Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 855 (2007) 121–126
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

The disposition into hair of new designer drugs; methylone, MBDB and methcathinone☆

Ruri Kikura-Hanajiri*, Maiko Kawamura, Kazuhiro Saisho, Yukio Kodama, Yukihiro Goda

National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya, Tokyo 158-8501, Japan

Received 5 February 2007; accepted 11 May 2007
Available online 29 May 2007

Abstract

The disposition into hair of methylone and other new designer drugs, methcathinone and MBDB, was studied with the animal model. Moreover, the incorporation rates of these drugs were compared with those of their related eight compounds previously studied in order to evaluate their incorporation tendency into hair and the usefulness of hair specimens for the retrospective confirmation of the use of these drugs. When the ratio of hair concentration to AUC in plasma ([Hair]/AUC) was represented as an index of the incorporation rate of drugs into hair, the [Hair]/AUC of methylone was 14 times higher than that of methcathinone. It might support earlier findings that the methylenedioxy group on the benzene ring leads to considerably higher incorporation rates. However, [Hair]/AUC of methylone was five-sevenths times lower in comparison with that of MDMA. This suggested that the beta-carbonyl group leads to lower incorporation rates. Although methylone has both groups in its structure, the positive effect of the methylenedioxy group may be stronger than the negative effect of the beta-carbonyl group. On the other hand, the [Hair]/AUC of MBDB, which has methylenedioxyphenyl-2-butanamine structure, was higher than that of MDMA while that of methcathinone, having beta-ketone in its structure, was extremely low. In conclusion, as with MA and MDMA, the incorporation tendency of methylone and MBDB (except for methcathinone) into hair is relatively high, and a hair sample would be a good specimen for the confirmation of retrospective use of these drugs.

Keywords: Methylone; Hair analysis; Drug disposition into hair; GC–MS

1. Introduction

It is difficult to evaluate the drug incorporation tendency into hair only by hair concentrations because they vary according to the total doses administered and the bioavailability of the drugs. Even if a defined dose is used in the experimental design, it may not be enough because there are large individual differences in both metabolism and bioavailability. One reasonable method for controlling these problems is to consider the total amount of drugs in whole blood or plasma. We have proposed that the drug incorporation rates into hair could be measured as the comparison between the total amount of drugs in the blood and those in the hair [1,2]. The total amount of drugs in the blood can be represented by area under the plasma concentration curve (AUC). On the other hand, in practice, it is difficult to estimate the total amount of drugs in the hair. Therefore, this measure may theoretically be replaced by drug concentrations in the hair. These concentrations would be determined by comparisons with equal lengths of hair that have grown for a definite duration. Based on the above reason, we have used the ratio of drug concentration in hair to plasma AUC ([Hair]/AUC) as one of the indexes of the incorporation rate of a drug from blood into hair in the animal experimental model [1,2].

In a previous report [2], we have studied the structural factors of drugs on the incorporation rate into hair from blood using 32 amphetamine analogs. As a result, [Hair]/AUC of 3,4-methylenedioxyamphetamine (MDA) which has a 3,4-methylenedioxy group to the benzene ring of amphetamine (AP) was 5.5 times higher than that of AP; 3,4-methylenedioxymethamphetamine (MDMA) corresponding to

☆ This paper was presented at the 44th Scientific Meeting of The International Association of Forensic Toxicologists (TIAFT), Ljubljana, Slovenia, 27 August–1 September 2006.

* Corresponding author. Tel.: +81 3 3700 8764; fax: +81 3 3707 6950.
E-mail address: kikura@nihs.go.jp (R. Kikura-Hanajiri).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.05.018

methamphetamine (MA) analog was 5.9 times higher than that of MA, and 3,4-methylenedioxyethylamphetamine (MDEA) corresponding to *N*-ethylamphetamine (EAP) analog was 6.1 times higher than that of EAP. Moreover, the [Hair]/AUC of 3-methoxy-4,5-methylenedioxyamphetamine (MMDA) was 2.3 times higher than that of MDA [3]. These results suggested that a methylenedioxy or a methoxy group on the benzene ring leads to considerably higher incorporation rates. On the other hand, the result of the [Hair]/AUC measurement of cathinone containing a carbonyl group at the benzyl position of AP gave unusually low values compared with that of AP. It is clear that the beta-ketoamphetamines (e.g., cathinone) would show very low incorporation rates.

