Simultaneous determination of dimethylamphetamine and its metabolites in rat hair by gas chromatography–mass spectrometry

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Abstract

In order to study the disposition of dimethylamphetamine (DMAP) and its metabolites, DMAP *N*-oxide, methamphetamine (MA) and amphetamine (AP), from plasma to hair in rats, a simultaneous determination method for these compounds in biological samples using gas chromatography–mass spectrometry with selected ion monitoring (GC–MS-SIM) was developed. As DMAP *N*-oxide partially degrades to DMAP and MA during GC–MS analysis, it was necessary to avoid conditions which co-extract the *N*-oxide in the sample preparation so as to assure no contribution of artifactual products from DMAP *N*-oxide in the detection of the other compounds. For confirmation of the satisfactory separation of DMAP *N*-oxide from the others, the internal standards used for quantification were labeled with different numbers of deuterium atoms. Determination of unchanged DMAP was performed without any derivatization, that of DMAP *N*-oxide was carried out after conversion into trifluoroacetyl-MA by reaction with trifluoroacetic anhydride, and MA and AP were quantified after trifluoroacetyl-derivatization.

After intraperitoneal administration of DMAP HCl to pigmented hairy rats (5 mg kg^{-1} day^{-1}, 10 days, $n=3$), concentrations of DMAP and its metabolites in urine, plasma and hair were measured by GC–MS-SIM. The area under the concentration versus time curves (AUCs) of DMAP, DMAP *N*-oxide, MA and AP in the plasma were 397.2±97.5, 279.7±68.3, 18.4±1.2 and 15.9±2.2 µg min ml^{-1}, while their concentrations in the hair newly grown for 4 weeks after administration were 4.82±0.67. 0.45±0.09, 3.25±0.36 and 0.89±0.05 ng mg^{-1}, respectively. This fact suggested that the incorporation tendency of DMAP *N*-oxide from plasma into hair was distinctly low in comparison with the other compounds. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Hair analysis; Dimethylamphetamine; Dimethylamphetamine *N*-oxide; Methamphetamine; Amphetamine; GC–MS

1. Introduction

Methamphetamine (MA) and amphetamine (AP) are strictly controlled drugs, the abuse of which is a serious problem worldwide. For identification of abusers, urinalysis is usually the method of choice in forensic laboratories. However, MA or AP are also detected in urine as metabolites after ingestion of some other drugs. Several amphetamine-like over-the-counter (OTC) drugs which are available in the United States, Europe or Japan, such as deprenyl [1], benzphetamine [2,3], furfenorex [2,3], mefenorex [4], fenproporex [5] and fenethylline [6,7], have been

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reported to be metabolized to AP and/or MA in the body. From a forensic toxicological point of view, it is required to detect the parent drug itself or specific metabolites of the drug in biological samples in order to distinguish legitimate use of these OTC drugs from illegal use of MA/AP.

Dimethylamphetamine (DMAP) is also metabolized to AP and MA in animals and humans [8–10] (Fig. 1). DMAP produces behavioral effects that are generally comparable to those of MA but with reduced potency [11]. It is not a legal drug, but DMAP and DMAP-MA mixtures have been sold illegally on the streets in Japan as stimulants and this confounded the toxicological investigation [10]. Therefore, it is important to develop a simultaneous determination of DMAP and its metabolites including MA and AP in biological samples. Jenner [8] showed that MA and AP, together with unchanged DMAP and DMAP N-oxide were excreted in human urine after oral administration of DMAP HCl. Beckett and Al-Sarraj briefly reported [9] the presence of some other hydroxylamines and deaminated metabolites in rabbit urine after intraperitoneal administration of the drug. Inoue and Suzuki have also reported [10] the metabolism of DMAP in rat and man. They showed that unchanged drug and five metabolites, DMAP N-oxide, MA, AP and their aromatic hydroxylated compounds (except p-hydroxyamphetamine), were excreted in human urine for three days after oral administration of the drug. Although the major metabolite was DMAP N-oxide, MA was the most abundant metabolite in urine on the second and third day after administration.
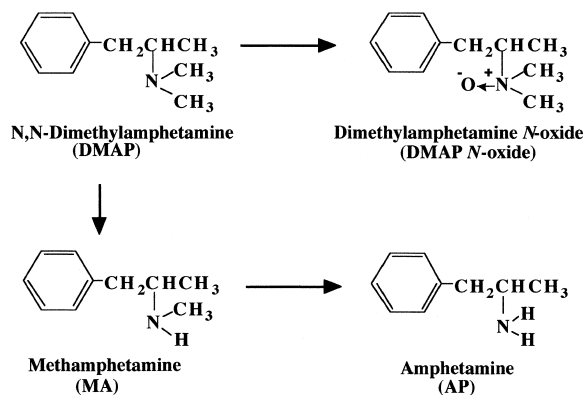
In recent years, hair analysis has rapidly progressed as a useful tool for detecting and monitoring drug intake over a long term. Hair analysis provides useful information about individual drug histories from a few days to years [12], while the information from blood and urine is limited by the elimination date. Moreover, we have reported that incorporation of drugs of abuse into hair mostly depends on their physicochemical properties [13,14], and the N-derivatives of AP, benzphetamine [15] and fenethylline [16], are more easily incorporated into hair than their polar metabolites. Therefore, it was expected that hair samples would be useful for the detection of parent compounds over a long time period.

