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Analysis of benzphetamine and its metabolites in rat urine by liquid chromatography–electrospray ionization mass spectrometry

Motoyasu Sato[a,*], Toshiyuki Mitsui[a], Hisamitsu Nagase[b]

[a]*Scientific Investigation Research Laboratory, Aichi Pref. Police H.Q., 2-1-1 Sannomaru, Naka-ku, Nagoya 460-8502, Japan*
[b]*Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502-8585, Japan*

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Abstract

An analytical method to identify and determine benzphetamine (BMA) and its five metabolites in urine was developed by liquid chromatography–electrospray ionization mass spectrometry (LC–ESI–MS) using the solid-phase extraction column Bond Elut SCX. Deuterium-labeled compounds, used as internal standards, were separated chromatographically from each corresponding unlabeled compound in the alkaline mobile phase with an alkaline-resistant ODS column. This method was applied to the identification and determination of BMA and its metabolites in rat urine collected after oral administration of BMA. Under the selected ion monitoring mode, the limit of quantitation (signal-to-noise ratio 10) for BMA, *N*-benzylamphetamine (BAM), *p*-hydroxybenzphetamine (*p*-HBMA), *p*-hydroxy-*N*-benzylamphetamine (*p*-HBAM), methamphetamine (MA) and amphetamine (AM) was 700 pg, 300 pg, 500 pg, 1.4 ng, 6 ng and 10 ng in 1 ml of urine, respectively. This analytical method for *p*-HBMA, structurally closer to the unchanged drug of all the metabolites, was very sensitive, making this a viable metabolite for discriminating the ingestion of BMA longer than the parent drug or other metabolites in rat. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Benzphetamine; Methamphetamine; Amphetamine

1. Introduction

Methamphetamine (MA) constitutes a large proportion of the drugs abused in Japan, and the abuse of MA has become a serious social problem. MA is detected in urine as a metabolite after the ingestion of other drugs [1–8]. Benzphetamine (BMA) is one of these drugs and is used as an anorectic drug in the U.S. and other countries, while in Japan its use is prohibited. In discriminating between MA and BMA use, it is important to analyze BMA or its metabolites, which are unique to BMA, with high sensitivity.

In previous reports [1,2], it was shown that MA and amphetamine (AM) are excreted in human urine after the administration of BMA. Niwaguchi and Inoue et al. reported several metabolites other than those excreted in rat and human urine [9,10]. In these papers, it was reported that the determination of *p*-hydroxy-*N*-benzylamphetamine (*p*-HBAM), a major metabolite, was most useful for the discrimination of BMA intake.

The analytical methods used in previous reports include fluorescence polarization immunoassay (FPIA) [11], gas chromatography (GC) [1,2], gas

*Corresponding author.

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chromatography–mass spectrometry (GC–MS) [9,10,12–14], high-performance liquid chromatography (HPLC) with ultraviolet detection [15,16] and micellar electrokinetic chromatography (MEKC) with a photodiode array [17]. FPIA, GC, HPLC and MEKC lack reliability for qualitative analysis, and GC and GC–MS require derivatization of the metabolites. In addition, the parent ions of BMA and its metabolites are not detected when using electron impact ionization (EI)-GC–MS and their EI mass spectra are very similar. Therefore, it is useful for more definite identification to confirm their quasi-molecular ions with chemical ionization (CI)-GC–MS.

Recently, liquid chromatography–mass spectrometry (LC–MS) has gained widespread recognition as a versatile technique in forensic toxicology. LC–MS is a more versatile technique than GC–MS, does not require derivatization, allows the acquisition of detailed structural information by in-source collisionally induced dissociation (CID) and shows a reduction of the detection limit by the injection of a large volume of sample at trace levels.

A corresponding deuterium-labeled analog has been widely used as an internal standard for the quantitative analysis of drugs and their metabolites by GC–MS, and the best quantitative results are obtained with deuterated internal standards. For qualitative analysis employing full-scan acquisition, an internal standard that can be separated chromatographically from an analyte is more suitable. Such an internal standard permits a simultaneous procedure for qualitative and quantitative analyses.

In this study, the analysis of BMA and its metabolites by LC–ESI–MS with solid-phase extraction was established with high sensitivity and reproducibility. Deuterium-labeled analogs were used as internal standards and separated from the corresponding unlabeled compounds on the total ion chromatogram (TIC). This method was applied to the urine of rats administered BMA.

2. Experimental

2.1. Chemicals and materials

Methamphetamine HCl (MA-HCl) was purchased from Dainippon Pharmaceutical (Osaka, Japan). Amphetamine $1/2H_2SO_4$ (AM-$1/2H_2SO_4$), methamphetamine-d_5 HCl (MA-d_5-HCl) and amphetamine-d_5 $1/2H_2SO_4$ (AM-d_5-$1/2H_2SO_4$) were synthesized as previously described [18]. Benzphetamine HCl (BMA-HCl), *N*-benzylamphetamine HCl (BAM-HCl), *p*-hydroxybenzphetamine HCl (*p*-HBMA-HCl) and *p*-hydroxy-*N*-benzylamphetamine HCl (*p*-HBAM-HCl) were essentially prepared by the method of Niwaguchi et al. [9]. Benzphetamine-d_7 HCl (BMA-d_7-HCl), *N*-benzylamphetamine-d_7 HCl (BAM-d_7-HCl), *p*-hydroxybenzphetamine-d_7 HCl (*p*-HBMA-d_7-HCl) and *p*-hydroxy-*N*-benzylamphetamine-d_7 HCl (*p*-HBAM-d_7-HCl) were synthesized in the same manner from benzyl chloride-d_7 instead of benzyl chloride. All synthetic compounds were identified and checked for purity by proton nuclear magnetic resonance and mass spectra.

A Bond Elut SCX cartridge (1 ml/100 mg, Varian, Harbor City, USA), a membrane filter (Millex-LH, 4 mm I.D., 0.5 μm, Nihon Millipore, Tokyo, Japan), β-glucuronidase (Type H-5, Sigma, St Louis, MO, USA) and benzyl-d_7 chloride (Aldrich, Milwaukee, USA) were used. All other chemicals used were of the highest purity available commercially.

2.2. Animals

Male Wistar rats weighting 135–181 g were used. An aqueous solution of BMA HCl was given orally to rats at a dose of 2.5 mg/kg. The animals were kept in individual metabolism cages and freely given water and food. The urine was collected every 24 h after administration of the drug and stored at −85°C until analyzed. The assembly used for collecting the urine was replaced by a clean one at each collection time.

