

## Hair Analysis for Drugs of Abuse. XI. Disposition of Benzphetamine and Its Metabolites into Hair and Comparison of Benzphetamine Use and Methamphetamine Use by Hair Analysis

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Received May 12, 1995; accepted August 25, 1995

In order to study the disposition of benzphetamine (BZP) and its metabolites, desmethyl benzphetamine (norBZP), *p*-hydroxy desmethyl benzphetamine (OHnorBZP), methamphetamine (MA) and amphetamine (AP), from plasma to hair in rats, an analytical method for identifying these drugs in plasma, urine and hair was developed with selected ion monitoring of gas chromatograph/mass spectrometry (GC/MS-SIM) results. After the intraperitoneal administration of BZP to rats (10 mg/kg/d, 10 d,  $n=3$ ), concentrations of BZP and its metabolites in rat hair newly grown for 4 weeks were compared to the areas under the concentration *versus* time curve (*AUCs*) of these drugs in the rat plasma.

The concentrations of BZP, norBZP, OHnorBZP, MA and AP in the rat hair were  $14.8 \pm 1.4$ ,  $6.1 \pm 0.3$ ,  $2.6 \pm 0.5$ ,  $2.3 \pm 0.1$  and  $9.2 \pm 0.3$  ng/mg, and the ratio of the concentrations in the hair to *AUCs* in the rat plasma was 3.0:0.1:0.1:0.6:0.2, respectively. This fact suggested that BZP tends to be readily incorporated into hair from blood.

The method was applied to the determination of the metabolites in scalp hair and pubic hair of humans who orally ingested BZP (30 mg/d, 5 d,  $n=2$ ). BZP, norBZP, MA and AP were detected at 0.14–0.56, 0.29–0.63, 0.10 and 1.06–1.66 ng/mg in the scalp hair and at 0.10–0.20, 0.13–0.18, trace–0.15 and 0.23 ng/mg in the pubic hair, respectively. It was shown that BZP use could be retrospectively distinguished from MA use by the detection of BZP and/or norBZP in hair.

**Key words** hair analysis; drug abuse; benzphetamine; methamphetamine; GC/MS; forensic toxicology

Benzphetamine (BZP, 1-phenyl-2-(*N*-methyl-*N*-benzylamino)propane; Didrex), a benzyl analog of methamphetamine (MA), is medically used as an anorexic which acts on noradrenergic and possibly dopaminergic receptors. BZP is extensively metabolized in animals and man to MA and amphetamine (AP) with little, if any, excreted as unchanged drug.<sup>1)</sup> Therefore, it is not easy to distinguish illegal MA use from legitimate BZP use by routine urinary analysis. In court, this has caused problems and an appropriate analytical method other than urinalysis should have been ready for discrimination between legal and illegal use.

It has been demonstrated<sup>2)</sup> that hair sample has many advantages as a useful diagnostic tool for the confirmation of past drug use and the monitoring of a long drug use history, whereas urine sample provides only short-term information of an individual's drug use. In recent years, hair analysis has been used as scientific evidence in court. Accordingly, in order to discriminate between illegal MA use and BZP use by hair analysis, the disposition of BZP and its metabolites into hair must be clarified.

In our previous reports, we have demonstrated that parent compounds such as MA,<sup>3)</sup> cocaine<sup>4)</sup> and 6-acetylmorphine<sup>5)</sup> are more rapidly and readily incorporated into hair than their polar metabolites. Thus, it is expected that unchanged parent drug which is not found in urine might be detected in hair.

In this study, a method for determining the presence of BZP and its metabolites in plasma, urine and hair has been developed, and the disposition of these drugs into hair from blood has been investigated by comparison between concentrations in rat hair and areas under the concentration *versus* time curve (*AUCs*) in rat plasma

following the administration of BZP. Our analytical method was applied to the determination of BZP and its metabolites in the scalp and pubic hair of humans who orally ingested BZP in order to estimate the possibility of discrimination between BZP use and MA abuse by hair analysis, and these results were compared with urinalysis for practical use.