In this work, we developed a gas chromatographic–mass spectrometric (GC–MS) method for the simultaneous identification and determination of DMAP and its metabolites (DMAP N-oxide, MA and AP) in rat plasma, urine and hair. In order to investigate the disposition of these drugs from plasma to hair, the hair concentrations and the area under the concentration versus time curves (AUCs) in the plasma were compared after administration of DMAP HCl to rats.

2. Experimental

2.1. Chemicals and reagents

MA was purchased from Dainippon (Osaka, Japan). Paraformaldehyde-d2 and formic acid-d2 were purchased from MSD Isotope (Montreal, Canada). DMAP [13] and AP [17] were prepared by previously reported methods. DMAP N-oxide was synthesized from DMAP by N-oxidation using hydrogen peroxide [18]. Deuterated compounds used as internal standards, 2-methylamino-1-phenylpropane-2,3,3,3-d4 (MA-d4) and 2-amino-1-phenylpropane-2,3,3,3-d4 (AP-d4), were also prepared according to the method reported by our group [19]. 2-Dimethylamino-1-phenylpropane-2,3,3,3-d4 (DMAP-d4) was synthesized from MA-d4 with formaldehyde and acetic acid, and 2-dimethylamino-d6-l-phenylpropane (DMAP-d6) from AP with paraformaldehyde-d2 and formic acid-d2 by N-methylation according to our previous report [20]. DMAP N-oxide-d6 was prepared from DMAP-d6 by N-

Fig. 1. Metabolic pathway of DMAP.

oxidation [18]. The structure and purity of each compound was confirmed by thin-layer chromatography, GC–MS and ^{1}H nuclear magnetic resonance. *N*-Oxides were additionally confirmed by high performance liquid chromatography/electrospray ionization–mass spectrometry (LC–MS). LC–MS analysis was performed on a HP Series 1100 LC/MSD (Hewlett-Packard, Palo Alto, CA, USA) with Eclipse XDB-C$_{18}$ column (2.1×150 mm, 5 μm, Hewlett-Packard). Analytical conditions were as follows. HPLC: mobile phase; methanol:0.l% acetic acid (10:90), flow-rate; 0.1 ml min^{-1}, oven temperature; 40°C, injection; 5 μl. MS: mass range; 50–550 amu, ionization; electrospray, fragmentor; 40V, nebulizer; nitrogen (30 p.s.i.), drying gas; nitrogen (10 l min^{-1}, 280°C), mode; positive. DMPA *N*-oxide and DMAP *N*-oxide-d6 eluted at 3.56 and 3.65 min as single peaks and their base peak ions were *m/z* 180.1 [*M*+H] and 186.1 [*M*+H], respectively.

Trifluoroacetic anhydride (TFAA) was purchased from Wako Chemicals (Tokyo, Japan). All other chemicals were of analytical reagent grade (Wako Chemicals).

2.2. Apparatus

GC–MS analysis was performed using a 6890 series gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) equipped with 6890 series auto-injector and 5973 MSD. The gas chromatograph was equipped with a 20 m×0.25 mm I.D., 0.25 μm film thickness cross-linked methylsilicone fused-silica column TC-1 (GL Sciences, Tokyo, Japan). The injection port temperature was 200°C (splitless mode) and helium was the carrier gas (5.5 p.s.i. head pressure; 1.16 ml min^{-1} flow-rate). The oven temperature was programmed from 60°C (held for 0.5 min) to 280°C at a rate of 20°C min^{-1}. The transfer line was maintained at 280°C. Target compounds in biological samples were analyzed by monitoring the selected ions (dwell time, 50 msec) as follows: *m/z* 72, 154 and 140 for DMAP, trifluoroacetyl (TFA)-MA and TFA-AP, and *m/z* 76, 144, 157 and 158 for DMAP-d4, TFA-AP-d4, TFA-MA-d3 (converted from DMAP *N*-oxide-d6 by reaction with TFAA as described in Section 2.4) and TFA-MA-d4, respectively.

2.3. Animal experiments

Male Dark Agouti (DA) pigmented rats, aged 5 weeks (100~120 g), were purchased from Japan SLC (Shirzuoka, Japan), and used for the experiments.

2.3.1. Hair

Prior to drug administration, the hair on the back of the rats was shaved with an animal electric shaver (Daitoh Electric Machine, Tokyo, Japan). The rats were intraperitoneally administered with DMAP HCl once a day (5 mg kg^{-1}) for ten successive days (*n*=3). The newly grown hair was collected by shaving 28 days after the first administration.

2.3.2. Plasma

Blood was collected on the day of the first administration at 5, 15, 30, 60, 120 and 360 min after injection from the orbital vein plexus using a capillary glass tube. The blood (500 μl) was collected into a plastic tube containing heparin and was cooled in ice. The plasma samples were obtained by centrifugation at 10 000 *g* for 3 min and were stored at −20°C until analysis. Plasma pharmacokinetic parameters were calculated as described previously [13].