2.3. LC–ESI–MS conditions

LC–ESI–MS was performed using a PU-980 pump (Jasco, Tokyo, Japan) connected to a Platform II electrospray ionization mass spectrometer (Micromass, Manchester, UK). Develosil ODS-UG-5 (150 mm×1.0 mm I.D., Nomura, Seto, Japan), which is resistant to alkaline conditions, with Develosil ODS-UG-S (10 mm×1.5 mm I.D.) as a guard column was used. The mobile-phase system was composed of acetonitrile and 10 m*M* ammonium acetate buffer (pH 9.0) and a stepwise gradient

elution was carried out as follows: 5–35% (5–11 min) to 55% (16–22 min) to 80% (26–45 min). In front of the injector, the mobile phase was split with a resistance column. The flow-rate of the mobile phase in the analytical column was 80–100 μl/min. The source temperature and capillary voltage were kept at 80°C and 3.5 kV, respectively. The cone voltages were 30 V for AM, AM-d_5, MA and MA-d_5, and 40 V for *p*-HBAM, *p*-HBAM-d_7, *p*-HBMA, *p*-HBMA-d_7, BAM, BAM-d_7, BMA and BMA-d_7 for the qualitative analysis. During quantitative analysis, the cone voltage was set as follows: 25 V for AM-d_5, 30 V for MA-d_5, *p*-HBAM, *p*-HBAM-d_7, BAM and BAM-d_7, and 35 V for AM, MA, *p*-HBMA, *p*-HBMA-d_7, BMA and BMA-d_7. The determination of BMA and its metabolites was carried out by monitoring the selected ions as follows: m/z 119, 119, 242, 256, 226 and 240 for AM, MA, HBAM, HBMA, BAM and BMA, respectively, and m/z 141, 155, 249, 263, 233 and 247 for AM-d_5, MA-d_5, *p*-HBAM-d_7, *p*-HBMA-d_7, BAM-d_7 and BMA-d_7, respectively.

2.4. GC–MS conditions

The GC–MS analysis was carried out on a HP 6890 GC (Hewlett-Packard, CA, USA) connected to a JMS-600W mass spectrometer (JEOL, Tokyo, Japan). The column was a DB-17 (30 m×0.25 mm I.D., film thickness 0.25 μm, J&W Scientific, CA, USA), and the column temperature was raised from 150 to 240°C at 10°C/min with a 1 min hold at 150°C and a 3 min hold at 240°C. Helium was used as the carrier gas at a flow-rate of 1 ml/min. The injection port was used at a split ratio of 1:30 and maintained at 270°C. The other operating conditions were as follows: electron impact ionization (EI), electron energy of 70 eV, ion source temperature at 200°C, interface temperature at 270°C.

2.5. Analytical procedure

One hundred microliters of an aqueous solution containing 208 ng/ml AM-d_5, 96.0 ng/ml MA-d_5, 71.8 ng/ml *p*-HBAM-d_7, 85.5 ng/ml *p*-HBMA-d_7 55.3 ng/ml BAM-d_7, and 72.3 ng/ml BMA-d_7 as the internal standards was added to 1 ml of rat urine or diluted urine with distilled water. Distilled water was then added to make a final volume of 3 ml. The mixture was adjusted to pH 5.0 with acetic acid and incubated with β-glucuronidase (1000 units/ml urine) at 37°C for 24 h. After incubation, the mixture was adjusted to pH 9.5 with 2.8% aqueous ammonia. A Bond Elut SCX cartridge was placed in a Vac-Elut system (Varian, Harbor City, USA). The SCX cartridge was activated by washing with 2 ml of methanol and 1 ml of 0.2 *M* ammonium acetate buffer (pH 9.5). A sample solution was applied and the SCX cartridge was rinsed with 1 ml of 0.2 *M* ammonium acetate buffer (pH 9.5) and 2 ml of 1 *M* acetic acid. After vacuum drying for 5 min, 2 ml of methanol was drawn through and the SCX cartridge was eluted with 2 ml of 1 N HCl–methanol. The eluate was evaporated at 60°C under a nitrogen stream. The residue was dissolved in 100 μl of 10 m*M* ammonium acetate buffer (pH 9.0)–acetonitrile (9:1, v/v) and 10 μl of 0.03% aqueous ammonia added. The mixture was passed through a 0.5 μm membrane filter and the entire volume of the eluate was injected into the LC–ESI–MS system. Due to the inorganic compounds in the sample solution which may plug the LC–ESI–MS interface, the ESI probe was connected to the mass spectrometer 8 min after sample injection.

2.6. Calibration curves for benzphetamine and its metabolites

Known concentrations of BMA and its metabolites dissolved in 100 μl methanol were added to tubes in which internal standards in 100 μl methanol and a small amount of acetic acid were present, then evaporated at 60°C under a nitrogen stream. The residue was dissolved in 100 μl of 10 m*M* ammonium acetate buffer (pH 9.0)–acetonitrile (9:1, v/v), and the entire volume was injected into the LC–ESI–MS system. The calibration curves were prepared using peak area ratios.

2.7. Precision and recovery test

Control urine was collected from the rat just before drug dosing. To investigate the accuracy and precision of the method, 100 μl of methanol solution containing BMA and its metabolites was placed in a tube. The methanol was evaporated and 1 ml of control urine was added. The concentration of the urine sample was much the same as each detection

limit of BMA and its metabolites under full-scan mode.

The recoveries of BMA and its metabolites were investigated in the same manner as above, except for the addition of the internal standards. One hundred microliters of a methanol solution containing internal standards was added to the eluate through the SCX cartridge.

3. Results and discussion

3.1. Selection of cone voltage

The cone voltage has a significant effect on fragmentation. The cone voltage was set to give the largest intensity of each monitoring ion for quantitative analysis and to produce a quasi-molecular ion and several fragment ions of each analyte for qualitative analysis. Due to the interference by contaminants in the selected ion monitoring (SIM) chromatograms of the quasi-molecular ions at m/z 136 for AM and m/z 150 for MA, the fragment ion at m/z 119 was monitored for both compounds. The quasi-molecular ions at m/z 141, 155, 242, 249, 256, 263, 226, 233, 240 and 247 were monitored for AM-d_5, MA-d_5, p-HBAM, p-HBAM-d_7, p-HBMA, p-HBMA-d_7, BAM, BAM-d_7, BMA and BMA-d_7, respectively. The effect of the cone voltage on ion intensity is shown in Fig. 1. The values relative to the ion intensities at a cone voltage of 30 V for AM and MA, and 20 V for p-HBAM, p-HBMA, BAM and BMA are plotted on the ordinate. The ion intensities for AM, MA, p-HBAM, p-HBMA, BAM and BMA were maximized at 35, 40, 30, 35, 30 and 35 V, respectively. The addition of a methyl group to the amino group produced an enhancement in the optimum voltage. Due to the use of the same ion at m/z 119, a cone voltage of 35 V was selected for both AM and MA, and those described above were used for the others. A cone voltage of 30 V for AM and MA, and 40 V for p-HBAM, p-HBMA, BAM and BMA was used in the qualitative analysis.

