Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International xxx (2007) xxx–xxx
Forensic Science International
www.elsevier.com/locate/forsciint

Time-resolved hair analysis of MDMA enantiomers by GC/MS-NCI

Liliane Ferreira Martins [a,*], Michel Yegles [a], Nele Samyn [b], Johannes G. Ramaekers [c], Robert Wennig [a]

[a] National Laboratory of Health, Toxicology Division, University of Luxembourg, 162A, avenue de la Faïencerie, L-1511 Luxembourg, Luxembourg
[b] National Institute of Criminalistics and Criminology, Federal Public Service Justice, Brussels, Belgium
[c] Department of Neurocognition, Faculty of Psychology, Maastricht University, Maastricht, The Netherlands

Received 8 September 2006; received in revised form 8 January 2007; accepted 13 January 2007

Abstract

The aim of the study was to determine the enantioselective disposition of 3,4-methylenedioxymethamphetamine (MDMA) and other amphetamine-type stimulants (ATS) in segmented hair specimens of self-declared ecstasy abusers, who took part in a double-blind placebo-controlled six-way crossover study during approximately 7 weeks, during which they received a 75 and a 100 mg dose of racemic MDMA twice.

Hair specimens were washed and cut into pieces of 2 cm length. After digestion and solid phase extraction, the enantiomers were derivatized with a chiral agent (2*S*,4*R*)-*N*-heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride, developed at the authors laboratory and quantified by gas chromatography coupled to mass spectrometry operating in the negative chemical ionization mode.

Most of the hair specimens that were tested positive for MDMA showed a predominance of the (*R*)-enantiomer. The *R*/*S* ratios of MDMA varied between 1.02 and 2.75 and total concentrations ranged from 0.1 to 20.1 ng/mg. The enantiomers of its metabolite 3,4-methylenedioxyamphetamine (MDA) were also quantified in most hair segments. The *R*/*S* ratios of MDA varied between 0.60 and 1.60, while the concentrations of the enantiomers ranged from 10 to 160 pg/mg hair. When segmental analysis was performed on single hair specimens, no inversion of the *R* versus *S* ratios of MDMA and MDA was observed. The predominance of (*R*)-MDMA in hair was in accordance with those already published for other matrices. Furthermore, both enantiomers of amphetamine (AM) were also detected in hair segments of four volunteers and the *R*/*S* ratios ranged from 1.00 to 1.47.

Keywords: MDMA; MDA; Amphetamine; Segmental hair analysis; Enantiomeric ratios

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), also commonly known as ecstasy, XTC or Adam, is a synthetic amphetamine-type stimulant (ATS) widely used as a recreational drug among youth. This designer drug is a central nervous system (CNS) stimulant, which increases the secretion of serotonin in the synapses and inhibits the reuptake of serotonin [1]. Besides the desired euphoric and stimulating effects, its abuse is responsible for severe or even fatal intoxications [2–4]. 3,4-Methylenedioxyamphetamine (MDA), also referred to as the ''Love Drug'' is the *N*-demethylated metabolite of MDMA [4].

MDMA is usually available at the illicit drug market as a racemic mixture in the form of capsules, loose powder or tablets [5–7]. As expected, the two enantiomers of MDMA show different pharmacological properties. The (*S*)-enantiomer of MDMA has more CNS stimulant activity than the (*R*)-MDMA [1,8,9]. In the case of MDA, both optical isomers cause long-term serotonin neurotoxicity, with (*R*)-MDA having a more hallucinogenic effect and (*S*)-MDA being more amphetamine-like [9,10].

Several studies have described the enantioselective metabolism and disposition of these compounds in the human body. Due to the longer elimination half-life of (*R*)-MDMA, MDMA showed a stereoselective disposition, which results in *R* versus *S* ratios > 1 for MDMA in urine, plasma or vitreous humour and in other human tissues like liver and bile [4–7,11–17]. In contrast, the concentrations of (*S*)-MDA exceeded in most cases those of (*R*)-MDA in urine or plasma due to the faster

* Corresponding author. Tel.: +352 46 66 44 6488; fax: +352 22 13 31.
E-mail address: liliane.martins@lns.etat.lu (L.F. Martins).

0379-0738/$ – see front matter © 2007 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2007.01.008

metabolisation of (*S*)-MDMA. However, according to a few pharmacokinetic studies in urine and plasma, approximately 36 h after consumption of racemic MDMA, either comparable enantiomeric amounts of its metabolite MDA or even a predominance of (*R*)-MDA were observed [5,7,13,18].