Methylone (2-amino-1-(3,4- methylenedioxyphenyl)propan-1-one) is a new ecstasy-type designer drug that recently appeared in the Japanese drug market as well as in Europe (Fig. 1). Although only a little is known about the harmfulness of this drug, risks common to MDMA cannot be excluded because of the similarities between these drugs [4–6]. Methylone has the methylenedioxy and the beta-carbonyl groups in its structure. As mentioned above, these two functional groups would have contrary effects on the incorporation rate into hair of the drug. Therefore, it may be difficult to guess the incorporation tendency compared to its corresponding compound, MA.

In this study, the dispositions into hair of methylone and other new designer drugs, methcathinone and *N*-methyl-1-(3,4-methylenedioxyphenyl)butan-2-amine (MBDB), were studied with the animal model. Moreover, the incorporation rates of these drugs were compared with those of the related compounds previously studied, cathinone, AP, MA, MDA, MDMA, MDEA and MMDA, in order to evaluate their incorporation tendency into hair and the usefulness of hair specimens for the confirmation of the retrospective use of these drugs. Its chemical structure and those of related drugs are shown in Fig. 1.

2. Experimental

2.1. Chemicals and reagents

MBDB hydrochloride and MBDB-d5 (1,2-dideutero-*N*-trideuteromethyl-1-(3,4-methylenedioxyphenyl)-2-butanamine) were obtained from Cerilliant (Round Rock, TX, USA). MDMA-d3 hydrochloride [3] and methamphetamine-d4 hydrochloride [7] used as internal standards were prepared as previously reported. Methcathinone hydrochloride was prepared by the oxidation of pseudoephedrine [8]. Methylone hydrochloride was synthesized according to the procedure of Kamata et al. [9]. Its structure and purity were confirmed by a melting point (degradation, 225 °C), TLC, GC–MS [9] and ^{1}H nuclear magnetic resonance. Solid-phase extraction column (Bond Elut Certify, 10 mL/300 mg) was obtained from Varian (Harbor City, CA, USA). Pentafluoropropionic anhydride was purchased from Aldrich (Steinheim, Germany). All other chemicals and solvents were of analytical reagent grade or HPLC grade (Wako Chemicals, Osaka, Japan).

2.2. Instrumentation

For the quantitative analysis of the drugs in plasma and hair samples, GC–MS in the electron impact (EI) mode at 70 eV of electron energy was used. The instrument consisted of a Hewlett-Packard 5890 series II plus GC with a 5972 mass selective detector. Helium was used as the carrier gas through the fused silica capillary column (DB35-MS capillary, 30 m × 0.25 mm i.d., 0.25 μm film thickness) at 1 mL/min. The injector temperature was 200 °C, and splitless injection was employed with a split valve for 1.0 min. The oven temperature was started at 100 °C (held for 1.0 min), followed by a 10 °C/min ramp to 280 °C (held for 5 min) for the analyses of methylone and methcathinone. It