3.2. Chromatograms and mass spectra of benzphetamine and its metabolites

TIC and mass chromatograms under acidic and alkaline mobile phase conditions are shown in Fig. 2. Under acidic conditions with 10 mM ammonium

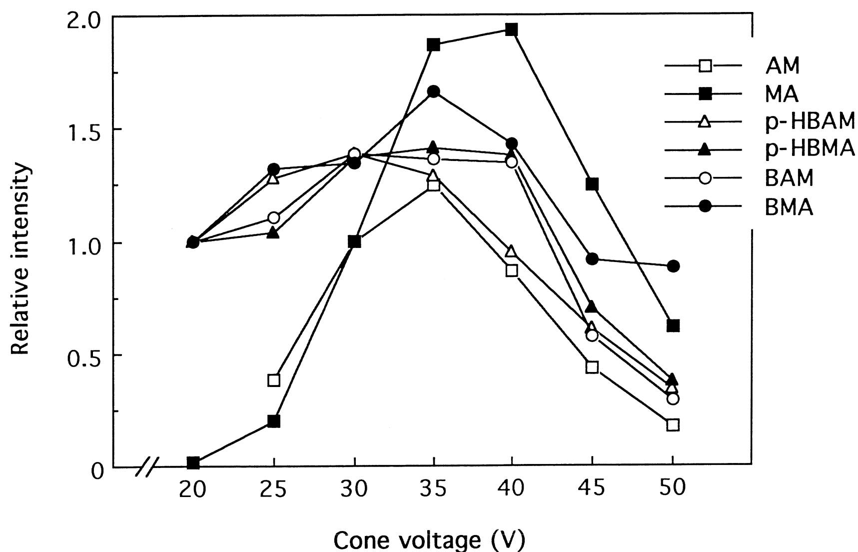

Fig. 1. Effect of cone voltage on ion intensities of BMA and its metabolites. The ions at m/z 119, 119, 242, 256, 226 and 240 were monitored for AM, MA, p-HBAM, p-HBMA, BAM and BMA, respectively. The values relative to the ion intensities at cone voltages of 30 V for AM and MA and 20 V for p-HBAM, p-HBMA, BAM and BMA are plotted on the ordinate.

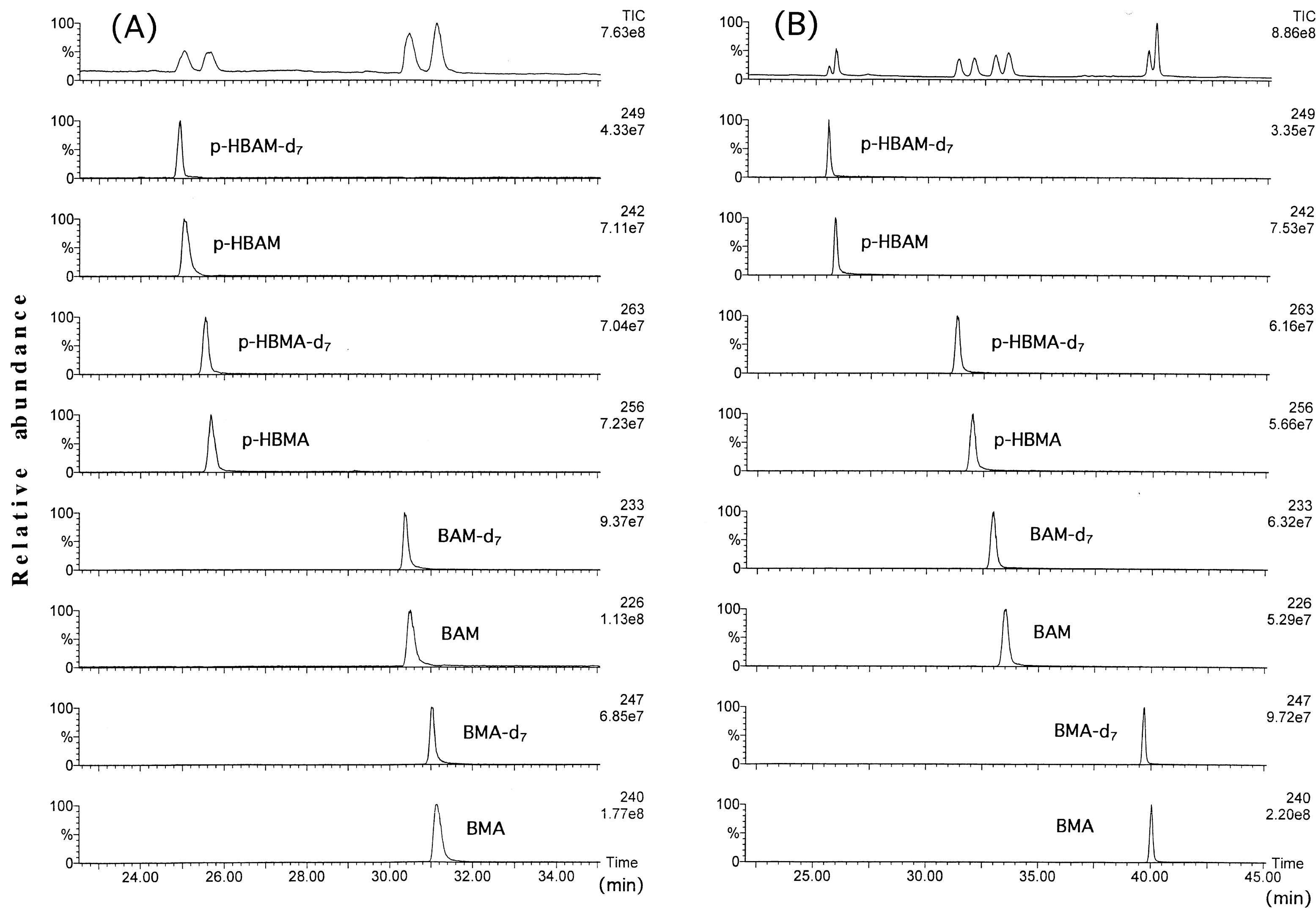

Fig. 2. Total ion chromatograms and mass chromatograms for BMA and its metabolites under acidic (A) and alkaline (B) mobile phase conditions. Acidic mobile phase: CH_3CN–10 m*M* NH_4OAc (pH 4.0) [5–60% (35 min)]. Alkaline mobile phase: CH_3CN–10 m*M* NH_4OAc (pH 9.0) [5–35% (5–11 min) to 55% (16–22 min) to 80% (26–45 min)].

acetate buffer (pH 4.0), the peaks of unlabeled compounds entirely overlap those of the corresponding labeled compounds. The separation of the unlabeled and labeled compounds using an alkaline mobile phase with 10 m*M* ammonium acetate buffer (pH 9.0) was sufficient to give satisfactory mass spectra of the unlabeled compounds. Resolutions of the unlabeled and labeled compounds were 1.13, 1.52, 1.09 and 1.23 for *p*-HBAM, *p*-HBMA, BAM and BMA, respectively. The alkaline mobile phase allowed a simultaneous procedure for identification and quantitation of BMA and its metabolites by the chromatographic separation of the unlabeled and labeled compounds. For AM and MA, the unlabeled and labeled compounds were eluted simultaneously under both conditions and could not be separated.

