*10*] extensively measured methamphetamine concentration in blood obtained in actual cases and presented a criterion on methamphetamine intoxication as a function of its blood levels: 4.5 to 6.0 ng/μL, fatal; 3.0 to 4.5 ng/μL, severe; 0.4 to 3.0 ng/μL, moderate; and < 0.3 ng/μL, weak. In comparison with these blood levels, it seems likely that methamphetamine in blood is actively accumulated in hair, since it levels in hair of some samples (Table 1) far exceed the fatal blood level.

Hair is advantageous as a forensic science material, since it is easily obtainable and drugs do not break down for a long period. Our method is so sensitive that subnanograms of methamphetamine or amphetamine can be measured accurately. The high sensitivity enables us to detect the stimulants in a single hair left on the spot of a case, and also to perform a segmental analysis of the stimulants with a long hair to specify the time of administration, considering the growth rate of hair (about 1.5 cm per month). The present method is also simple and rapid; it was possible to complete the assays of ten samples within 4 h. We can recommend our method for analyses of methamphetamine or amphetamine in hair in actual forensic science practice.

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