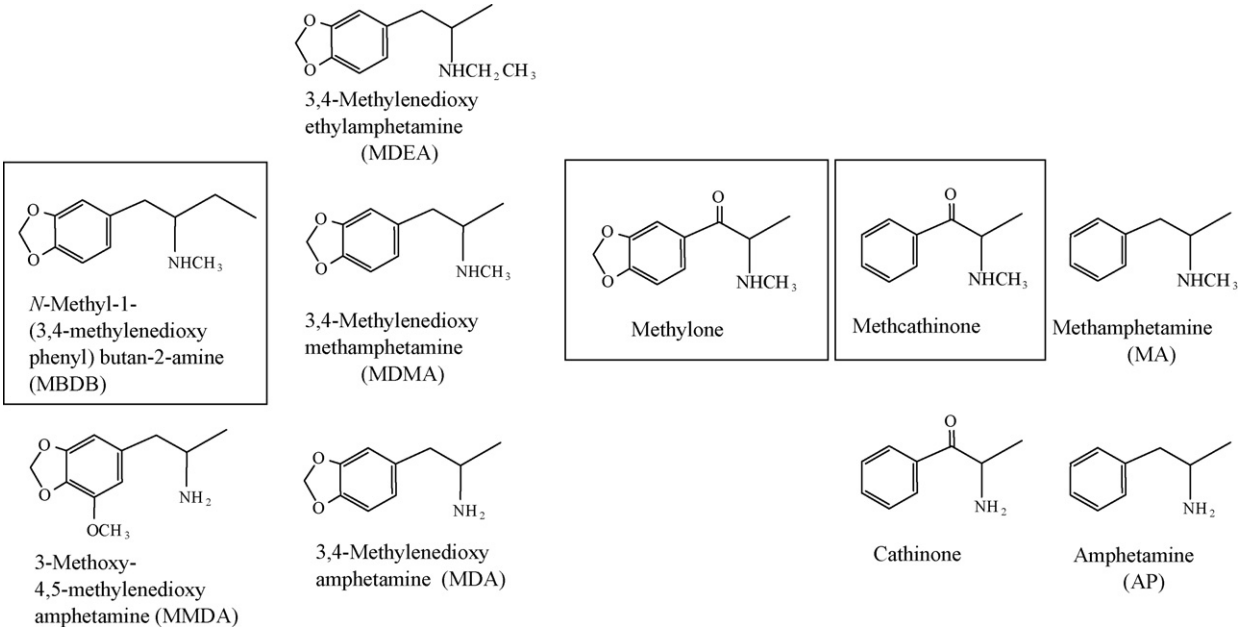

Fig. 1. Chemical structures of methylone, MBDB, methocathinone and its related compounds.

was programmed from 100 °C (3 min hold) to 280 °C (5 min) at a rate of 20 °C/min for the analysis of MBDB. The mass selective detector was kept at 280 °C. Drugs in biological specimens were investigated by monitoring the selected ions as follows: *m/z* 149 (a base peak ion), 204, 121, 160, 353 (M^+) for pentafluoropropionyl (PFP)–methylone, *m/z* 218 (a base peak ion), 176, 135, 353(M^+) for PFP–MBDB, *m/z* 204 (a base peak ion), 105, 160, 77, 119 for PFP–methcathinone, *m/z* 207 for PFP–MDMA-d3 (as an internal standard for the analysis of methylone), *m/z* 222 for PFP–MBDB-d5 (for the analysis of MBDB) and *m/z* 208 for MA-d4 (for the analysis of methcathinone).

2.3. Animal experiments

The animal experimental model was designed to compare the ratio of hair to AUC in many kinds of drugs as shown in our previous report [1,2]. The experimental drugs were administered to male dark agouti (DA) pigmented rats ($n = 3$), which were 5 weeks old and around 100 g mean weight (Japan SLC, Shizuoka, Japan). The drugs were given once a day at 5 mg/kg by intraperitoneal injection for 10 successive days. Two hundreds μL of Blood samples were collected 5, 15, 30, 60 120 and 360 min after the drug administration and plasma samples were prepared. The AUC was calculated by the conventional method [1]. Each animal had been shaved on the back just before the first drug administration. The newly growing hair samples were collected 28 days after the first administration. The ratio of the drug concentration in the hair to the AUC of the plasma was calculated as the index of the incorporation rate into hair.

2.4. Analytical methods

2.4.1. Stock solution

An individual standard solution of 1.0 mg/mL of each drug, methylone, methathinone and MBDB was prepared in methanol and distilled water, and stored at 4 °C. The IS solutions of 10 μg/mL of MDMA-d3, 1 μg/mL of MA-d4 and 5 μg/mL of MBDB-d5 in methanol for the analysis of hair samples and those of 1 μg/mL of MDMA-d3, 2 μg/mL of MA-d4 and 1 μg/mL of MBDB-d5 in distilled water for plasma samples were also prepared.