In the GC–MS analysis, BMA and the trifluoroacetyl derivatives of its metabolites could not be separated satisfactorily from their corresponding labeled compounds using the DB-5MS capillary column (30 m×0.32 mm I.D., film thickness 0.5 μm, J&W Scientific, CA, USA). A better separation was pursued using a more polar DB-17 column. As shown in Fig. 3, this strategy led to a good separation of *p*-HBAM-2TFA, BAM-TFA and the corresponding labeled compounds on TIC. The separation of BMA, *p*-HBMA-TFA and the deuterated compounds was insufficient on TIC. The GC–MS analysis was inferior to the LC–ESI–MS analysis in the separation of labeled and unlabeled compounds.

The positive ESI mass spectra obtained with the alkaline mobile phase are shown in Fig. 4. Each

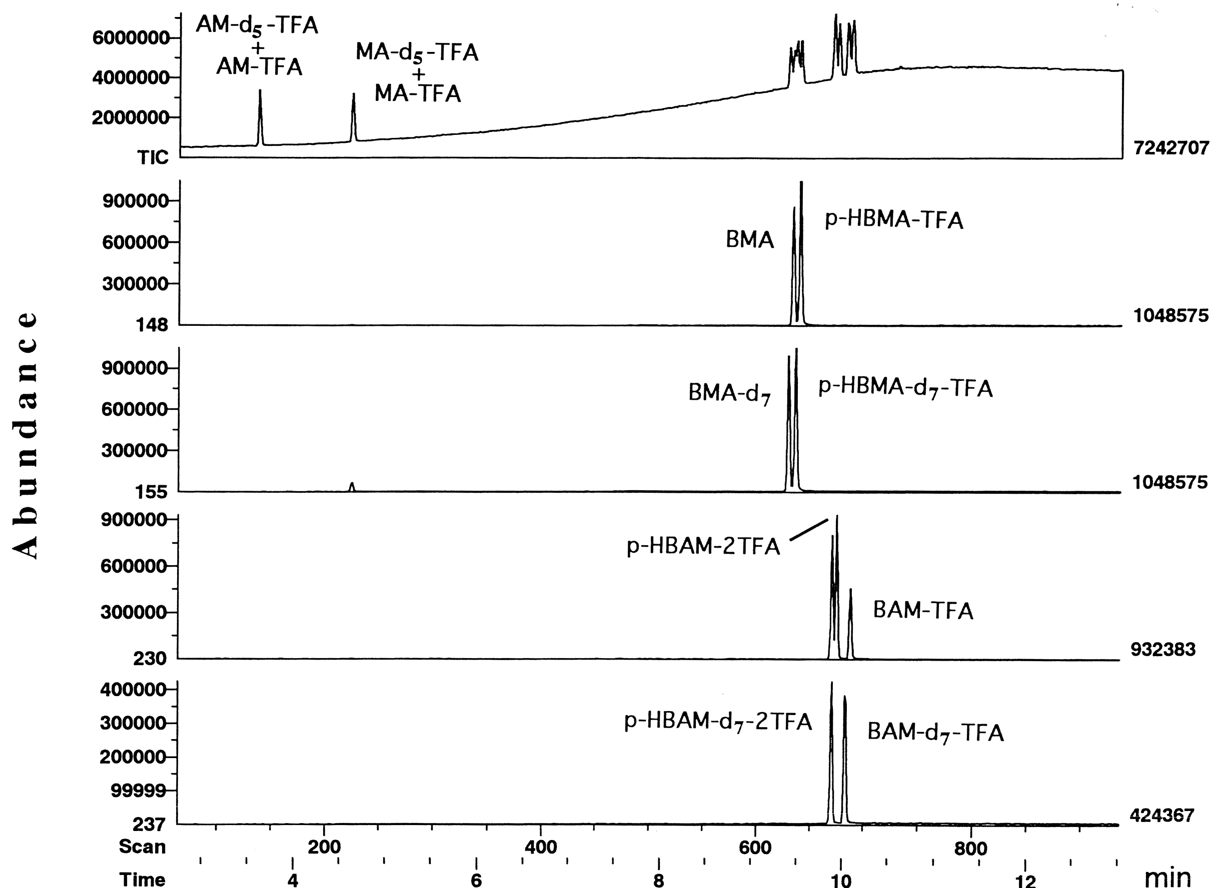

Fig. 3. Total ion chromatogram and mass chromatograms of trifluoroacetyl derivatives of BMA and its metabolites by EI-GC–MS. TFA, trifluoroacetyl derivative.