### MATERIALS AND METHODS

**Materials** BZP-HCl was obtained from Upjohn Company (Kalamazoo, MI, U.S.A.). MA-HCl was purchased from Dainippon Pharmaceutical Co. (Osaka, Japan). AP sulfate was prepared as previously reported.<sup>6)</sup> Desmethyl BZP (norBZP) and *p*-methoxy desmethyl BZP (*p*-methoxy norBZP) were prepared from AP and *p*-methoxy AP using benzaldehyde and sodium boron hydride by a modified Boissier and Ratouis method.<sup>7)</sup> *p*-Hydroxy desmethyl BZP (OHnorBZP) was synthesized by treatment with the hydrobromic acid of *p*-methoxy norBZP.

Deuterated compounds, MA-*d*<sub>4</sub> and AP-*d*<sub>4</sub>, were synthesized by previously described methods.<sup>3)</sup> BZP-*d*<sub>5</sub> was synthesized from MA and benzyl chloride-*d*<sub>5</sub> with sodium carbonate using the Heinzelman and Aspergren method.<sup>8)</sup> The structures of BZP, its metabolites and internal standards are shown in Fig. 1.

Trifluoroacetic anhydride (TFAA) was purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and Bond Elut Certify from Varian sample preparation products (Harbor City, CA, U.S.A.).

**Apparatus** Gas chromatograph-mass spectrometry (GC/MS) analysis was performed using a Hewlett Packard

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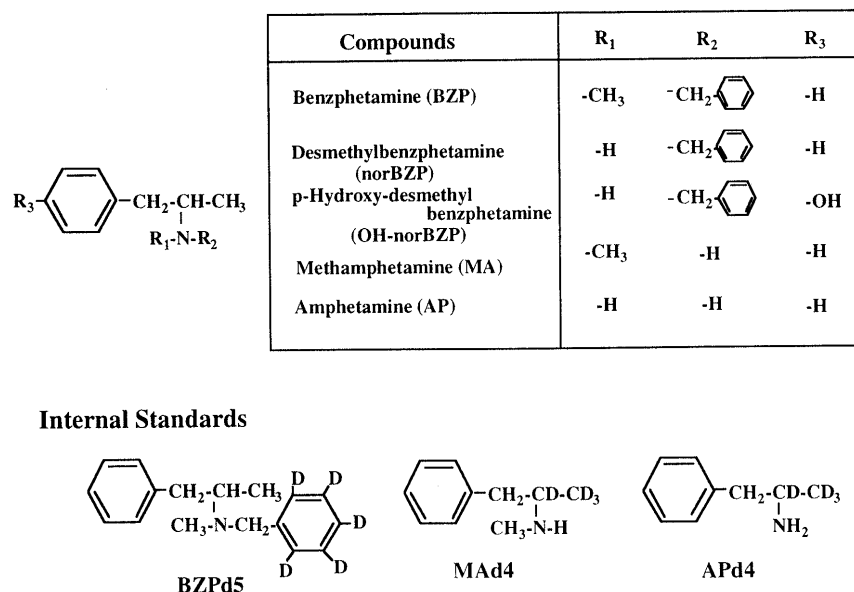


Fig. 1. Structures of BZP and Its Metabolites Investigated in This Study

5890 series-II gas chromatograph equipped with a 7673A autosampler and MSD 5971. The gas chromatography (GC) was carried out with a 20 m × 0.25 mm i.d., 0.25 μm cross-linked methylsilicone fused silica column TC-1 (GL Sciences Inc., Tokyo, Japan). The injection port temperature was 200 °C (splitless mode) and helium was the carrier gas (5.5 psi head pressure). The oven temperature was programmed to increase from 60 °C (held for 0.5 min) to 280 °C at a rate of 20 °C/min. Drugs in biological specimens were investigated by monitoring the selected ions as follows: *m/z* 148, 91 for BZP, *m/z* 230, 118, 91 for trifluoroacetyl (TFA)-norBZP, *m/z* 230, 203 for TFA-OHnorBZP, *m/z* 154, 118, 110 for TFA-MA, *m/z* 140, 118 for TFA-AP, and *m/z* 153, 158 and 144 for BZP-*d*<sub>5</sub>, TFA-MA-*d*<sub>4</sub> and TFA-AP-*d*<sub>4</sub>, respectively.