2.4.2. Plasma

To 100 μL of plasma sample was added 50 μL of the IS aqueous solution and 3 mL of 0.1 M potassium hydrogen phosphate buffer (pH 6.0). After a Bond Elut Certify was pre-activated with methanol and the buffer, the sample solution was applied to the Bond Elut Certify and the column was washed with 1 mL of distilled water and 1 M acetic acid, successively. The column was dried under vacuum for 5 min. After the column was rinsed with 1 mL of methanol and dried under vacuum for 2 min, 3 mL of mixed solution of dichloromethane, methanol and hydrochloric acid (60:40:1) was passed through the column to elute the target drugs. Following evaporation of the solvent under a nitrogen stream, the residue was dissolved in 200 μL of PFPA/ethylacetate (1:1) and heated at 60 °C for 20 min. The reaction solution was evaporated with a nitrogen stream, and the residue was re-dissolved in 50 μL of ethylacetate. One μL of the solution was automatically injected into the GC–MS.

2.4.3. 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 condition. After the sample was dried under a nitrogen stream at room temperature, approximately 15 mg of finely cut hair was precisely weighed and extracted with 3 mL of methanol/5 M hydrochloric acid mixed solution (20:1) containing 100 μL of each IS methanol solution for 1 h under ultrasonication. Following storage at room temperature overnight, the hair was filtered off, and the filtrate was evaporated with a nitrogen stream and the residue was dissolved in 3 mL of 0.1 M potassium hydrogen phosphate buffer (pH 6.0). The solution was treated with Bond Elut Certify, derivatized and analyzed as above.

2.5. Calibration curves

The drug concentrations in the samples were calculated using the peak-area ratios of the base peak ions of the target compounds versus IS. The calibration curves for the determination were constructed by analyzing extracted drug-free control samples spiked with the standard solution as described above. The calibration samples containing 0, 10, 50, 100, 500, 1000, 2000 ng/mL (methylone or methcathinone) and 0, 50, 100, 250, 500, 1000, 2000 ng/mL (MBDB) for the rat plasma samples were prepared just before analysis. The samples containing 0, 5,10, 25, 50, 100, 200 ng/mg (methylone or MBDB) and 0, 1.0, 2.5, 5.0, 10, 25, 50 ng/mg (methcathinone) for the hair samples were also prepared. The limit of quantitation of each drug was chosen to be the concentration of the lowest calibration standard with an acceptable limit of 20% for both precision and accuracy.

2.6. Precision and accuracy of the method

The precision and accuracy of the method were evaluated by analyzing triplicates of the plasma samples which were spiked with the standard solutions containing 10, 50, 2000 ng/mL (methylone or methcathinone) and 50, 500, 2000 ng/mL (MBDB), respectively. For the analyses of hair, the control samples, spiked with the standard solutions each containing 5, 50, 200 ng/mg (methylone or MBDB) and 1, 10, 50 ng/mg (methcathinone), were evaluated. Accuracy, expressed as bias, was calculated as the difference between the amount of each drug added and recovered.

3. Results and discussion

3.1. Accuracy and precision of the methods

Under the chromatographic conditions used, there was no interference with all drugs or the deuterated internal standards

Table 1
Validation of results of the GC–MS analysis of methylone, MBDB and methcathinone for rat plasma and hair samples