In recent years, hair analysis for drugs of abuse has rapidly emerged as a useful tool for detecting and monitoring drugs over a long time period [19,20]. Hair analysis provides useful information on individual's drug history from weeks to months. However, it is important to study the mechanisms of drug incorporation into the hair matrix in order to evaluate correctly the results obtained from the hair analysis. There exist very limited data about the stereoselective incorporation and the disposition of MDMA enantiomers in human hair. Tagliaro et al. analyzed one hair specimen obtained from a single real ecstasy user by capillary electrophoresis (CE) and observed a predominance of the (*R*)-MDMA, while the electropherogram of its metabolite MDA clearly showed comparable enantiomeric concentrations [21]. Martins et al. observed also in one hair specimen an *R* versus *S* ratio > 1 for MDMA and *R*/*S* ratio < 1 for its metabolite MDA [22]. Additionally, to the best of our knowledge, no data about the enantioselective disposition of MDMA and MDA in human segmented hair have been published yet.

The present study was designed to determine the concentrations of MDMA, MDA, AM, methamphetamine (MA) and 3,4-methylenedioxyethlyamphetamine (MDEA) in hair segments from self-declared ecstasy abusers, who participated in a controlled study at the University of Maastricht.

2. Materials and methods

2.1. Reagents and material

Standard solutions of 1 mg/mL of racemic amphetamine (AM), methamphetamine (MA), MDMA, MDA, 3,4-methylenedioxyethylamphetamine (MDEA), (*R*)-AM and (*S*)-AM in methanol and methanolic deuterated standards of racemic AM-d_5, MA-d_5, MDA-d_5, MDMA-d_5 and MDEA-d_5 were purchased from Radian Corporation (Austin, TX). Cyclohexane and sodium hydroxide (NaOH) were purchased from Sigma–Aldrich (Bornem, Belgium). Sodium carbonate (Na_2CO_3, 99.5%), sodium hydrogenocarbonate ($NaHCO_3$, 95.5%) were obtained from Merck (Overijse, Belgium). Clean Screen® C18 ZSDAU020 columns were obtained from United Chemical Technologies (Bristol, USA). All chemicals were of analytical grade. The derivatization reagent (2*S*,4*R*)-*N*-heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride [(*S*,*R*)-HFBOPCl] was synthesized according to the literature [22].

2.2. Hair specimens collection

Drug free hair specimens, collected from healthy subjects, were used for preparation of calibration specimens.

The authentic hair specimens were obtained from 14 self-declared ecstasy abusers following a double-blind placebo-controlled six-way crossover study realized in Maastricht (The Netherlands). The study was conducted under the guidelines for the protection of human subjects and each volunteer provided informed consent.

The aim of this study was to assess the acute effects of MDMA and alcohol, alone and in combination, on behavioural measures of impulsivity and risk-taking behaviour [23]. The treatments consisted of MDMA 0, 75 or 100 mg with and without alcohol (Table 1). The mean period between the successive administrations was 1 week. The participating volunteers were subjected to a urine drug test for the most frequently used illicit drugs (including amphetamines/methamphetamines) at the beginning of each test day. The total duration of this double-blind placebo-controlled six-way crossover study was 7 weeks (mean value). It has to be noted that no detailed report about the ecstasy use of these subjects before the test period exists.

Table 1
Ingestion data of the double-blind placebo-controlled six-way crossover study

Period[a]	Group 1 ($n = 2$)[b]	Group 2 ($n = 3$)	Group 3 ($n = 2$)	Group 4 ($n = 4$)	Group 5 ($n = 1$)	Group 6 ($n = 2$)
	Administration					
1	a	b	c	e	f	d
2	e	f	d	a	c	b
3	c	d	f	b	a	e
4	d	c	b	f	e	a
5	f	e	a	d	b	c
6[c]	b	a	e	c	d	f

Abbreviations: a, placebo; b, 75 mg MDMA; c, 100 mg MDMA; d, alcohol; e, 75 mg MDMA + alcohol; f, 100 mg MDMA + alcohol.
[a] Mean period between successive treatments was 1 week.
[b] Number of volunteers per group.
[c] Collection of hair specimens.

Hair specimens were collected during the last administration day. Thus, as the growth rate of hair is 1–1.3 cm/month [24], the proximal hair segment of 2 cm corresponding to a period of 10 weeks before the hair cut, would cover the major period of the controlled study and a mean of 3 weeks of ''uncontrolled'' ATS consumption.