**Animal Experiments** Before BZP was administered, the hair on the back of rats was shaved with an animal electric shaver (Daitoh Electric Machine Co., Tokyo, Japan). Male dark agouti (DA) pigmented rats, aged 5 weeks (100–120 g), were intraperitoneally administered once a day with BZP-HCl (10 mg/kg) for 10 successive days (*n*=3). The newly grown back hair was collected by the electric shaver 28 d after the first administration. Blood was collected at 2, 5, 15, 45, 120 and 420 min after injection with a capillary glass tube from the orbital vein plexus following the first administration. The blood was collected into a plastic tube containing heparin cooled in ice. The plasma samples were obtained by centrifugation at 10000 × *g* for 3 min and stored at –20 °C until analysis. AUCs were calculated by the trapezoidal rule over the time of measurement with the remainder of the curve estimated as  $\beta \cdot C_{\text{last}}$  where  $\beta$  is the terminal rate constant and  $C_{\text{last}}$  is the concentration of the last observed time point.

**Human Experiments** One healthy male (subject YN, 49 years) and one female (subject RK, 28 years) ingested 30 mg of BZP-HCl orally once a day for 5 successive days. Urine samples were collected at 0, 1, 3, 6, 9, 12, 24, 48 and 72 h after the last dosage. Scalp hair from the posterior vertex region and pubic hair were both cut near the skin

3 weeks (21 d) after the first dosage. A section 1 cm from the root side of the hair was used for analytical experiments.

**Analytical Methods** Plasma: To 200 μl of plasma of DA rat were added 100 μl of internal standard (IS) aqueous solution containing MA-*d*<sub>4</sub>, AP-*d*<sub>4</sub> and BZP-*d*<sub>5</sub> at 1 μg/ml each and 500 μl of 0.1 M potassium hydrogen phosphate buffer (pH 6.0). After a Bond Elut Certify was pre-activated with 2 ml of MeOH and 2 ml of 0.1 M potassium hydrogen phosphate buffer (pH 6.0), the test solution was applied to the Bond Elut Certify and the column was washed with 1 ml of water, 1 ml of 0.1 M acetic acid and 1 ml of water, successively. The column was dried under a vacuum for 5 min. After the column was rinsed with 1 ml of methanol and dried under a vacuum for 2 min, 3 ml of MeOH–5 M HCl (20:1) was passed through the column to elute the target drugs. Following the evaporation of the solvent under a nitrogen stream, the residue was dissolved in 200 μl of TFAA–ethylacetate (1:1) and heated at 60 °C for 20 min. The reaction solution was evaporated with a nitrogen stream and the residue was redissolved in 50 μl of ethylacetate. Two μl of the ethylacetate solution was automatically injected into the GC/MS.

Urine: To human urine samples (0.1–3 ml) were added 100 μl of IS aqueous solution containing MA-*d*<sub>4</sub>, AP-*d*<sub>4</sub> and BZP-*d*<sub>5</sub> at 1 μg/ml each and 2 ml of 0.1 M phosphate buffer (pH 6.0). The solution was treated with Bond Elut Certify, derivatized and analyzed as above.