	Compounds	Linear range	Linearity	Precision (%)		Accuracy (%)	
				Concentration	%	Concentration	%
Plasma (ng/mL)	Methylone	10–2000	$y = 0.0031x + 0.0815$, $R^2 = 0.9966$	10	11.6	10	7.3
				500	11.4	500	−1.0
				2000	17.3	2000	−2.7
	MBDB	50–2000	$y = 0.0019x + 0.0051$, $R^2 = 0.9999$	50	2.6	50	13.2
				500	0.2	500	−1.3
				2000	2.8	2000	0.9
	Methcathinone	10–2000	$y = 0.0011x + 0.0141$, $R^2 = 0.9992$	10	10.6	10	4.3
				500	2.4	500	−0.9
				2000	4.4	2000	6.5
Hair (ng/mg)	Methylone	5.0–200.0	$y = 0.0193x + 0.0358$, $R^2 = 0.9966$	5.0	1.8	5.0	14.0
				50.0	9.6	50.0	−1.5
				200.0	2.3	200.0	−1.6
	MBDB	5.0–200.0	$y = 0.0267x + 0.0649$, $R^2 = 0.9987$	5.0	13.9	5.0	4.2
				50.0	0.9	50.0	−1.4
				200.0	1.8	200.0	−4.4
	Methcathinone	1.0–50.0	$y = 0.0906x + 0.0059$, $R^2 = 0.9999$	1.0	5.1	1.0	13.3
				10.0	4.4	10.0	4.8
				50.0	4.7	50.0	−2.1

by any extractable endogenous materials in the control rat plasma and control rat hair. The calibration curves were linear over the concentration range 10–2000 ng/mL (except for MBDB; 50–2000 ng/mL) for rat plasma and 5.0–200.0 ng/mg (except for methcathinone; 1.0–50.0 ng/mg) for rat hair with good coefficients of determination of $r^2 \geq 0.997$. The precision and accuracy data of the analytical procedure for rat plasma and hair samples, spiked with standard solution of methylone, MBDB and methcathinone, are presented in Table 1. The precision of these drugs ranged from 0.2 to 17.3% and the accuracy ranged from −4.4 to 14.0%. These values are almost below 15% although the precision data for methylone in the plasma sample at 2000 ng/mL are a little higher.

3.2. Drug concentrations in rat plasma

After intraperitoneal administration of methylone hydrocloride, MBDB hydrocloride and methcathinone hydrocloride to DA rats at 5 mg/kg, the concentrations of each drug in rat plasma over 360 min were monitored using GC–MS-SIM. Time courses of the rat plasma concentrations of these drugs are shown in Fig. 2. Half-lives of methylone, MBDB and methcathinone in the plasma were 120, 170 and 220 min, respectively. Average peak plasma concentrations of these drugs were approximately 700–1500 ng/mL 15 or 30 min after the administration. The plasma AUCs of methylone, MBDB and methcathinone were 147 ± 12, 151 ± 17 and 191 ± 59 μg min/mL, respectively, and

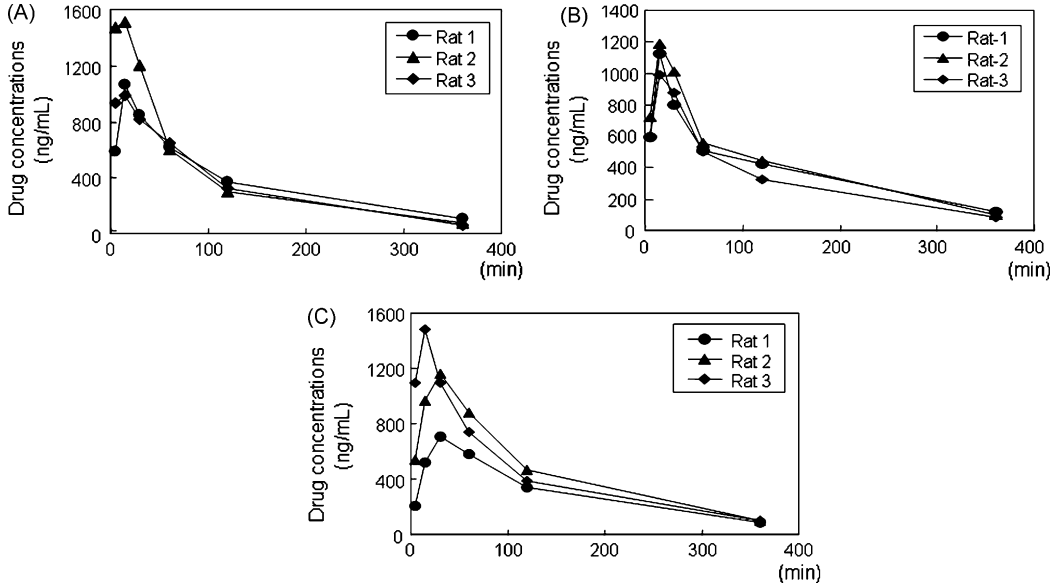

Fig. 2. The time courses of the rat plasma concentrations after administration of (A) methylone HCl, (B) MBDB HCl and (C) methcathinone HCl (i.p., 5 mg/kg, $n = 3$).