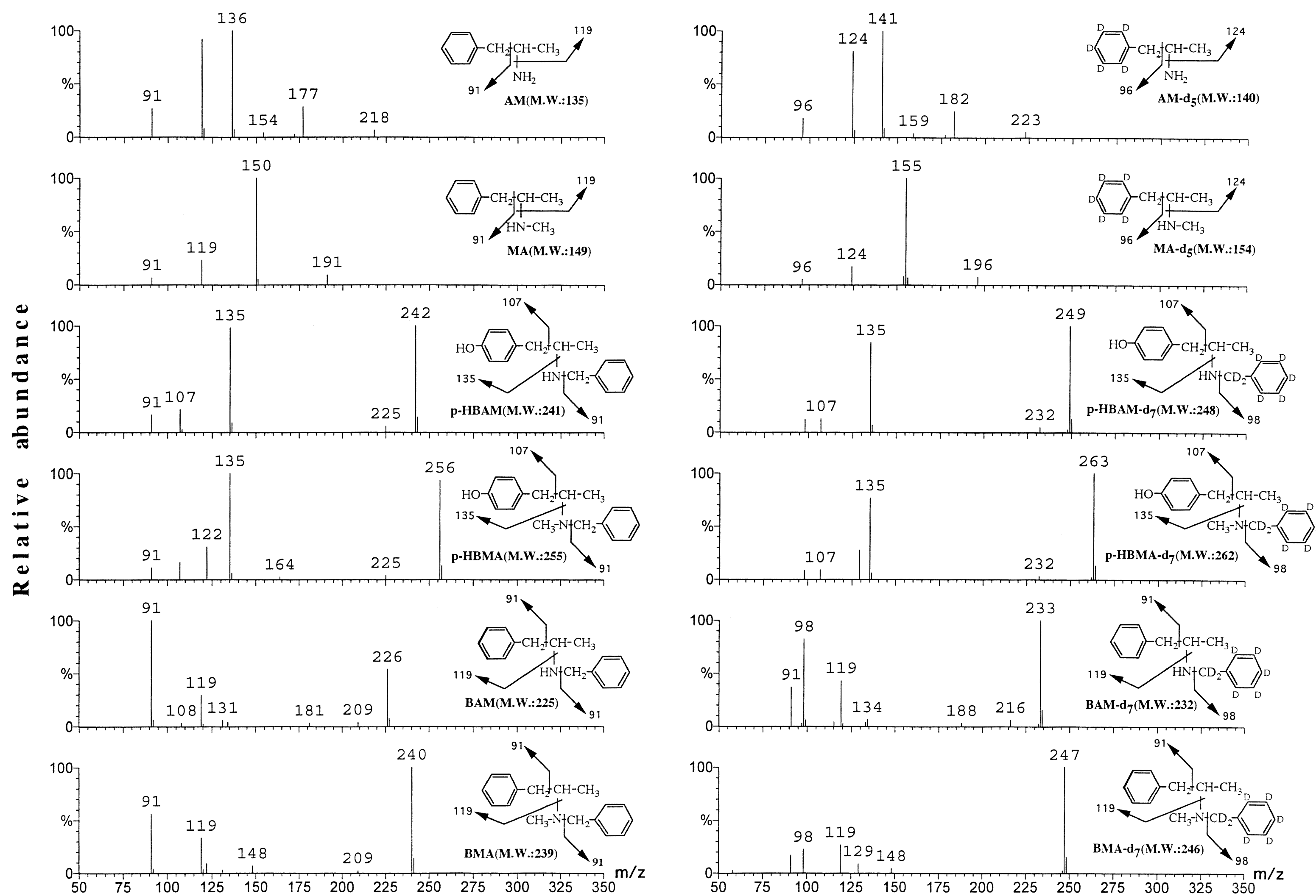

Fig. 4. Positive ESI mass spectra of BMA and its metabolites and those of deuterium-labeled compounds.

mass spectrum shows a quasi-molecular ion and several fragment ions which enabled us to accomplish a reliable identification.

3.3. Limit of detection and limit of quantitation

BMA and its metabolites were spiked in the control urine and analyzed under full-scan mode and SIM mode. Fig. 5 shows the TICs of the extracts from 1 ml of control urine with and without 200 ng/ml AM, 120 ng/ml MA, 28 ng/ml *p*-HBAM, 10 ng/ml *p*-HBMA, 6 ng/ml BAM and 14 ng/ml BMA, which are the limits of detection in the full-scan mode. Under the SIM mode, the limit of quantitation (signal-to-noise ratio 10) for AM, MA, *p*-HBAM, *p*-HBMA, BAM and BMA was 10 ng, 6 ng, 1.4 ng, 500 pg, 300 pg and 700 pg in 1 ml of urine, respectively.

The calibration curves for BMA and its metabolites were all linear as follows. AM, $y = 4.7942 \cdot 10^{-2} + 2.0252 \cdot 10^{-2}X$ (50 ng–5 μg/ml, $r = 0.999$); MA, $y = -2.9225 \cdot 10^{-2} + 4.0439 \cdot 10^{-2}X$ (50 ng–5 μg/ml, $r = 0.999$); *p*-HBAM, $y = -2.0087 \cdot 10^{-2} + 0.11626X$ (10 ng–1 μg/ml, $r = 0.999$); *p*-HBMA, $y = 1.4544 \cdot 10^{-2} + 0.12869X$ (2 ng–200 ng/ml, $r = 0.999$), $y = 0.25109 + 0.10497X$ (5 ng–100 ng/ml, $r = 0.998$); BAM, $y = 4.4854 \cdot 10^{-3} + 0.17571X$ (2 ng–200 ng/ml, $r = 0.999$), $y = -3.5696 \cdot 10^{-2} + 0.17954X$ (50 ng–1 μg/ml, $r = 0.999$); BMA, $y = -5.7607 \cdot 10^{-3} + 0.12863X$ (2 ng–200 ng/ml, $r = 0.999$), $y = -0.11512 + 0.13905X$ (50 ng–1 μg/ml, $r = 0.999$).

3.4. Intra- and inter-day assay precision

The day-to-day reproducibility ($n = 5$) of the retention time for BMA and its metabolites was between 0.6 and 1.7%. The mean data and relative standard deviation (RSD) for the quantitative analysis of five urine samples are shown in Table 1. The intra- and inter-day RSDs were between 3.1 and 6.5% and between 4.8 and 8.8%, respectively.

3.5. Recoveries of benzphetamine and its metabolites

The recoveries of BMA and its metabolites are shown in Table 1. The recoveries of the five compounds except for BMA were >83.8%, which was sufficient to measure. It is believed that BMA was retained more than the other compounds in the packing material of the SCX cartridge.

3.6. Analysis of BMA and its metabolites in rat urine

BMA and its metabolites in rat urine were collected every 24 h after the administration of BMA (2.5 mg/kg) and identified and quantified using the presented technique. The TIC obtained from 0.1 ml of urine on the first day is shown in Fig. 6. *p*-HBAM and *p*-HBMA were in higher abundance than the others in the TIC. The mass spectrum of *p*-HBAM could be obtained exactly at the top of the peak in spite of the closeness of the next peak. The mass spectrum of the peak appearing about 0.75 min after the occurrence of *p*-HBAM was the same as that of *p*-HBAM and is thus regarded as its isomer, *m*-hydroxy-*N*-benzylamphetamine. The mass spectrum of the peak adjacent to *p*-HBMA was in agreement with that of *p*-HBMA and is regarded as its isomer, *m*-hydroxy-benzphetamine. Under full-scan mode, the feasible periods of identification were up to the first day for AM, MA, BAM and BMA, the second day for *p*-HBAM and the third day for *p*-HBMA.

The SIM chromatograms obtained from 0.5 ml of urine on the second day are shown in Fig. 7. BMA and its metabolites could be determined without interference from other peaks.