2.3. Sample preparation

The hair pre-treatments and the derivatization procedure were realized according to the methods described by Martins et al. [22,25].

Hair specimens were washed with water (1 min) and two times with acetone (1 min). After drying with warm air, the hair specimens were completely cut into segments of 2 cm. For one volunteer (subject 1), sufficient sample material was available to perform a different segmentation: the proximal hair segment of 5 cm was cut into segments of 1 cm and the distal hair segment (from 5 to 11 cm) was cut into segments of 2 cm.

The digestion and extraction of the hair specimens were performed according to the procedure previously described by Martins et al. [25]. Hair specimens were digested with 1 M sodium hydroxide at 100 °C during 30 min and extracted by a solid phase procedure using Cleanscreen ZSDAU020. The ATS were eluted with 3 mL of dichloromethane/2-propanol/ammonia (80:20:2, v/v). After the addition of 10 μL of 1% (volume fraction) HCl in methanol, the solvent was removed under nitrogen at 37 °C.

Drug enantiomers were converted into their diastereomeric derivatives by dissolving the dry residue into 200 μL of an 5% aqueous carbonate buffer (70 g/L $NaHCO_3$–30 g/L Na_2CO_3; pH 9.5) followed by the addition of 20 μL of a 0.2 M solution of (*S*,*R*)-HFBOPCl [22]. The mixture was left on a rotary shaker at room temperature for 15 min. Thereafter, 100 μL of cyclohexane were added and the reaction vial was left again on the rotary shaker for 1 min. The phases were separated by centrifugation (10,500 × *g* for 4 min), and the upper phase was transferred to an autosampler vial for analysis.

2.4. Instrumentation

An Agilent gas chromatography coupled to mass spectrometry (GC/MS) instrument equipped with an 7673A automatic sampler, a 6890 series II gas chromatograph and a 5973 mass selective detector (Agilent Technologies, Brussels, Belgium) was used. The gas chromatograph was equipped with a Hewlett-Packard HP-5MS (crosslinked 5% phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 μm film thickness). The injector temperature was 260 °C, the GC/MS interface temperature was 280 °C; the helium carrier gas flow rate was 1 mL/min. Injection volume was 3 μL. Initial temperature was 150 °C for 2 min, followed by an increase of 20 °C/min

to 220 °C, 5 °C/min from 220 to 260 °C, 30 °C/min to 305 °C. The mass spectrometer was running in the negative chemical ionization (NCI) mode with methane (standard purity > 99.99%) as reagent gas (flow of 40%) using pulsed splitless injection mode. The following ions were monitored in the selected-ion monitoring (SIM) mode: AM 640, 451, **620**; AM-d_5 645, 456, **625**; MA 654, 614, **634**; MA-d_5 659, 619, **639**; MDA 644, 684, **664**; MDA-d_5 649, 689, **669**; MDMA 698, 658, **678**; MDMA-d_5 703, 663, **683**; MDEA 712, 672, **692** and MDEA-d_5 717, 677, **697**. The ions marked in bold were used as target ions for quantitation.

2.5. Quantitation procedure

For calibration, 10 mg of drug-free hair were spiked with ATS (AM, MA, MDA, MDMA and MDEA), each enantiomer covering the range from 0.002 to 20 ng/mg. The internal standards (IS) ATS-d_5 were added at a fixed concentration of 2.5 ng/mg. Assuming a 1:1 ratio between the enantiomers of each analyte, the calibration curves were obtained for each enantiomer by plotting the peak-area ratios of the spiked calibrations standards versus their concentrations. The regression line was calculated using a weighted ($1/x$) least-square regression model.

The enantiomers of MDMA, MDA and of the other ATS tested (AM, MA and MDEA) in abusers' hair were quantitated by comparison of their peak-area ratios (enantiomer of analyte versus corresponding enantiomers of IS) to calibration curves. The full validation data of the analytical method has been published previously [22].

3. Results and discussion

The enantioselective disposition of ATS has been examined in hair segments from 14 self-declared ecstasy abusers who had been administered racemic ecstasy via the oral route [23]. A total of 47 hair segments of 2 cm length were analyzed for the presence of MDMA and MDA enantiomers as well as for the optical isomers of AM, MA and MDEA.