Hair: Rat hair samples (15 mg), human scalp hair samples (1.6, 2.1 mg), and pubic hair samples (6.0, 4.0 mg) were washed three times with 0.1% sodium dodecyl sulfate (SDS) and water under ultrasonication. After the samples were dried under a nitrogen stream and precisely weighed, they were extracted with 2 ml of MeOH–5 N HCl (20:1) containing each IS as 100 ng of MA-*d*<sub>4</sub>, AP-*d*<sub>4</sub> and BZP-*d*<sub>5</sub> for rat hair samples and 5 ng of MA-*d*<sub>4</sub>, AP-*d*<sub>4</sub> and BZP-*d*<sub>5</sub> for human hair samples for 1 h under ultrasonication, and then the solution was left to stand at room temperature overnight. The hair was filtered off, the

filtrate was evaporated with a nitrogen stream and the residue was dissolved in 1 ml of 0.1 M phosphate buffer (pH 6.0). The solution was treated with Bond Elut Certify, derivatized and analyzed as above.

## RESULTS AND DISCUSSION

**Simultaneous Determination of BZP and Its Metabolites in Biological Specimens** The simultaneous determination of BZP, norBZP, OHnorBZP, MA and AP in plasma, urine and hair was studied with the solid phase extraction method and selected ion monitoring of GC/MS (GC/MS-SIM) using a stable isotope dilution method.

The calibration curves for the determination of BZP, norBZP, OHnorBZP, MA and AP were constructed by analysis of the control specimens spiked with the standard solutions of these drugs and the deuterated internal standards. The calibration curves for their drugs were linear over a concentration range of 10–1000 ng/ml in rat plasma or human urine and 0.1–25 ng/mg in hair, with correlation coefficients of  $r=0.999$ . The coefficients of variation of analysis of human control scalp hair spiked with the standard solution of these drugs ( $n=3$ ) were in the range 2.9–7.1% at 0.5 ng/mg and 3.9–8.9% at 0.1 ng/mg. Values less than 10 ng/ml in rat plasma or human urine and 0.1 ng/mg in hair were not used.

**Concentrations of BZP, norBZP, OHnorBZP, MA and AP in Rat Plasma** After the intraperitoneal administration of BZP-HCl to rats at 10 mg/kg, changes in drug concentration in the rat plasma over 420 min were

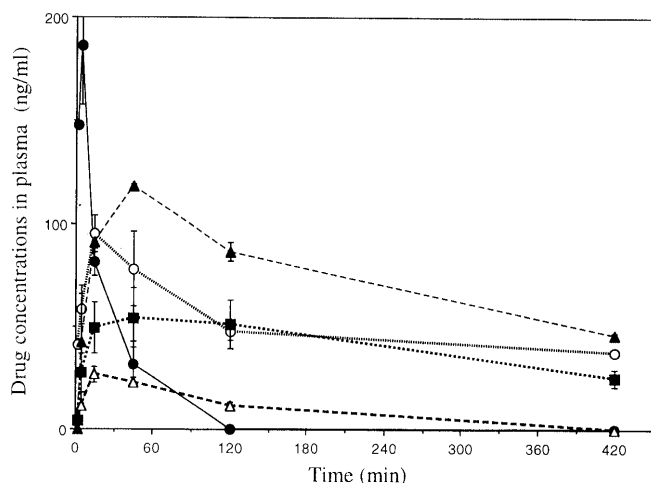


Fig. 2. Time Courses of Rat Plasma Concentrations of BZP, norBZP, OHnorBZP, MA and AP after Administration of BZP-HCl (i.p., 10 mg/kg,  $n=3$ )

●, BZP; ○, norBZP; ■, OHnorBZP; △, MA; ▲, AP.

monitored with GC/MS. The time courses of the rat plasma concentrations of BZP and its metabolites over 420 min are shown in Fig. 2. BZP rapidly disappeared from the plasma. The half-life of BZP was very short (6.5 min) and it could be detected in the plasma only until 60 min after administration. The average peak plasma concentrations of MA (15 min) and AP (45 min) were half as much as that of BZP (5 min), and the half-lives of these drugs were 46.7 and 306.7 min, respectively.