The time courses of the urinary excretion of BMA and its metabolites are shown in Table 2. Niwaguchi et al. reported that unchanged BMA and BAM were not detected in rat urine [9], while both BMA and BAM were identified by this method. In a single dose of 50 mg of BMA-HCl, Inoue and Suzuki reported that unchanged BMA was observed in human urine [10], but there is no description of its detection period. In a multidose of 30 mg of BMA-HCl per day for 5 successive days, Kikura and Nakahara reported that BMA was detected in the 1 h urine of one of two subjects [12]. Cody and Valtier reported that BMA was not present in appreciable amounts following a single 50 mg oral dose of BMA-HCl [14]. BMA was either detected at low levels for a short time or not detected in these human studies. In our study involving rats, BMA was

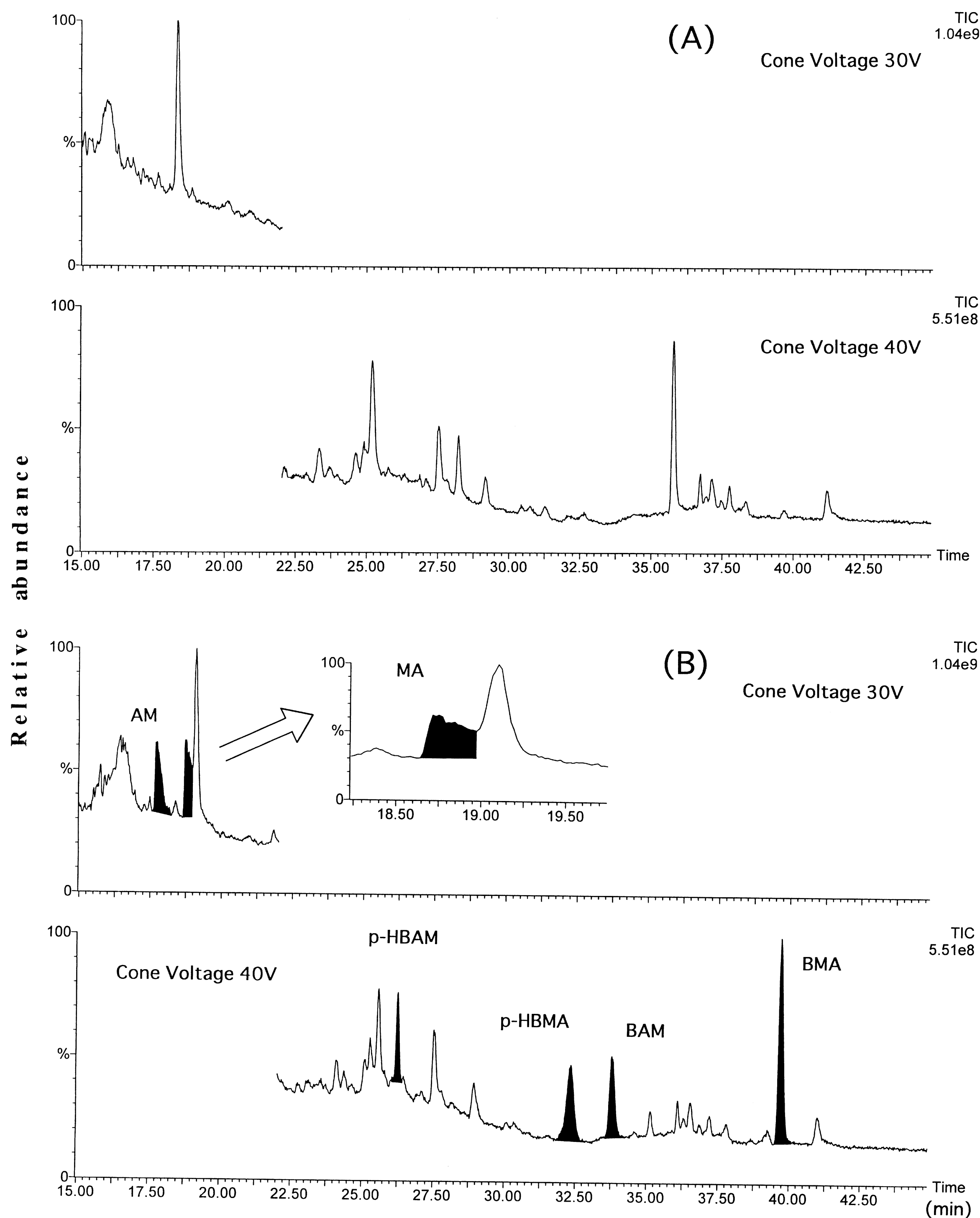

Fig. 5. Total ion chromatograms of extracts from 1 ml of rat control urine (A) and that spiked with BMA and its metabolites (B). The concentrations of AM, MA, *p*-HBAM, *p*-HBMA, BAM and BMA were 200, 120, 28, 10, 6 and 14 ng/ml, respectively.

Table 1
Accuracy and precision for determination and recovery of BMA and its metabolites

Compound	Actual value[a] (ng/ml)	Recovery ($n = 5$, %)	Measured value ($n = 5$, ng/ml)	
			Intra-day	Inter-day
Amphetamine (AM)	209.0	92.1±8.9[c]	205.0 (3.8)[b]	189.1 (8.8)[b]
Methamphetamine (MA)	118.2	95.1±2.9	116.9 (3.4)	114.7 (5.4)
1-(*p*-Hydroxyphenyl)-2-(*N*-benzylamino)propane (*p*-HBAM)	27.36	96.3±1.3	26.38 (3.1)	25.92 (7.2)
1-(*p*-Hydroxyphenyl)-2-(*N*-methyl-*N*-benzylamino)propane (*p*-HBAM)	10.76	93.6±2.1	11.37 (6.1)	11.40 (6.2)
Desmethylbenzphetamine (BAM)	6.04	83.8±7.2	6.60 (6.5)	6.96 (6.3)
Benzphetamine (BMA)	14.05	67.7±2.7	13.24 (4.4)	15.20 94.8)

[a] Concentrations in urine spiked with BMA and its metabolites.
[b] Mean data from five experiments and relative SD (%) in parentheses.
[c] Mean data from five experiments±SD.

detected at very low levels even in the 24–48 and 48–72 h urine samples. This discrepancy in detection time may be due to the differences in the detection limits, dosing amounts or metabolic patterns between humans and rats.

p-HBAM was the most abundant metabolite on the first day. After the first day, the excreted amount of *p*-HBMA was the largest among the excreted metabolites. A large portion of BMA and its metabolites were excreted on the first day and the amounts