2.3.3. Urine

The urine samples were collected at 0–2, 2–6, 6–12, 12–24, 24–48, 48–72 and 72–96 h after single intraperitoneal administration of 10 mg kg^{-1} of DMAP HCl, and stored at −20°C until analysis.

2.4. Analytical methods

2.4.1. Internal standard (I.S.) solution

I.S. aqueous solution was prepared by dissolving the hydrochlorides of DMAP-d4, MA-d4 and AP-d4 in distilled water. The concentration of each drug was 1 μg ml^{-1} as free base. The I.S. solution of DMAP *N*-oxide was prepared by dissolving DMAP *N*-oxide-d6 (10 μg ml^{-1}) in methanol. This I.S. solution was prepared freshly for each analysis.

2.4.2. Hair

Hair samples were washed three times with 0.1% sodium dodecyl sulfate (SDS) under ultrasonication, followed by washing three times with water under

the same conditions. After the sample was dried under a nitrogen stream at room temperature, approximately 20 mg of finely cut hair was precisely weighed. Ten microliters of DMAP N-oxide-d6 solution was evaporated to dryness in an appropriate analytical glass tube, and the hair sample added. To this, 1 ml of 1 M NaOH and 100 µl of the I.S. aqueous mixed solution were added, and the sample was completely digested with shaking for 15 h at room temperature. After filtration of digested solution, the filtrate was extracted by a liquid–liquid extraction procedure reported previously [10]. The solution was extracted three times with diethyl ether and the combined ether fraction was back extracted into 3 ml of 0.2 M HCl, which was subsequently basified with 5 M NaOH and reextracted with 3 ml of diethyl ether. The ether extract was divided into two aliquots. The first aliquot was concentrated under a nitrogen stream at room temperature and the residue was redissolved in 50 µl of ethyl acetate. Two microliters of the solution was automatically injected into the GC–MS for determination of DMAP. Another aliquot of ether extract was also evaporated to dryness under a nitrogen stream at 40°C after the addition of 50 µl of MeOH–5 M HCl (20:1) to prevent loss of amines. The residue was dissolved in 200 µl of TFAA–ethyl acetate (1:1, v/v) and heated at 60°C for 20 min. The reaction solution was concentrated under a nitrogen stream at room temperature, the residue redissolved in 50 µl of ethyl acetate and 2 µl applied to GC–MS for determination of MA and AP as their TFA derivatives. The aqueous phase remaining after the first diethyl ether extraction in the above procedure was neutralized with HCl, adjusted to pH 9 with sodium carbonate and then extracted three times with chloroform–isopropanol (3:1, v/v). The combined organic layer was evaporated to dryness under a nitrogen stream at 30°C and reacted with TFAA as described above for the conversion of DMAP N-oxide into MA-TFA. The reaction solution was concentrated under a nitrogen stream at room temperature, the residue redissolved in 50 µl of ethyl acetate, and 2 µl applied to GC–MS for determination of DMAP N-oxide as MA-TFA.

2.4.3. Plasma

Ten microliters of DMAP N-oxide-d6 solution was evaporated to dryness in an analytical glass tube and

100 µl of plasma sample added. To this, 20 µl of 5 M NaOH and 100 µl of the I.S. aqueous mixed solution were added, and the sample extracted and analyzed as described above.

2.4.4. Urine

Ten microliters of DMAP N-oxide-d6 solution was evaporated to dryness in an analytical glass tube and 50–100 µl of urine sample added. To this, 20 µl of 5 M NaOH and 100 µl of the I.S. aqueous mixed solution were added, and the sample extracted and analyzed as described above.

2.5. Calibration curves

The drug concentrations in the biological samples were calculated using the peak-area ratios of the ions monitored for the target drugs versus their deuterated compounds. The calibration curves for determination of concentrations were constructed by analyzing extracted and derivatized drug-free control samples spiked with the standard solution as described above. Calibration samples containing 0.1, 1.0, 5.0, 10.0, 50.0, 100.0 µg ml^{-1} of DMAP, DMAP N-oxide, MA and AP for rat urine and plasma samples, or 0.1, 0.5, 1.0, 5.0, 10.0, 20.0 ng mg^{-1} (except DMAP; 0.5, 1.0, 5.0, 10.0, 20.0 ng mg^{-1}) for rat hair samples were prepared just before analysis. The limit of quantitation of each drug was chosen to be the concentration of the lowest calibration standard with an acceptable limit of variance.