The concentrations of the enantiomers and the *R* versus *S* ratios of the ATS detected during segmental hair analysis from the 14 subjects are summarized in the Tables 2 and 3, respectively. Both enantiomers of MDMA have been detected in the proximal 2 cm segment, which in major part corresponded to the time when these volunteers participated in the

Table 2
MDMA, MDA and AM enantiomer concentrations determined after segmental hair analysis

Subject	ATS in hair (ng/mg)	0–2 (cm)		2–4 (cm)		4–6 (cm)		6–8 (cm)		8–10 (cm)		10–12 (cm)	
		R	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
1	MDMA	0.88	0.51	1.23	0.77	1.07	0.64	0.73	0.42	0.44	0.26	0.30	0.18
	MDA	0.04	0.05	0.06	0.07	0.06	0.06	0.04	0.04	0.02	0.03	0.02	0.02
	AM	0.25	0.17	0.36	0.26	0.37	0.28	0.26	0.19	0.20	0.15	0.15	0.11
2	MDMA	0.23	0.09	0.22	0.08	na		na		na		na	
3	MDMA	0.22	0.09	na		na		na		na		na	
4	MDMA	10.12	9.97	8.88	8.58	6.67	6.46	5.07	4.77	na		na	
	MDA	0.06	0.10	0.07	0.10	0.07	0.10	0.07	0.10	na		na	
	AM	0.19	0.17	0.26	0.22	0.31	0.28	0.34	0.32	na		na	
5	MDMA	0.76	0.41	0.18	0.10	na		na		na		na	
	MDA	0.03	0.03	nd	nd	na		na		na		na	
6	MDMA	0.49	0.25	0.18	0.11	na		na		na		na	
	MDA	0.02	0.03	nd	nd	na		na		na		na	
7	MDMA	0.94	0.41	1.03	0.55	1.21	0.77	1.34	0.85	na		na	
	MDA	0.04	0.04	0.04	0.04	0.05	0.04	0.06	0.04	na		na	
	AM	nd	nd	nd	nd	0.06	0.06	0.09	0.09	na		na	
8	MDMA	0.14	0.06	1.13	0.49	na		na		na		na	
	MDA	nd	nd	0.05	0.06	na		na		na		na	
9	MDMA	2.60	1.01	2.92	1.16	na		na		na		na	
	MDA	0.13	0.09	0.16	0.10	na		na		na		na	
	AM	3.67	2.76	2.92	2.26	na		na		na		na	
10	MDMA	0.51	0.22	0.17	0.08	0.14	0.06	0.11	0.06	0.10	0.04	na	
	MDA	0.02	0.02	0.01	0.01	0.01	0.01	nd	nd	nd	nd	na	
11	MDMA	1.24	0.87	0.40	0.21	0.36	0.17	0.35	0.17	0.25	0.14	0.16	0.08
	MDA	0.03	0.05	0.02	0.02	0.02	0.02	0.01	0.01	nd	nd	nd	nd
12	MDMA	0.51	0.22	nd	nd	nd	nd	nd	nd	nd	nd	na	
13	MDMA	0.17	0.07	nd	nd	na		na		na		na	
14	MDMA	0.54	0.22	nd	nd	na		na		na		na	

na, Hair segment not available; nd, not detected.

Table 3
Determination of the enantiomeric ratios R vs. S of MDA, MDMA, AM after segmental hair analysis

Subject	ATS in hair	*R* vs. *S* ratios in hair segments					
		0–2 (cm)	2–4 (cm)	4–6 (cm)	6–8 (cm)	8–10 (cm)	10–12 (cm)
1	MDMA	1.73	1.60	1.67	1.74	1.70	1.67
	MDA	0.80	0.86	1.00	1.00	0.67	1.00
	AM	1.47	1.39	1.32	1.37	1.33	1.36
2	MDMA	2.56	2.75	na	na	na	na
3	MDMA	2.44	na	na	na	na	na
4	MDMA	1.02	1.04	1.03	1.06	na	na
	MDA	0.60	0.70	0.70	0.70	na	na
	AM	1.12	1.18	1.11	1.06	na	na
5	MDMA	1.85	1.80	na	na	na	na
	MDA	1.00	nd	na	na	na	na
6	MDMA	1.92	1.64	na	na	na	na
	MDA	0.67	nd	na	na	na	na
7	MDMA	2.29	1.85	1.57	1.58	na	na
	MDA	1.00	1.00	1.25	1.50	na	na
	AM	nd	nd	1.00	1.00	na	na
8	MDMA	2.33	2.31	na	na	na	na
	MDA	nd	0.83	na	na	na	na
9	MDMA	2.60	2.52	na	na	na	na
	MDA	1.44	1.60	na	na	na	na
	AM	1.33	1.29	na	na	na	na
10	MDMA	2.32	2.13	2.33	1.83	2.50	na
	MDA	1.00	1.00	1.00	nd	nd	na
11	MDMA	1.43	1.91	2.12	2.06	1.76	2.00
	MDA	0.60	1.00	1.00	1.00	nd	nd
12	MDMA	2.32	nd	nd	nd	nd	na
13	MDMA	2.43	nd	na	na	na	na
14	MDMA	2.46	nd	na	na	na	na

na, Not available; nd, not detected.