The AUCs of BZP, norBZP, OH norBZP, MA and AP in the rat plasma were  $4.9 \pm 1.0$ ,  $43.2 \pm 4.7$ ,  $27.9 \pm 5.4$ ,  $4.0 \pm 0.4$  and  $50.1 \pm 1.9 \mu\text{g} \cdot \text{min}/\text{ml}$ , and the ratio of their AUCs was approximately 1:8.8:5.7:0.8:10.2, respectively (Table 1).

**Drug Concentrations in Rat Hair and Drug Incorporation Rates into Hair** GC/MS-SIM chromatograms of extracts (TFA-derivatives) from control rat hair spiked with IS and the hair of the rats administered BZP are shown in Fig. 3. All five drugs were detected in the rat hair, and the concentrations of BZP, norBZP, OHnorBZP, MA and AP were  $14.8 \pm 1.4$ ,  $6.1 \pm 0.3$ ,  $2.6 \pm 0.5$ ,  $2.3 \pm 0.1$  and  $9.2 \pm 0.3 \text{ ng}/\text{mg}$ , respectively. The ratio of their concentrations in the hair, 1:0.4:0.2:0.2:0.6, shows that the concentration of BZP in the hair was 2–5 times higher than those of any other compounds.

We have proposed<sup>2b,4a,b,5)</sup> that the incorporation rate of a drug into hair from plasma is represented by the ratio of drug concentration in hair to AUC in plasma ( $[\text{hair}]/[\text{AUC}]$ ). According to our proposal, those of BZP, norBZP, OHnorBZP, MA and AP were 3.0, 0.1, 0.1, 0.6 and 0.2, respectively, as shown in Table 1.

In our previous reports,<sup>4,5,9)</sup> we have already found that there are obvious differences in the incorporation rates of parent drugs and their metabolites. In the congeners, the more hydrophilic metabolites have smaller incorporation rates into hair. In 20 drugs examined in our previous study, cocaine and phencyclidine have the highest incorporation rates (2–3), 6-acetylmorphine and MA have medium incorporation rates (0.1–1) and morphine, deprenyl and benzoylecgonine low rates ( $<0.1$ ). In the case of BZP, its  $[\text{hair}]/[\text{AUC}]$  was 3.0, which was 5–30 times greater than those of the other hydrophilic metabolites. It is concluded that BZP is a drug readily incorporated into hair, and that a hair sample is a good specimen for the confirmation of BZP use.

**Time Course of Excretion of BZP and Its Metabolites into Human Urine** After administrating oral doses of BZP (30 mg/d) to two human subjects for 5 d, the subjects' urine samples were collected at 0, 1, 3, 6, 9, 12, 24, 48 and 72 h

Table 1. Comparison between AUCs in Rat Plasma and Drug Concentrations in Rat Hair after Administration with BZP-HCl (i.p. 10 mg/kg  $\times$  10 d)

	BZP	norBZP	OHnorBZP	MA	AP
AUC ( $\infty$ ) in plasma ( $\mu\text{g} \cdot \text{min}/\text{ml}$ ) <sup>a)</sup>	$4.9 \pm 1.0$	$43.2 \pm 4.7$	$27.9 \pm 5.4$	$4.0 \pm 0.4$	$50.1 \pm 1.9$
Drug concentrations in hair (ng/mg) <sup>a)</sup>	$14.8 \pm 1.4$	$6.1 \pm 0.3$	$2.6 \pm 0.5$	$2.3 \pm 0.1$	$9.2 \pm 0.3$
Ratio in plasma ( $[\text{BZP}]=1.0$ )	1.0	8.8	5.7	0.8	10.2
Ratio in hair ( $[\text{BZP}]=1.0$ )	1.0	0.4	0.2	0.2	0.6
$[\text{hair}]/[\text{AUC}]$ <sup>b)</sup>	3.0	0.1	0.1	0.6	0.2

a) Mean data from three experiments  $\pm$  S.E.M. are presented. b)  $[\text{hair}]/[\text{AUC}]$  means the ratio of drug concentration in hair to AUC in plasma.