3. Results and discussion

3.1. Quantitative analysis of DMAP N-oxide using GC–MS

Fig. 2(A) shows the GC–MS total ion chromatogram obtained after injection of DMAP N-oxide. Four peaks of degradation products formed during GC analysis appeared. Two of them at retention times of 4.32 and 4.80 min were attributed to MA and DMAP by comparing their retention times and mass spectra with those of the standard compounds, respectively (Fig. 2(B) and (C)). The other two peaks could not be identified. It has been reported that clozapine N-oxide, a metabolite of the antipsychotic agent clozapine, also significantly thermally
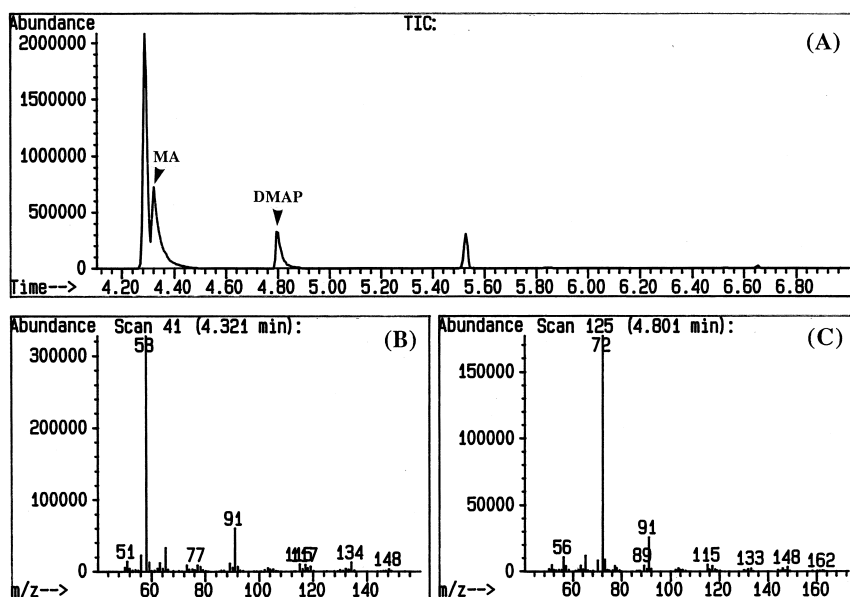
Fig. 2. GC–MS total ion chromatogram obtained after an injection of DMAP N-oxide (A). Spectra (B) and (C) correspond to the degradation products appearing at 4.32 and 4.80 min, respectively.

degrades to its parent drug during GC analysis [21]. From these results, we concluded that it was necessary to convert DMAP N-oxide into a stable compound for GC–MS analysis.

Although GC–MS analysis of DMAP N-oxide after reduction to its parent compound, DMAP, with titanium (III) chloride has been reported previously [8–10], this method required additional extraction steps after the reduction and it was not satisfactorily sensitive because DMAP was not derivatized with the reagents commonly used for AP analogs. In this paper, by considering the rearrangement reaction of a tertiary amine with trifluoroacetic anhydride, in which one of the alkyl groups attached to the nitrogen is cleaved and corresponding N,N-disubstituted trifluoroacetamide was formed (Potier-Polonovski reaction) [22], DMAP N-oxide was analyzed after conversion into TFA-MA by reaction with TFAA. The standard methanol solutions containing 0.01, 0.1, 0.5, 1.0, 5.0 and 10.0 µg of DMAP N-oxide and 0.1 µg of DMAP N-oxide-d6 were taken, evaporated to dryness under a nitrogen stream, treated with TFAA, and analyzed with GC–MS as described above. The calibration curve of DMAP N-oxide between the measured peak area ratios (y) (analyte to the internal standard) and the spiked concentrations (x) for standard solutions was linear over the concentration range used ($y = 0.0283 + 0.0123x$) and gave good correlation coefficients of $r^2 = 0.999$. The coefficients of variation for triplicate analysis were 1.24 and 0.57% at 1.0 and 50.0 µg ml^{-1}, respectively. These results suggest that this analytical procedure is useful for the measurement of DMAP N-oxide using GC–MS.

3.2. Separation of DMAP N-oxide from DMAP, MA and AP

As mentioned above, DMAP N-oxide partially degraded to DMAP and MA during GC–MS analysis. In addition, while MA was measured as TFA-derivative, the N-oxide was also measured as MA-TFA produced by reaction with TFAA in our method. Therefore, it is important to avoid conditions which co-extract DMAP N-oxide in the sample preparation for DMAP and its metabolites so as to assure no contribution of artifactual products from the N-oxide in the detection of the other compounds. The conversion of an amine to its N-oxide leads to a remarkable increase in polarity and a drop in the pKa value, If extraction of a biological sample is carried out with diethyl ether under strong

alkali condition, the basic drugs (including DMAP, MA and AP) will favor the ether phase while the non basic DMAP *N*-oxide will remain in the aqueous phase. Separation of DMAP *N*-oxide from the other drugs at this stage, thus, eliminates any doubt as to the origin of any DMAP *N*-oxide. For confirmation of the satisfactory separation of DMAP *N*-oxide from the other compounds by the liquid–liquid extraction method, their deuterated compounds were used as internal standards.The internal standard compounds of DMAP, MA and AP were substituted with four deuterium atoms at the 2,3,3,3 positions of their phenylpropane structures (Fig. 3). The DMAP-d4, TFA-MA-d4 and TFA-AP-d4 gave base peak ions of m/z 76, 158 and 144 in GC–MS analysis, respectively. On the other hand, DMAP *N*-oxide-d6 had six deuterium atoms at its *N*-dimethyl group. DMAP-d6, its thermal degradation product, gave a base peak ion of m/z 78, and TFA-MA-d3, its reaction product

with TFAA, gave that of m/z 157 (Fig. 3). Therefore, it is possible to differentiate between unchanged DMAP and the degraded product of DMAP *N*-oxide, or MA derivatized with TFAA and TFA-MA formed from DMAP *N*-oxide by reaction with TFAA by ascertaining the base ion peaks of their deuterated compounds used as I.S. on each GC–MS-SIM chromatogram.