study. Median concentrations of (*R*)-MDMA and (*S*)-MDMA in the proximal segment were 0.54 and 0.24 ng/mg, respectively, ranging from 0.14 to 10.12 ng/mg for (*R*)-MDMA and from 0.06 to 9.97 ng/mg for (*S*)-MDMA. No correlation could be established between the doses of MDMA ingested under controlled conditions and the concentration of MDMA found in the proximal hair segment. One reason may be that the 2 cm proximal segment collected did not cover the whole period of the controlled study and that the MDMA concentrations observed could also be the result of the ''uncontrolled use'' before the study. Furthermore, although urine tests for the most frequently used illicit drugs had only been performed at each administration day, an undeclared consumption of MDMA cannot be excluded during the controlled study.

In total, 39 of the 47 hair segments analyzed (all hair segments) contained MDMA enantiomers and the total concentration of MDMA ranged from 0.2 to 20.1 ng/mg. In each hair segment, the concentration of (*R*)-MDMA exceeded those of the (*S*)-MDMA with ratios varying from 1.02 to 2.75. MDA enantiomers were also detected during segmental hair analysis (Table 2), but their concentrations were lower than those of the non-metabolized MDMA (metabolite/parent drug MDA/MDMA ratio < 0.09), which suggests that MDA is principally the result of the metabolisation of MDMA rather than the result of its consumption as a parent drug [26,27]. The enantiomeric ratios of MDA varied from 0.60 to 1.60 in the hair segments tested positive for MDA; most MDA positive hair segments presented comparable enantiomeric concentrations (Table 2). Furthermore, most MDA positive hair segments from one individual presented comparable enantiomeric ratios.

The presence of both enantiomers of AM in the proximal hair segment and/or in the distal hair segments of four subjects indicated that they had also consumed amphetamine. Fig. 1 represents the chromatograms of two hair segments from subject 9, who has been tested positive for both enantiomers of MDMA, MDA and AM. The *R*/*S* ratios of AM determined during this segmental hair analysis ranged from 1.00 to 1.47 with higher concentrations of (*R*)-AM in most hair segments (Table 3). Although the subjects were tested by means of a urine screening just before the administration, the presence of AM enantiomers in the proximal hair segment of some volunteers may be the result of an undeclared consumption of AM between the administration sessions and/or of an AM ingestion before the controlled study.

To the best of our knowledge, this study describes for the first time a distribution of the MDMA enantiomers and those of its metabolite MDA by segmental hair analysis. The predominance of the (*R*)-MDMA in the hair segments confirms the results of Tagliaro et al. [21] and Martins et al. [22] obtained after analysis of a single real case ''ecstasy user''. Furthermore, the stereoselective disposition of MDMA enantiomers ($R/S > 1$) observed in the hair segments is in accordance with those

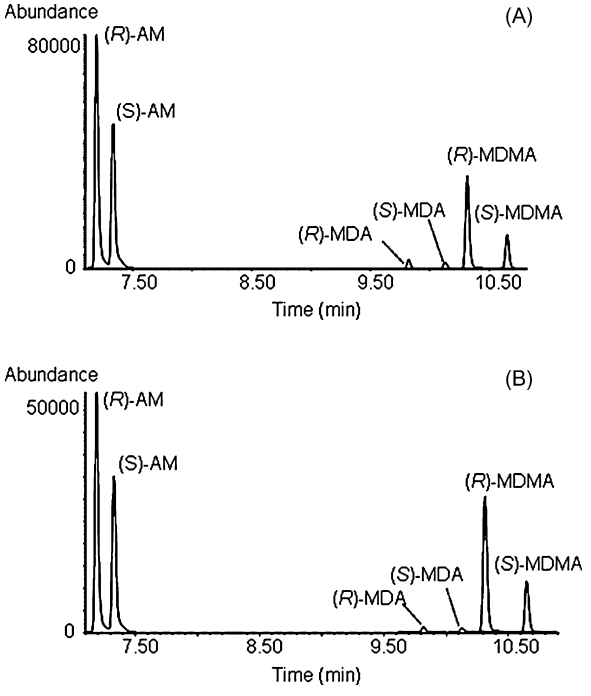

Fig. 1. Chromatograms of two extracts of hair segments from volunteer 9 who tested positive for AM, MDA and MDMA enantiomers. (A) Segment from 0 to 2 cm; (B) hair segment from 2 to 4 cm. Single ion monitoring at $m/z = 620$ for AM, $m/z = 664$ for MDA and $m/z = 678$ for MDMA.