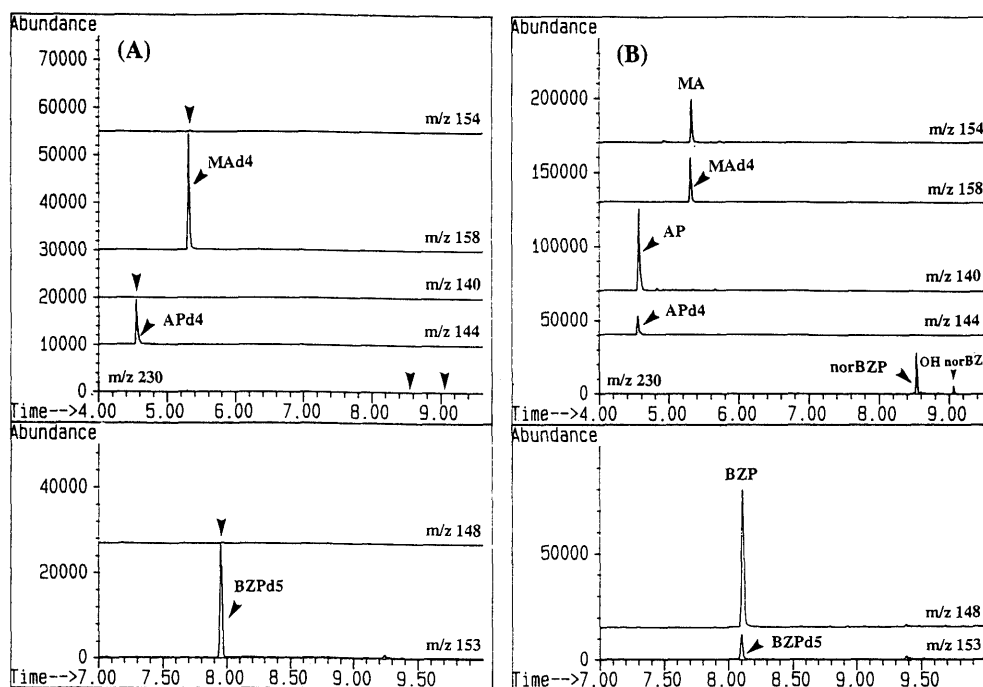


Fig. 3. GC/MS-SIM Chromatograms of TFA-Derivatized Extracts from (A) Control Rat Hair Spiked with IS Compounds and (B) the Rat Hair 4 Weeks after Administration of BZP-HCl (i.p., 10 mg/kg  $\times$  10 d)

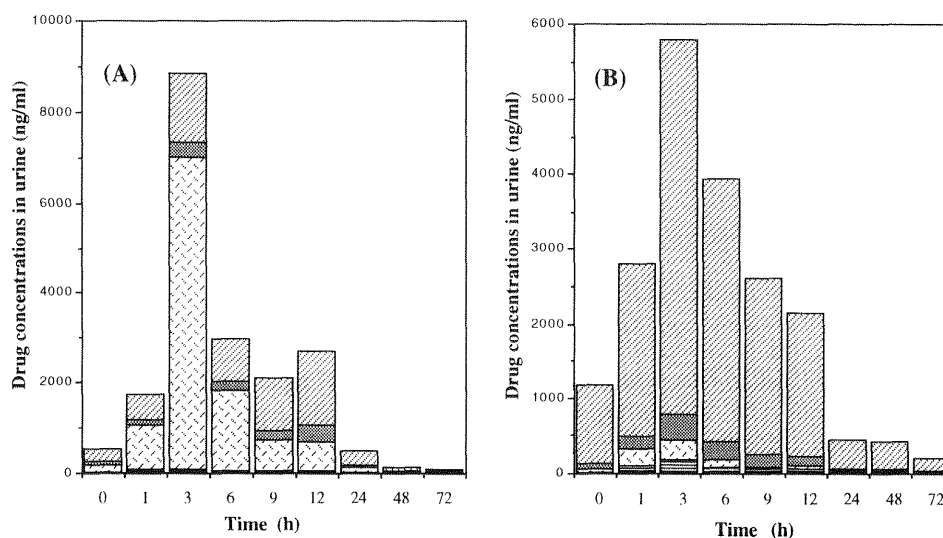


Fig. 4. Time Courses of Excretion of BZP, norBZP, OHnorBZP, MA and AP into the Urine of Two Subjects ((A), YN; (B), RK) after the Last Dosage of BZP-HCl (Oral, 30 mg)