Ten microliters of methanol solutions of DMAP *N*-oxide (10 μg ml^{-1}) and DMAP *N*-oxide-d6 (10 μg ml^{-1}) were taken and evaporated to dryness in an analytical glass tube, and 20 mg of control hair sample was added. To this, 1 ml of 1 *M* NaOH, 100 μl of the I.S. mixed aqueous solution and standard drug mixed aqueous solution (DMAP, MA, and AP, each 1 μg ml^{-1}) were added, and then hair sample was completely digested, extracted and analyzed using GC–MS-SIM as described above. The GC–MS-SIM chromatograms of the extracts from this
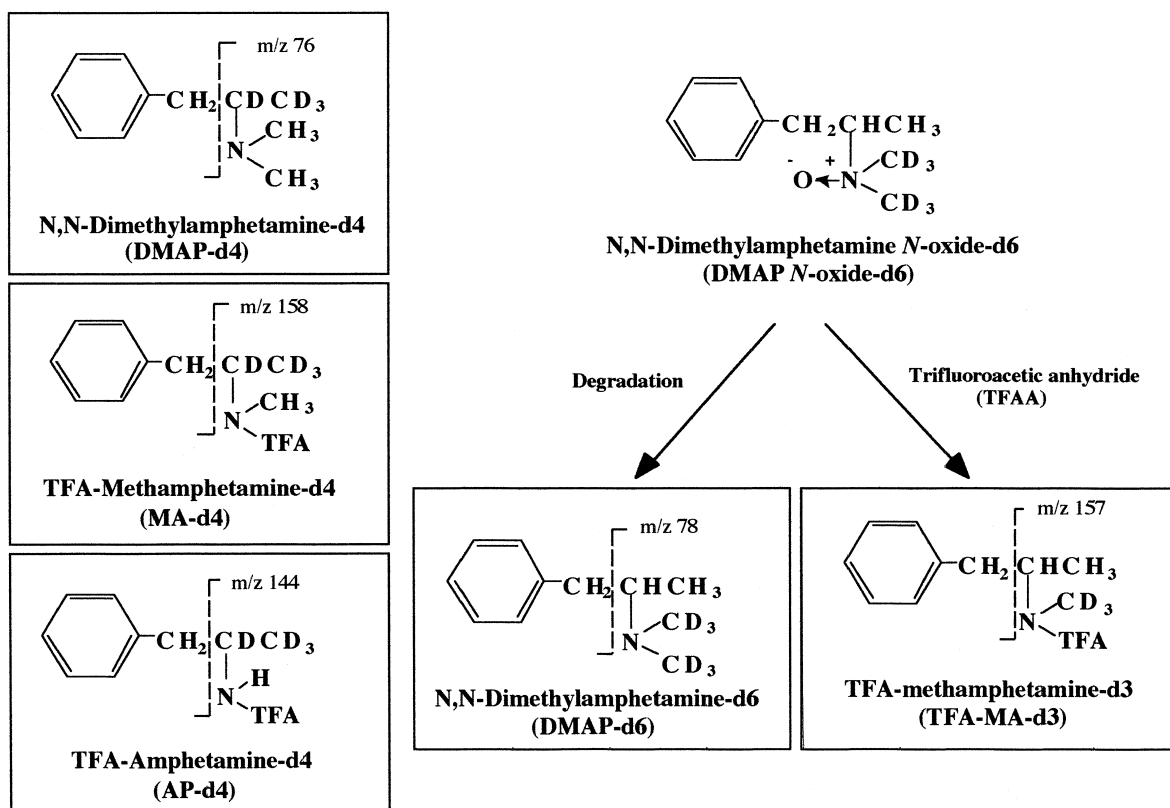

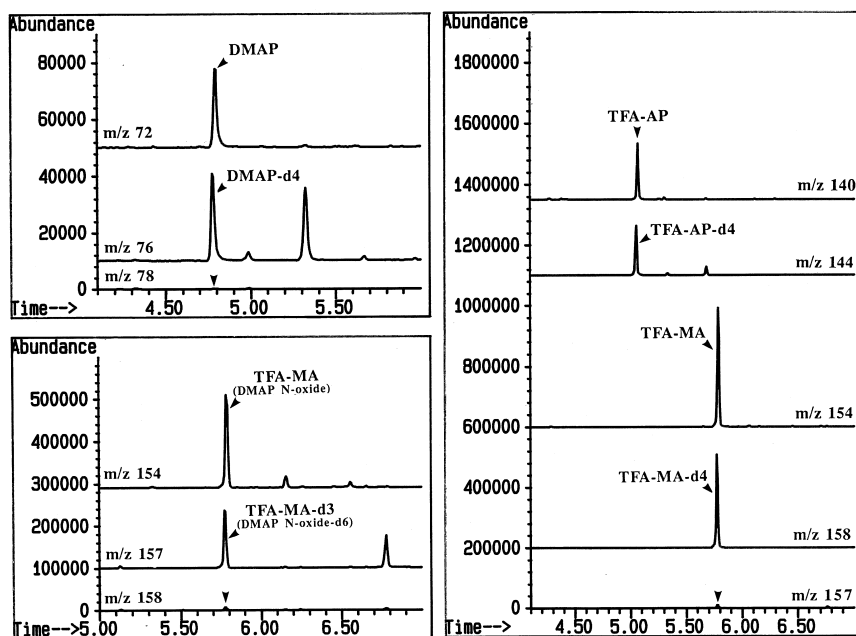Fig. 3. Deuterated I.S. compounds used in this study.