Table 2
Rat plasma AUCs, drug concentrations in rat hair and incorporation rates ([Hair]/AUC)

Compounds	Plasma AUC (μg min/mL)	Hair (ng/mg)	[Hair]/AUC
Cathinone*	291 ± 20	4.2 ± 0.9	0.01 ± 0.00
Methcathinone	191 ± 59	6.6 ± 1.9	0.04 ± 0.01
AP*	182 ± 7	18.1 ± 0.1	0.10 ± 0.02
MA*	125 ± 21	16.3 ± 2.3	0.13 ± 0.02
MDA*	411 ± 59	121.9 ± 27.5	0.30 ± 0.05
Methylone	147 ± 12	79.8 ± 22.3	0.55 ± 0.19
MDMA*	121 ± 16	93.4 ± 10.9	0.77 ± 0.05
MDEA*	165 ± 10	138.4 ± 4.4	0.85 ± 0.03
MMDA*	183 ± 50	215.0 ± 20.5	1.24 ± 0.06
MBDB	151 ± 17	164.5 ± 25.0	1.10 ± 0.25

$n = 3$.
* The data is from [3].

these values were close to those of their related compounds (AP, MA, MDMA, MDEA and MMDA) whose data have been reported in our previous study, as shown in Table 2.

3.3. Drug concentrations in rat hair

The concentrations of each drug in the rat hair are shown in Table 2. The results of GC–MS analyses of PFP-derivatized extracts from the control rat hair and the hair of rats after intraperitoneal administration of methylone hydrochloride at 5 mg/kg for 10 successive days were shown in Fig. 3. The concentrations of methylone and MBDB in the hair were 79.8 ± 22.3 and 164.5 ± 25.0 ng/mg, respectively. The value of MBDB was the second highest of all drugs shown in Table 2. However, the concentration of methcathinone in the hair was extremely low (6.6 ± 1.9 ng/mg) in spite of its relatively high AUC.

3.4. Incorporation rates of drugs into rat hair from rat plasma

As mentioned, we propose that the ratio of drug concentration in hair to AUC in plasma ([Hair]/AUC) could be one of the indexes that indicate the incorporation rate of drug into hair from plasma [1,2]. According to our proposal, [Hair]/AUC of methylone, MBDB and methcathinone were 0.55, 1.10 and 0.04, respectively. When these values were compared with those of their related compounds that have been studied previously [2,3], the order of the [Hair]/AUC was cathinone < methcathinone < AP < MA < EAP < MDA < methylone < MDMA < MDEA < MBDB < MMDA (Fig. 4). The [Hair]/AUC of MDA, MDMA and methylone was 3–40 times higher than those of AP, MA and methcathinone, suggesting that the methylenedioxy group on the benzene ring leads to consid-