■, BZP; □, norBZP; ▨, OHnorBZP; ▩, MA; ▤, AP.

after the last dosage, and the concentrations of drugs in the urine were determined (Fig.4).

OHnorBZP or AP were a major metabolites in the urine of both subjects. Especially, AP was detected until 72 h after the last dosage. However, BZP was not detected in any of the urine samples except for the 1 h urine of one subject. A small amount of norBZP was detected in the urine of both subjects until 12 h. In the urine of YN (A) and RK (B), the peak concentrations of these metabolites were 0.09 (A) and 0.18 (B)  $\mu$ g/ml at 3 h for norBZP, 6.91 (A) and 0.25 (B)  $\mu$ g/ml at 3 h for OHnorBZP, 1.65  $\mu$ g/ml (A) at 12 h and 5.01  $\mu$ g/ml (B) at 3 h for AP, and 0.33 (A) and 0.35 (B)  $\mu$ g/ml at 3 h for MA.

#### Detection of BZP and Its Metabolites from Human Scalp

**Hair and Pubic Hair after BZP Intake** Human subjects took BZP orally (30 mg/d) for 5 d, and their scalp hair and pubic hair samples were collected 3 weeks after the first dosage. Figure 5 shows the SIM chromatograms of the TFA-derivatized extract from the human control scalp hair spiked with IS and from the subject's scalp hair. MA (0.1 ng/mg), AP (1.06—1.66 ng/mg), norBZP (0.29—0.63 ng/mg) and BZP (0.14—0.56 ng/mg) were detected in both of the subjects' scalp hair samples. It was demonstrated that the detection of BZP and/or norBZP in scalp hair confirmed past BZP use. However, OH norBZP was not detected in the hair, despite being one of the major metabolites in human urine (Table 2).