Fig. 4. GC–MS-SIM chromatograms of the extract from drug-free control rat hair spiked with standard solutions (each 5 ng mg^{-1}) of DMAP, DMAP N-oxide, MA and AP, and their deuterated I.S. solutions (each 5ng mg^{-1}).

control sample are shown in Fig. 4. Neither the base ion peak of DMAP-d6 (m/z 78) nor that of TFA-MA-d3 (m/z 157), derived from DMAP N-oxide-d6, were found in the chromatograms of the fractions for DMAP, or MA and AP. Moreover, the base ion peak of TFA-MA-d4 (m/z 158) was not found in the chromatogram of the fraction for DMAP N-oxide. These results suggest that DMAP N-oxide is not contaminated with the fractions for DMAP, MA and AP after the extraction. It was thus concluded that the concentrations of DMAP, DMAP N-oxide, MA and AP in biological samples could be estimated without overestimation of DMAP and MA due to artificial contribution from DMAP N-oxide by the proposed method.

3.3. Selectivity, linearity and precision of the method

Under the chromatographic conditions used for the experiments, there were almost no interference to the target compounds or their I.S.s from any extractable endogenous materials in the control rat urine, plasma or hair samples. The calibration curves for these compounds were linear over the concentration range 0.1–100 μg ml^{-1} for rat urine and plasma, and from 0.1 to 20.0 ng mg^{-1} (except DMAP; 0.5–20.0 ng mg^{-1}) for rat hair samples with good correlation coefficients of $r^2 \geq 0.998$. The intra- and inter-day precision and accuracy data of the analytical procedure for rat hair sample spiked with standard solution of DMAP, DMAP N-oxide, MA and AP are presented in Table 1. The precision and accuracy were evaluated by coefficient of variation and relative error, respectively. The intra-day precision and accuracy were estimated from three series standards analyzed within the same day, and the inter-day values were from the triplicate analysis of three samples on three separate days. The precision of these drugs ranged from 0.3 to 10.4% for intra-day, and 0.3 to 12.6% for inter-day, respectively. These accuracy ranged from −5.8 to 17.0% and −10.0 to 18.0%, respectively. The precision and accuracy values were not more than 13 and 18%, respectively, at concentrations of 0.5 μg mg^{-1} for DMAP and 0.1 ng mg^{-1} for DMAP N-oxide, MA and AP. At all other concentrations up to 20.0 ng mg^{-1}, these values were always less than 10%. From these

Table 1
Accuracy and precision study of DMAP and its metabolites in rat hair samples spiked with standard solution

	Concentration (ng mg^{-1} hair)	Intra-day ($n=3$)		Inter-day ($n=9$)	
		Precision (%)[a]	Accuracy (%)[b]	Precision (%)[a]	Accuracy (%)[b]
DMAP	0.5	10.4	17.0	12.6	18.0
	5.0	4.4	−4.5	6.2	−10.0
	10.0	3.0	1.1	3.6	−9.5
DMAP N-oxide	0.1	6.9	−5.8	10.3	−8.6
	0.5	2.1	−1.5	1.6	−4.0
	5.0	1.4	0.9	1.9	0.8
	10.0	0.3	−0.7	0.3	0.5
MA	0.1	5.5	4.8	8.5	8.3
	0.5	1.9	3.0	7.4	5.4
	5.0	1.7	4.2	1.1	8.1
	10.0	1.7	1.7	1.4	1.9
AP	0.1	9.6	10.0	11.4	10.2
	0.5	4.1	1.4	3.2	7.3
	5.0	3.0	−0.7	2.8	6.3
	10.0	3.0	0.1	0.7	4.7

[a] Assessed by expressing the standard deviation of the measurement as a percentage of the mean value.
[b] Defined as: [(measured conc.−target conc.)/target conc.]×100 (%).

results, it is suggested that the method was reliable within that range, and 0.5 ng mg^{-1} for DMAP and 0.1 ng mg^{-1} for the other drugs were defined to be the lower quantitation limits.

3.4. Time course of excretion of DMAP and its metabolites into rat urine

After intraperitoneal administration of DMAP HCl to 3 rats at 10 mg kg^{-1}, the concentrations of DMAP and its metabolites in rat urine were monitored using GC–MS-SIM. The time course of excretion of DMAP, DMAP N-oxide, MA and AP in the urine over 96 h are shown in Fig. 5. The major metabolite excreted in the rat urine was DMAP N-oxide, accounting for about 21% of the dose in the five days after administration. Maximum excretion occurred within the first 2 h in the urine (114.0±23.5 μg). Although unchanged DMAP was detected in all urine samples collected up to 96 h after administration, its amount was relatively low. The N-demethylated compounds, MA and AP, were minor metabolites of DMAP in the rat urine and accounted for about 6.0% of the dose. However, the amount of MA and AP was higher than that of DMAP in the urine samples collected from 6 h to 96 h after administration.