Table 4
Concentrations and enantiomeric ratios of MDA, MDA and AM determined after a different segmental hair analysis of the hair bundle of volunteer 1

Segment (cm)	(*R*)-MDMA (ng/mg)	(*S*)-MDMA (ng/mg)	*R* vs. *S* ratio	(*R*)-MDA (ng/mg)	(*S*)-MDA (ng/mg)	*R* vs. *S* ratio	(*R*)-AM (ng/mg)	(*S*)-AM (ng/mg)	*R* vs. *S* ratio
0–1	0.64	0.38	1.68	0.03	0.04	0.75	0.25	0.17	1.47
1–2	1.11	0.64	1.73	0.05	0.06	0.83	0.24	0.16	1.50
2–3	1.27	0.78	1.63	0.06	0.07	0.86	0.33	0.24	1.37
3–4	1.19	0.75	1.59	0.06	0.07	0.86	0.39	0.29	1.34
4–5	1.07	0.64	1.67	0.06	0.06	1.00	0.37	0.28	1.32
5–7	0.75	0.45	1.67	0.04	0.04	1.00	0.29	0.22	1.32
7–9	0.50	0.29	1.72	0.02	0.03	0.67	0.20	0.16	1.25
9–11	0.35	0.21	1.67	0.01	0.01	1.00	0.21	0.16	1.31

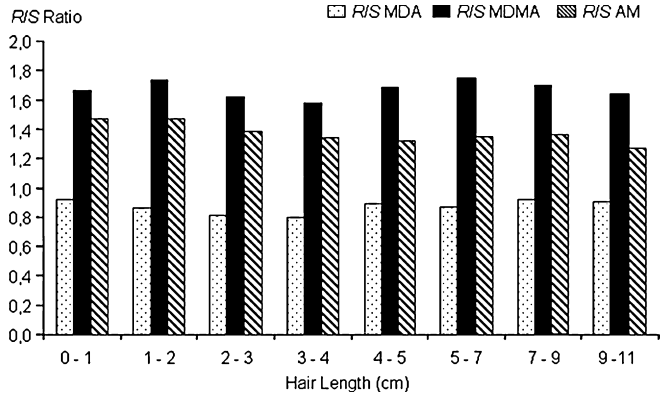

Fig. 2. Distribution of the enantiomeric ratios *R* vs. *S* of MDMA, MDA and AM after segmental hair analysis of the hair bundle of subject 1.

determined in other human matrices like plasma or urine [4–7,11–17]. Inter-individual differences observed for the disposition of the MDA enantiomers were also reported by some authors for other human matrixes [5,7,13,18].

The higher concentration of (*R*)-AM determined in our study was also confirmed by Nyström et al. [28] in hair specimens from one patient receiving racemic AM. These results seem also to reinforce the assumption that the mechanism of incorporation of ATS in hair is not enantioselective, implying that no inversion of the R/S ratio was observed during the sequestration into the keratin hair matrix. Thus, the disposition of ATS enantiomers may be the consequence of the hepatic stereoselective metabolism, the faster metabolism and/or the faster renal elimination of the (*S*)-isomers [29].

The enantiomeric ratios of MDMA, MDA and AM remained relatively stable in all the segments of one single hair bundle (Tables 3 and 4). Even when a "1 cm" segmentation was done for one hair specimen (Table 4; Fig. 2), the enantiomeric disposition of the MDMA, MDA and AM followed the same trend from the proximal hair segment to the distal hair segment and no inversion of the enantiomeric ratios was observed, which may indicate that the corresponding (*R*)- and (*S*)-enantiomers present the same stability after incorporation in the hair specimens.