It has been reported<sup>10)</sup> that the average ratio of the

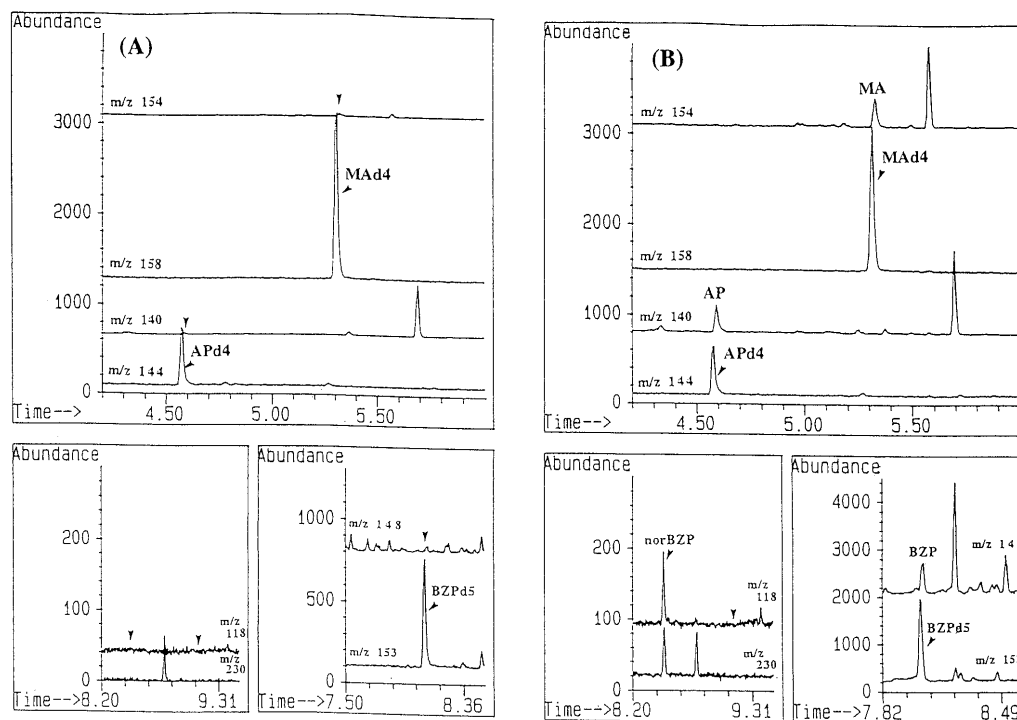


Fig. 5. GC/MS-SIM Chromatograms of TFA-Derivatized Extracts from (A) Human Control Scalp Hair Spiked with IS Compounds and (B) Subject YN's Scalp Hair 3 Weeks after the First Dosage of BZP-HCl (Oral, 30 mg  $\times$  5 d)

Table 2. Levels of BZP and Its Metabolites in Scalp Hair and Pubic Hair after Administration with BZP-HCl (Oral 30 mg  $\times$  5 d)

Hair	Subjects	Drug concentrations in hair (ng/mg)				
		BZP	norBZP	OH norBZP	MA	AP
Scalp hair	YN	0.56	0.63	ND <sup>a)</sup>	0.10	1.06
	RK	0.14	0.29	ND <sup>a)</sup>	0.10	1.66
Pubic hair	YN	0.20	0.18	ND <sup>a)</sup>	0.15	0.23
	RK	0.10	0.13	ND <sup>a)</sup>	TR <sup>b)</sup>	0.23

a) Not detected. b) Trace level.

concentrations of AP to MA ([AP]/[MA]) in scalp hair of 39 MA abusers is  $0.11 \pm 0.05$ . The ratio rarely exceeds 0.2, even though it increases according to the duration and frequency of drug use. In this study, the concentrations of AP in the scalp hair of both BZP users were 10 times as high as those of MA. These data suggested that [AP]/[MA] in scalp hair might be useful information for discrimination between BZP use and MA use.

Those drugs were also detected in the pubic hair, but the concentrations were lower than those in the scalp hair. The concentrations of BZP, norBZP, MA and AP in the pubic hair were 0.10–0.20, 0.13–0.18, trace–0.15 and 0.23 ng/mg, respectively (Table 2).

Since BZP and/or norBZP were detected in the pubic hair of BZP users, it can be said that pubic hair sample may also become a useful specimen for discrimination between BZP use and MA use when scalp hair sample is unavailable.

## CONCLUSION

In rat hair newly grown for 28 d following intra-peritoneal administrations of BZP-HCl (10 mg/kg  $\times$  10,  $n=3$ ), the concentrations of BZP, norBZP, OH norBZP,

MA and AP were 14.8, 6.1, 2.6, 2.3 and 9.2 ng/mg, and the ratio of the concentrations in the hair to AUCs in the plasma was 3.0:0.1:0.1:0.6:0.2, respectively. This fact suggested that BZP was a drug readily incorporated into hair, and thus a hair sample is a good specimen for the confirmation of BZP use.

Our analytical method was applied to the determination of the metabolites in the scalp hair and pubic hair of two human subjects who orally ingested BZP (30 mg/d, 5 d,  $n=2$ ). The parent compound and its metabolites were detected in scalp hair and pubic hair in the order of concentrations as follows: AP > BZP  $\approx$  norBZP > MA. However, OHnorBZP, which is one of the major metabolites in human urine, was not detected in both the hair samples.

It was shown here that BZP use could be retrospectively distinguished from MA use by the detection of BZP and/or norBZP in hair.

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