Inoue and Suzuki similarly reported [10] that DMAP N-oxide was the major metabolite (30% of the dose) in urine after oral administration of DMAP HCl to Wister rats at 20 mg kg^{-1}, and with the minor metabolic routes of demethylation accounting for 13% of the dose and aromatic hydroxylation 9% of the dose.
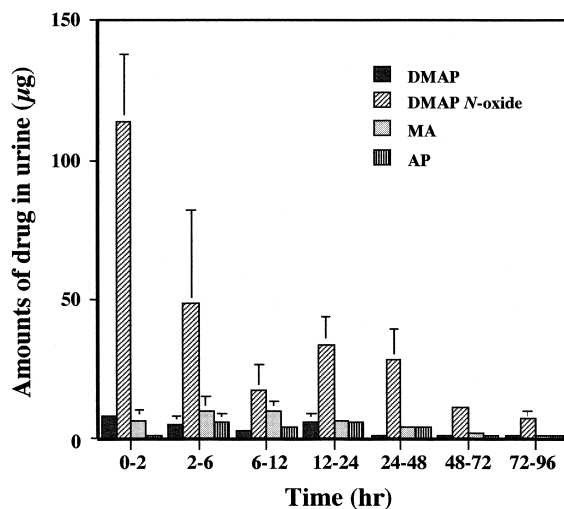

Fig. 5. Time course of excretion of DMAP and its metabolites into urine of rats after administration of DMAP HCl ($n=3$, 10 mg kg^{-1}, i.p.).
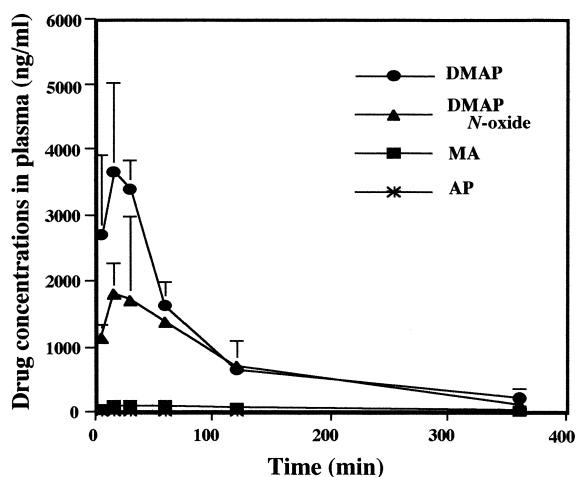
Fig. 6. Time course of rat plasma concentrations of DMAP and its metabolites after the first administration of DMAP HCl ($n=3$, 5 mg kg^{-1}, i.p.).

3.5. Concentrations of DMAP and its metabolites in rat plasma

Fig. 6 shows the time course of the rat plasma concentrations of DMAP and its metabolites over 360 min after administration of DMAP HCl at 5 mg kg^{-1} ($n=3$). The DMAP concentration in the plasma showed a peak (3670 ± 1160 ng ml^{-1}) within 15 min. The plasma half-life for DMAP was 150 min, and DMAP could hardly be detected in the plasma at 360 min after administration. The major metabolite, DMAP N-oxide, also had a maximum (1800 ± 450 ng ml^{-1}) at 15 min. The concentrations of MA a rid AP were extremely low and their peak concentrations were 96.7 ± 20.3 ng mg^{-1} at 30 min and 25.7 ± 5.2 ng ml^{-1} at 60 min, respectively. The AUC values of DMAP, DMAP N-oxide, MA and AP in the rat plasma were 397.2 ± 97.5, 279.7 ± 68.3, 18.4 ± 1.2 and 15.9 ± 2.2 μg min ml^{-1}, respectively, as shown in Table 2. The AUC values of DMAP and

DMAP N-oxide were more than ten times larger than those of MA and AP.

3.6. Drug concentrations in rat hair and incorporation rates of drugs into rat hair

GC–MS-SIM chromatograms of the extracts from the hair of the rats administered with DMAP are shown in Fig. 7. DMAP, DMAP N-oxide, MA and AP were detected in the rat hair at concentrations of 4.82 ± 0.67, 0.45 ± 0.09, 3.25 ± 0.36 and 0.89 ± 0.05 ng mg^{-1}, respectively (Table 2), showing that the concentrations of DMAP N-oxide in the hair were more than ten times lower than that of DMAP in spite of its larger AUC value.