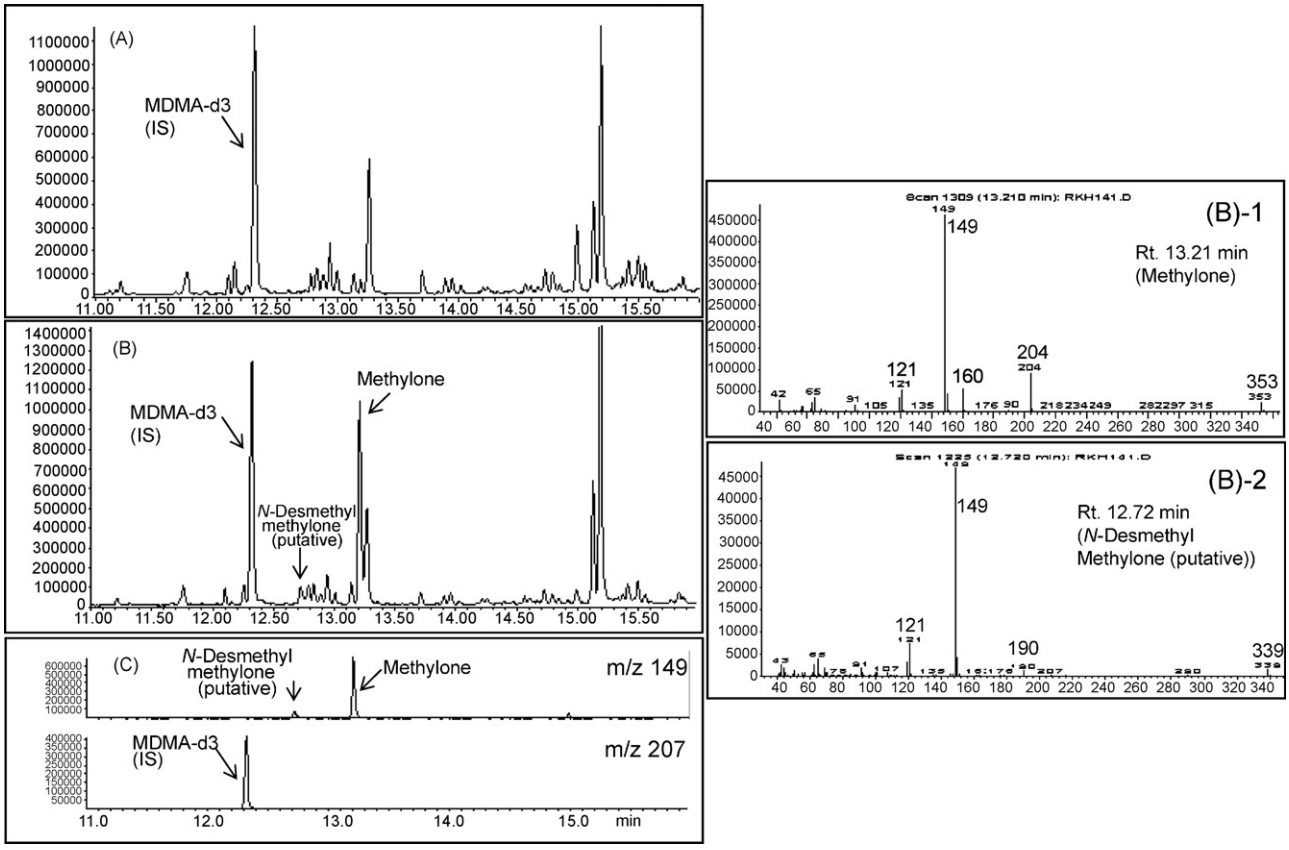

Fig. 3. GC–MS total ion chromatograms of PFP-derivatized extracts from (A) the control rat hair and (B) the hair of rat administered methylone HCl (i.p., 5 mg/kg × 10 days). Mass spectra of the peaks at 13.21 and 12.72 min of the chromatogram (B) are shown in (B)-1 (PFP-methylone) and (B)-2 (PFP-*N*-desmethylmethylone (putative)), respectively. The chromatograms of the selected monitoring ions of *m/z* 149 (the base peak ions of PFP-methylone and PFP-*N*-desmethylmethylone (putative)) and *m/z* 207 (the base peak ion of PFP-MDMA (IS)) are also shown in (C).