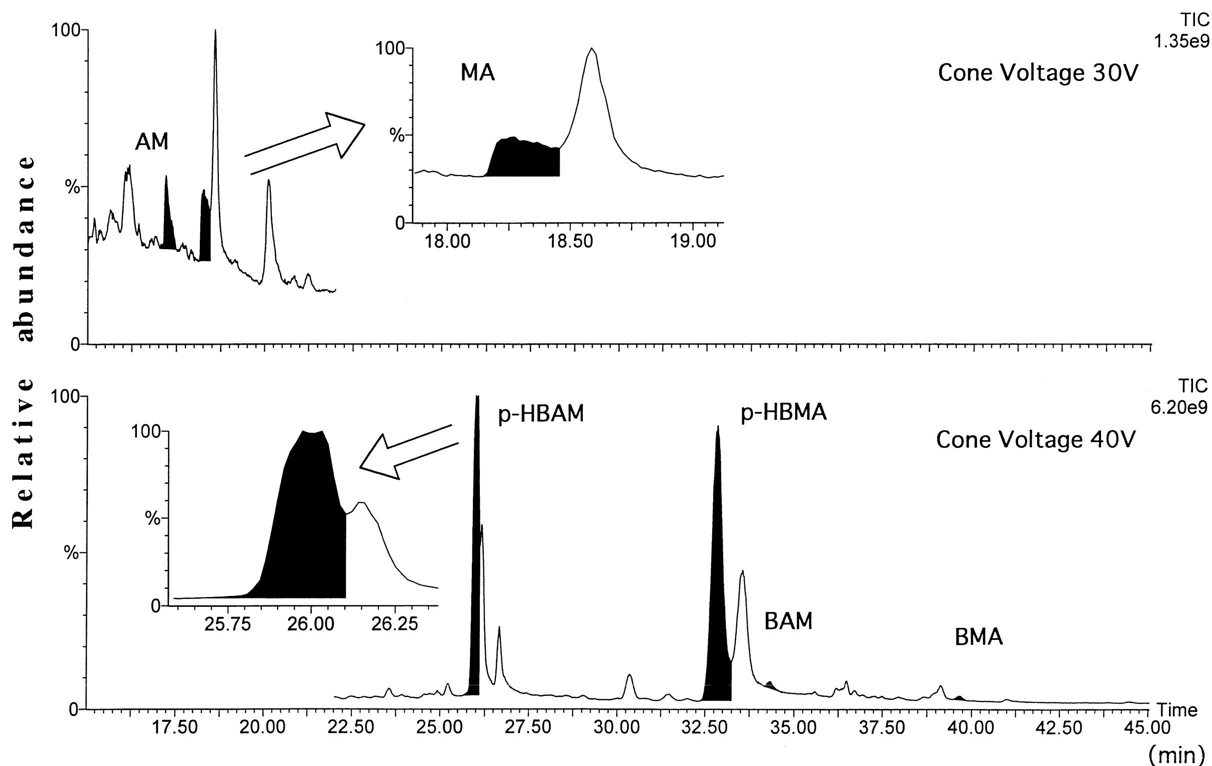

Fig. 6. Total ion chromatogram of extract from rat urine on the first day. The concentrations of AM, MA, *p*-HBAM, *p*-HBMA, BAM and BMA were 178, 171, 1100, 650, 16.1 and 24.5 ng/0.1 ml, respectively.

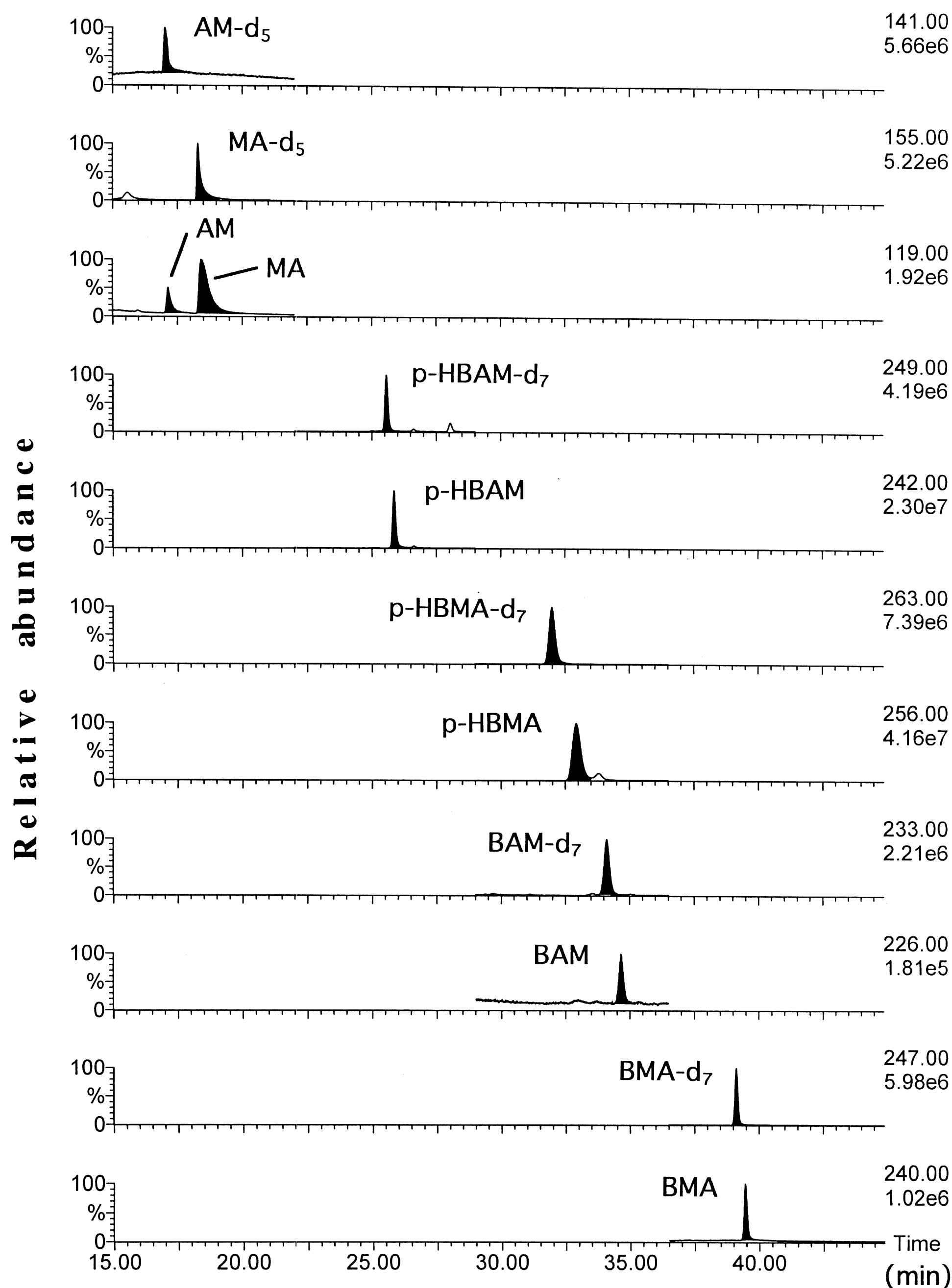

Fig. 7. SIM chromatograms of extract from rat urine on the second day. The concentrations of AM, MA, *p*-HBAM, *p*-HBMA, BAM and BMA were 11.2, 22.5, 55.1, 87.7, 0.701 and 3.22 ng/0.5 ml, respectively.

excreted significantly decreased after the first day. *p*-HBAM and *p*-HBMA could be determined even on the fifth day. In a single dose of 10 mg of BMA-HCl, Fujinami et al. reported that both *p*-HBAM and *p*-HBMA were detected up to 30–33 and 24–27 h in the urine of two human subjects [16].