We have proposed [13,14] that the ratio of drug concentration in hair to AUC value in plasma ([Hair]/[AUC]) could be used as an index of the incorporation tendency of drug into hair from plasma. With this approach, the [Hair]/[AUC]s of DMAP, DMAP N-oxide, MA and AP were calculated to be 0.012, 0.002, 0.177 and 0.056, respectively. Thus the [Hair]/[AUC] was in the order, MA > AP > DMAP ≫ DMAP N-oxide, which indicated that the incorporation tendency of DMAP N-oxide was distinctly low compared with the others. In our previous study [13], we reported that the extent of incorporation of basic and lipophilic drugs such as cocaine into rat pigmented hair after drug administration is generally greater than that of neutral, acidic or polar drugs. It has also been reported [14], using more than 30 amphetamine analogs, that structural factors relating to lipophilicity and basicity greatly affect the drug incorporation into pigmented hair from blood. It was demonstrated that the [Hair]/[AUC] of MA derivatives containing N-formyl and N-acetyl groups (N-formyl MA and N-acetyl MA) were nearly zero, indicating that the concentrations

Table 2
Comparison between AUCs in rat plasma and drug concentrations in rat hair after DMAP intraperitoneal administration at 5 mg kg^{-1} ($n=3$)

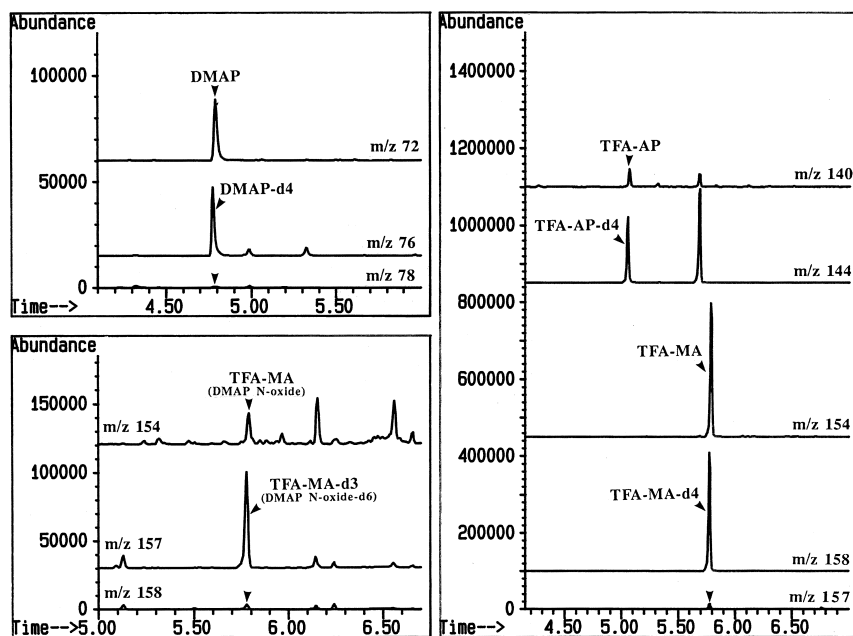|	DMAP	DMAP N-oxide	MA	AP
Plasma AUC∞ (μg·min ml^{-1})	397.2 ± 97.5	279.7 ± 68.3	18.4 ± 1.2	15.9 ± 2.2
Hair concentrations (ng mg^{-1})	4.82 ± 0.67	0.45 ± 0.09	3.25 ± 0.36	0.89 ± 0.05

Fig. 7. GC–MS-SIM chromatograms of the extract from the rat hair collected 4 weeks after the first administration of DMAP HCl ($n=3$, 5 mg kg^{-1}×10 days, i.p.).

in hair were very low in spite of their relatively high AUCs. This is thought to be due to the fact that the basicity of the drugs was lost by the N-acylation. For the same reason, it is expected that the [Hair]/[AUC] of DMAP N-oxide is extremely low because of loss of its basicity. In addition, the concentration of DMAP in the rat hair was in the same level as that of MA, although the rat plasma AUC of DMAP was more than 10 times larger than that of MA in this study. We have also described [14] that the [Hair]/[AUC] decreased in the order of secondary amine> primary amine>tertiary amine, presumalby related to their lipophilicity and pKa values. The [Hair]/[AUC] of DMAP could be lower than those of MA and AP in the same manner. It is generally accepted that the ability of melanin contained in hair to accumulate and retain drugs is remarkable [23,24]. Melanin is considered to be composed of polymers of indole quinone units capable of interaction with basic drugs that have diffused into the hair matrix. Furthermore, it is said that the isoelectric point of hair is about pH 4 [25], and the pH gradient that exists between blood at pH 7.4 and hair matrix could

facilitate diffusion of basic drugs across the membrane between the hair root and blood [15].

In summary, we have successfully developed an analytical method for DMAP and its metabolites in biological samples, including hair, using GC–MS-SIM, which is sensitive, specific and reproducible. In particular, the use of deuterated compounds with different numbers of deuterium atoms as internal standards made it possible to assure no contribution of artifactual products from DMAP N-oxide in the detection of the other compounds during GC–MS analysis. DMAP, DMAP N-oxide, MA and AP were detected in newly grown rat hair 28 days following i.p. administration of DMAP HCl (5 mg kg^{-1}×10), and the concentration of DMAP N-oxide in the hair was approximately one tenth of those of DMAP and MA despite the drug concentrations in the plasma and urine being relatively high, compared with the others. From these results, it was thought that the incorporation tendency of DMAP N-oxide from plasma into hair was distinctly low.

This method could be applied to the case of retrospective distinction between DMAP use and

MA use by the detection of DMAP in hair in the field of forensic toxicology.

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