4. Conclusion

This study has provided for the first time important information about the incorporation and the stability of MDMA, MDA and AM enantiomers in hair. The collected data have shown a stereoselective disposition of MDMA, MDA and AM in hair similar to other biological specimens. Furthermore, the quite stable distribution of the enantiomeric ratios during segmental analysis of single hair shafts showed that both enantiomers were comparably stable after their incorporation into the hair matrix.

References

[1] G. Rudnick, S.C. Wall, The molecular mechanism of "ecstasy" [3,4-methylenedioxymethamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 1817–1821.
[2] J.A. Henry, K.J. Jeffreys, S. Dawling, Toxicity and deaths from 3,4-methylenedioxymethamphetamine ("ecstasy"), Lancet 340 (1992) 384–387.
[3] G.P. Dowling, E.T. McDonough, R.O. Bost, 'Eve' and 'Ecstasy'. A report of five deaths associated with the use of MDEA and MDMA, JAMA 257 (1987) 1615–1617.
[4] K.A. Moore, A. Mozayani, M.F. Fierro, A. Poklis, Distribution of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) stereoisomers in a fatal poisoning, Forensic Sci. Int. 83 (1996) 111–119.
[5] J.K. Fallon, A.T. Kicman, J.A. Henry, P.J. Milligan, D.A. Cowan, A.J. Hutt, Stereospecific analysis and enantiomeric disposition of 3,4-methylenedioxymethamphetamine (ecstasy) in humans, Clin. Chem. 45 (1999) 1058–1069.
[6] T. De Boer, L.P. Tan, P. Gorter, R.M.A. Van de Wal, J.J. Kettenes-van den Bosch, E.A. De Bruijn, R.A.A. Maes, Gas chromatographic/mass spectrometric assay for profiling the enantiomers of 3,4-methylenedioxyamphetamine and its chiral metabolites using positive chemical ionization ion trap mass spectrometry, J. Mass Spectrom. 32 (1997) 1236–1246.
[7] D. Hensley, J.T. Cody, Simultaneous determination of amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), and methylenedioxyethylamphetamine (MDEA) enantiomers by GC-MS, J. Anal. Toxicol. 23 (1999) 518–523.
[8] F. Sadeghipour, J.-L. Veuthey, Enantiomeric separation of four methylenedioxylated amphetamines on beta-cyclodextrin chiral stationary phases, Chromatographia 47 (1998) 285–288.
[9] F. Benazzi, M. Mazzoli, Psychiatric illness associated with "ecstasy", Lancet 338 (1991) 1520.
[10] C.J. Schmidt, Acute administration of methylenedioxymethamphetamine: comparison with the neurochemical effects of its *N*-desmethyl and *N*-ethyl analogs, Eur. J. Pharmacol. 136 (1987) 81–88.
[11] F.T. Peters, N. Samyn, C.T. Lamers, W.J. Riedel, T. Kraemer, G. De Boeck, H.H. Maurer, Drug testing in blood:validated negative-ion chemical ionization gas chromatographic-mass spectrometric assay for enantioselective measurement of the designer drugs 3,4-methylenedioxyamphetamine,

3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine and its application to samples from a controlled study with MDMA, Clin. Chem. 51 (2005) 1811–1822.

[12] F.T. Peters, N. Samyn, M. Wahl, T. Kraemer, G. De Boeck, H.H. Maurer, Concentrations and ratios of amphetamine, methamphetamine, MDA, MDMA, and MDEA enantiomers determined in plasma samples from clinical toxicology and driving under the influence of drugs cases by GC-NICI-MS, J. Anal. Toxicol. 27 (2003) 552–559.

[13] N. Pizarro, M. Farré, M. Pujadas, A.M. Peiró, P.N. Roset, J. Joglar, R. De La Torre, Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites in human samples including the catechol-type metabolite (3,4-dihydroxymethamphetamine), Drug Metab. Dispos. 32 (2004) 1001–1007.

[14] N. Pizarro, J. Ortuno, M. Farré, C. Lopz-Hernandéz, M. Pujadas, A. Llebaria, J. Joglar, P.N. Roset, M. Mas, J. Segura, J. Cami, R. De La Torre, Determination of MDMA and its metabolites in blood and urine by GC-MS and analysis of enantiomers by capillary electrophoresis, J. Anal. Toxicol. 26 (2002) 157–165.

[15] M. Brunnenberg, K.-A. Kovar, Stereospecific analysis of ecstasy-like *N*-ethyl-3,4-methylenedioxyamphetamine and its metabolites in humans, J. Chromatogr. B 751 (2001) 9–18.