Table 2
Time courses of urinary excretion of BMA and its metabolites

Compound	Dose excreted in 0–120 h urine (%)				
	0–24 h	24–48 h	48–72 h	72–96 h	96–120 h
Amphetamine (AM)	2.84[a] (±1.22)	$3.93 \cdot 10^{-2}$ ($\pm 1.97 \cdot 10^{-2}$)	n.d.[b]	n.d.	n.d.
Methamphetamine (MA)	4.04 (±2.90)	$3.44 \cdot 10^{-2}$ ($\pm 3.20 \cdot 10^{-2}$)	$2.64 \cdot 10^{-2}$ ($\pm 3.80 \cdot 10^{-3}$)	Trace	Trace
1-(*p*-Hydroxyphenyl)-2-(*N*-benzylamino)propane (*p*-HBAM)	11.8 (±1.39)	$1.57 \cdot 10^{-1}$ ($\pm 7.00 \cdot 10^{-2}$)	$2.86 \cdot 10^{-2}$ ($\pm 8.15 \cdot 10^{-3}$)	$1.24 \cdot 10^{-2}$ ($\pm 2.78 \cdot 10^{-3}$)	$8.06 \cdot 10^{-3}$ ($\pm 8.80 \cdot 19^{-4}$)
1-(*p*-Hydroxyphenyl)-2-(*N*-methyl-*N*-benzylamino)-propane (*p*-HBMA)	5.55 ($\pm 9.97 \cdot 10^{-1}$)	$2.40 \cdot 10^{-1}$ ($\pm 8.35 \cdot 10^{-2}$)	$3.83 \cdot 10^{-2}$ ($\pm 8.30 \cdot 10^{-3}$)	$1.90 \cdot 10^{-2}$ ($\pm 2.11 \cdot 10^{-3}$)	$9.93 \cdot 10^{-3}$ ($\pm 1.28 \cdot 10^{-3}$)
Desmethylbenzphetamine (BAM)	$1.44 \cdot 10^{-1}$ ($\pm 1.23 \cdot 10^{-1}$)	$2.83 \cdot 10^{-3}$ ($\pm 3.61 \cdot 10^{-3}$)	$1.01 \cdot 10^{-3}$ ($\pm 4.60 \cdot 10^{-4}$)	Trace	Trace
Benzphetamine (BMA)	$1.86 \cdot 10^{-1}$ ($\pm 1.47 \cdot 10^{-1}$)	$4.39 \cdot 10^{-3}$ ($\pm 4.51 \cdot 10^{-3}$)	$2.00 \cdot 10^{-3}$ ($\pm 1.76 \cdot 10^{-3}$)	Trace	Trace

[a] Mean data from five experiments±SD.
[b] n.d., not detected.

Rats have the metabolic patterns of amphetamines removed from humans, and it is well known that the excretion of aromatic hydroxylated metabolites in humans is somewhat less than that in rats [19]. Although this study was performed using rats, this analytical method may be used for the discrimination of BMA users because of its high sensitivity for BMA and its metabolites.

4. Conclusion

The analytical separation of BMA and its metabolites by LC–ESI–MS following solid-phase extraction with an SCX cartridge was established and applied to the urine of rats administered BMA. Excellent accuracy and precision were obtained by the use of labeled compounds as internal standards. The use of an alkaline mobile phase enabled us to carry out simultaneous identification and quantification by the chromatographic separation of the unlabeled and labeled compounds. Injection of the entire volume of sample extract in LC–ESI–MS provided high sensitivity. Each mass spectrum of BMA and its metabolites on LC–ESI–MS showed a quasi-molecular ion and several fragment ions and enabled us to accomplish a reliable identification. *p*-HBAM was the most abundant metabolite, accounting for about 12% of the dose 5 days after administration. The amount of *p*-HBMA was greater than the other metabolites excreted after 24 h. This very sensitive analysis for *p*-HBMA, structurally closer to the unchanged drug, offers a longer detection period in rat urine to help discriminate the ingestion of BMA. The present study suggests that this analytical method is useful for the discrimination of BMA users because of its high sensitivity for BMA and its metabolites.

References

[1] A.H. Beckett, G.T. Tucker, A.C. Moffat, J. Pharm. Pharmacol. 19 (1967) 273.
[2] J. Marsel, G. Döring, G. Remberg, G. Spiteller, Z. Rechtsmed. 70 (1972) 245.
[3] J. Mallol, L. Pitarch, R. Coronas, A. Pons Jr., Arzneim.-Forsch. 24 (1974) 1301.
[4] T. Inoue, S. Suzuki, Xenobiotica 17 (1987) 965.
[5] R. Mrongovius, M. Neugebauer, G. Rücker, Eur. J. Med. Chem. 19 (1984) 161.
[6] Y. Yoo, H. Chung, H. Choi, J. Anal. Toxicol. 18 (1994) 265.
[7] T. Ono, M. Takahashi, Y. Nakamura, H. Ohde, J. Morita, H. Tsubouci, T. Takahara, Y. Hayashi, T. Tsuzuku, Y. Ueyama, N. Ohtani, C. Kamekou, Rinsho Iyaku 7 (1991) 1475.
[8] J.T. Cody, Forensic Sci. Rev. 5 (1993) 109.

[9] T. Niwaguchi, T. Inoue, S. Suzuki, Xenobiotica 12 (1982) 617.
[10] T. Inoue, S. Suzuki, Xenobiotica 16 (1986) 691.
[11] M.J. Choi, J. Choi, J. Park, S.A. Eremin, J. Immunoassay 16 (1995) 263.
[12] R. Kikura, Y. Nakahara, Biol. Pharm. Bull. 18 (1995) 1694.
[13] C. Battu, P. Marquet, A.L. Fauconnet, E. Lacassie, G. Lachatre, J. Chromatogr. Sci. 36 (1998) 1.
[14] J.T. Cody, S. Valtier, J. Anal. Toxicol. 22 (1998) 299.
[15] A. Fujinami, T. Miyazawa, Y. Kobayashi, F. Watanabe, Rinsho Kagaku 25 (1996) 243.
[16] A. Fujinami, T. Miyazawa, Y. Kobayashi, Ann. Clin. Biochem. 35 (1998) 775.
[17] A. Fujinami, T. Miyazawa, N. Tagawa, Y. Kobayashi, Biol. Pharm. Bull. 21 (1998) 1207.
[18] M. Sato, T. Mitsui, J. Pharm. Biomed. Anal. 16 (1997) 139.
[19] J. Caldwell, Drug Metab. Rev. 5 (1976) 219.