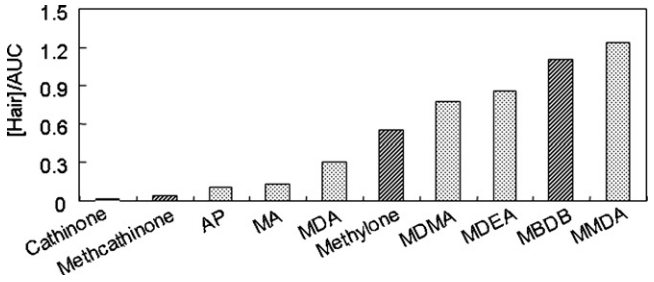

Fig. 4. Incorporation rates ([Hair]/AUC) of methylone, MBDB, methocathinone and their related compounds previously studied.

erably higher incorporation rates. However, the [Hair]/AUC of beta-ketoamphetamines, cathinone, methcathinone and methylone was 1/2–1/10 times lower in comparison with those of their corresponding amphetamines, AP, MA and MDMA, respectively. It is expected that the beta-carbonyl group might lead to lower incorporation rates. The [Hair]/AUC of methylone was lower than that of MDMA but higher than MA. Although methylone has both groups in its structure, the positive effect of the methylenedioxy group may be stronger than the negative effect of the beta-carbonyl group. On the other hand, the [Hair]/AUC of MBDB, which has methylenedioxyphenyl-2-butanamine structure, was higher than that of MDMA and the second highest to MMDA, although that of methcathinone, having beta-ketone in its structure, was extremely low.

4. Conclusions

As a result of an animal experiment, the incorporation rate ([Hair]/AUC) of MBDB was 1.4 times higher than that of MDMA, and a phenyl-2-butanamine structure may be more easily incorporated into hair than a phenyl-2-propamine. On the other hand, the value of methcathinone was unusually low, similar to cathinone. It is thought that these compounds, which have beta-carbonyl groups, may not be suitable for hair analysis because of their relatively low concentrations in the hair. The incorporation rate of methylone was 0.55. This value was four times larger than that of MA, but a little smaller than that of MDMA. It is suggested that the positive effect of the methylenedioxy group may be stronger than the negative effect of the beta-carbonyl group, in this case.

In our previous study [1], we determined melanin affinity and lipophilicity of 20 abused drugs, and these values were compared to their [Hair]/AUCs as an index of the incorporation tendency into hair. As a result, the combination of melanin affinity and lipophilicity showed a high correlation with the [Hair]/AUCs. In consideration of that result, those physico-chemical properties of methylone, methcathinone and MBDB could be significantly related to their [Hair]/AUCs.

In conclusion, as with MA and MDMA, the incorporation tendency of new designer drugs, methylone and MBDB (except for methcathinone) into hair is relatively high, and a hair sample would be a good specimen for the confirmation of the retrospective use of these drugs.

Acknowledgement

This research was supported by a Health Sciences Research Grant from the Ministry of Health, Labor and Welfare in Japan.

References

[1] Y. Nakahara, K. Takahashi, R. Kikura, Biol. Pharm. Bull. 18 (9) (1995) 1223.
[2] Y. Nakahara, R. Kikura, Arch. Toxicol. 70 (1996) 841.
[3] R. Kikura, Y. Nakahara, T. Mieczkowski, F. Tagliaro, Forensic Sci. Int. 84 (1997) 165.
[4] T.A. Dal Cason, R. Young, R.A. Glennon, Pharmacol. Biochem. Behav. 58 (4) (1997) 1109.
[5] N.V. Cozzi, M.K. Sievert, A.T. Shulgin, P. Jacob III, A.E. Ruoho, Eur. J. Pharmacol. 381 (1) (1999) 63.
[6] M.G. Bossong, J.P. Van Dijk, R.J. Niesink, Addict. Biol. 10 (4) (2005) 321.
[7] Y. Nakahara, K. Takahashi, M. Shimamine, Y. Takeda, J. Forensic Sci. 36 (1991) 70.
[8] R.A. Glennon, R. Young, B.R. Martin, T.A. Dal Cason, Pharmacol. Biochem. Behav. 50 (4) (1995) 601.
[9] H.T. Kamata, N. Shima, K. Zaitsu, T. Kamata, A. Miki, M. Nishikawa, M. Katagi, H. Tsuchihashi, Xenobiotica 36 (8) (2006) 709.