[16] N. Pizarro, A. Llebaria, S. Cano, J. Joglar, M. Farré, J. Segura, R. De La Torre, Stereochemical analysis of 3,4-methylenedioxymethamphetamine and its main metabolites by gas chromatography/mass spectrometry, Rapid Commun. Mass Spectrom. 17 (2003) 330–336.

[17] M. Lanz, R. Brenneisen, W. Thormann, Enantioselective determination of 3,4-methylene-dioxymethamphetamine and two of its metabolites in human urine by cyclodextrin-modified capillary zone electrophoresis, Electrophoresis 18 (1997) 1035–1043.

[18] J. Buechler, M. Schwab, G. Mikus, B. Fischer, l. Hermle, C. Marx, G. Grön, M. Spitzer, K.-A. Kovar, Enantioselective quantitation of the ecstasy compound (*R*)- and (*S*)-*N*-ethyl-3,4-methylenedioxyamphetamine and its major metabolites in human plasma and urine, J. Chromatogr. B 793 (2003) 207–222.

[19] W.A. Baumgartner, V.A. Hill, W.H. Blahd, Hair analysis for drugs of abuse, J. Forensic Sci. 34 (1989) 1433–1453.

[20] M.R. Moeller, P. Fey, R. Wennig, Simultaneous determination of drugs of abuse (opiates, cocaine and amphetamine) in human hair by GC/MS and its application to a methadone treatment program, Forensic Sci. Int. 63 (1993) 185–206.

[21] F. Tagliaro, G. Manetto, S. Bellini, D. Scarcella, F.P. Smith, M. Marigo, Simultaneous chiral separation of 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethylamphetamine (MDE), ephedrine, amphetamine and methamphetamine by capillary electrophoresis in uncoated and coated capillaries with native beta-cyclodextrin as the chiral selector. Preliminary application to the analysis of urine and hair, Electrophoresis 19 (1998) 42–50.

[22] L.F. Martins, M. Yegles, H.-S. Chung, R. Wennig, Sensitive, rapid and validated gas chromatography/negative ion chemical ionization-mass spectrometry assay including derivatization with a novel agent for the enantioselective quantification of amphetamine-type stimulants in hair, J. Chromatogr. B 842 (2006) 98–105.

[23] J.G. Ramaekers, K.P. Kuypers, Acute effects of 3,4-methylenedioxymethamphetamine (MDMA) on behavioral measures of impulsivity: alone and in combination with alcohol, Neuropsychopharmacology 31 (2006) 1048–1055.

[24] M.R. Harkey, Anatomy and physiology of hair, Forensic Sci. Int. 63 (1993) 9–18.

[25] L. Martins, M. Yegles, H.-S. Chung, R. Wennig, Simultaneous enantioselective determination of amphetamine and congeners in hair specimens by negative chemical ionization gas chromatography-mass spectrometry, J. Chromatogr. B 825 (2005) 57–62.

[26] M. Rothe, F. Pragst, K. Spiegel, T. Harrach, K. Fischer, J. Kunkel, Hair concentrations and self-reported abuse history of 20 amphetamine and ecstasy users, Forensic Sci. Int. 89 (1997) 111–128.

[27] G.A. Cooper, D.L. Allen, K.S. Scoot, J.S. Oliver, J. Ditton, I.D. Smith, Hair analysis: self-reported use of ''speed'' and ''ecstasy'' compared with laboratory findings, J. Forensic Sci. 45 (2000) 400–406.

[28] I. Nyström, T. Trygg, P. Woxler, J. Ahlner, R. Kronstrand, Quantitation of *R*-(−)- and *S*-(+)-amphetamine in hair and blood by gas chromatography-mass spectrometry: an application to compliance monitoring in adult-attention deficit hyperactivity disorder treatment, J. Anal. Toxicol. 29 (2005) 682–688.

[29] B.D. Paul, J. Jemionek, D. Lesser, A. Jacobs, Enantiomeric separation and quantification of (±)-amphetamine, (±)-methamphetamine, (±)-MDA, (±)-MDMA and (±)-MDEA in urine specimens by GC-EI-MS after derivatization with (*R*)-(−)- or (*S*)-(+)-α-methoxy-α-(trifluromethy)phenylacetyl cholride (MTPA), J. Anal. Toxicol. 28 (2004) 449–455.

Please cite this article in press as: L.F. Martins et al., Time-resolved hair analysis of MDMA enantiomers by GC/MS-NCI, Forensic Sci. Int. (2007), doi:10.1016/j.forsciint.2007.01.008