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Simultaneous enantioselective determination of amphetamine and congeners in hair specimens by negative chemical ionization gas chromatography–mass spectrometry

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Abstract

Enantioselective quantification of amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDEA) enantiomers in hair using gas chromatography–mass spectrometry (GC–MS) is described. Hair specimens were digested with 1 M sodium hydroxide at 100 °C for 30 min and extracted by a solid phase procedure using Cleanscreen ZSDAU020. Extracted analytes were derivatised with (*S*)-heptafluorobutyrylprolyl chloride and the resulting diastereoisomers were quantified by GC–MS operating in the negative chemical ionization mode. Extraction yields were between 73.0 and 97.9%. Limits of detection varied in the range of 2.1–45.9 pg/mg hair, whereas the lowest limits of quantification varied between 4.3 and 91.8 pg/mg hair. Intra- and inter-assay precision and respective accuracy were acceptable. The enantiomeric ratios (*R* versus *S*) of AM, MA, MDA, MDMA and MDEA were determined in hair from suspected amphetamine abusers. Only MA and AM enantiomers were detectable in this collective and the quantification data showed in most cases higher concentrations of (*R*)-MA and (*R*)-AM than those of the corresponding (*S*)-enantiomers.

Keywords: Hair analysis; Amphetamine; Amphetamine derivatives; Enantiomeric ratio

1. Introduction

Amphetamine (AM), methamphetamine (MA) and the amphetamine-derived designer drugs like 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDEA) are a major class of central nervous system stimulants (CNS) widely abused in many countries. All of these compounds contain a chiral center and their enantiomers show different pharmacological properties. Thus, the (*S*)-isomers of AM, MA and MDMA, have more CNS stimulant activity of than the (*R*)-isomers [1,2]. While for MDA, MDMA and MDEA no legal precursor drugs exist, it is known that several therapeutic drugs contain or are metabolized to AM and MA. For example, selegiline for treatment of Parkinson's disease, is metabolized to (*R*)-MA and (*R*)-AM [3–6] and Vicks Inhaler, a nasal decongestant, contains (*R*)-MA [7]. For correct interpretation of the positive results, the separation and identification of enantiomers might be helpful to differentiate therapeutic from illicit ingestion of AM, MA, or one of their precursor drugs [4–6].

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 [8,9]. Various methods have been published for the detection of AM, MA, and methylenedioxylated amphetamines in hair. Analytical procedures used

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for the enantioselective separation in hair were capillary electrophoresis [10,11] and liquid chromatography methods (HPLC) [12–14]. However, these developed methods generally lack sensitivity.

Recently, a negative chemical ionization gas chromatography–mass spectrometry (GC/NCI–MS) assay was developed for the quantification and the determination of enantiomeric ratios of AM, MA, MDA, MDMA or MDEA enantiomers in plasma [15–17] and oral fluid [18] after derivatization by (*S*)-heptafluorobutyrylprolyl chloride ((*S*)-HFBPCl) [19].

This feasibility study describes a method for the simultaneous determination of AM, MA, MDA, MDMA and MDEA enantiomers in hair by GC/NCI–MS, using the (*S*)-HFBPCl as chiral derivatization reagent. The method was applied to control specimens for validation purposes and finally to 11 suspected amphetamine abusers' hair specimens.

2. Material and methods

2.1. Chemicals and equipment

Standard solutions of 1 mg/mL of racemic AM, MA, MDA, MDMA and MDEA 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). *S*-(−)-1-phenylethylamine ((*S*)-PEA; $S{:}R \geq 99.5{:}05$) and *R*-(+)-1-phenylethylamine ((*R*)-PEA, $S{:}R \geq 99.5{:}05$) were obtained from Sigma–Aldrich (Bornem, Belgium). Clean Screen® C_{18} ZSDAU020 columns were obtained from United Chemical Technologies (Bristol, USA). The ball mill used was purchased from Retsch (Haan, Germany). All reagents, solvents and substances were of analytical grade.

2.2. Hair specimens

Drug free hair specimens were collected from healthy subjects in the authors' laboratory. As in Luxembourg no sufficient amphetamines positive hair specimens were available, 11 suspected amphetamines abusers' hair specimens from South Korea were analyzed.

2.3. Hair specimen extraction for GC/NCI–MS analysis

Total length of the hair specimens were washed with water (1 min) and two times with acetone (1 min) to reduce external contaminants [9,20]. After drying with warm air, hair, with lengths varying between 2 and 4 cm, was pulverized in a ball mill. Depending on hair specimen, 5–30 mg hair were then transferred to a glass tube and 2 mL of sodium hydroxide 1 M and 40 μL of a methanolic solution of racemic internal standard (IS) AM-d_5, MA-d_5, MDA-d_5, MDMA-d_5 and MDEA-d_5 (10 μg mL^{-1} each) were added. The tubes were shaken and incubated at 100 °C for 30 min. After digestion, the pH was adjusted to 6 with HCl 2 M. The solid phase extraction (SPE) was done with Clean Screen® C_{18} ZSDAU020 columns previously conditioned with 3 mL of methanol, 3 mL of purified water and 1 mL of phosphate buffer (KH_2PO_4 0.1 M, pH 6). After specimen addition, the columns were washed with 3 mL of purified water, 1 ml of acetic acid 1 M and 3 mL of methanol. The columns were dried and were eluted with 3 mL of dichloromethane/2-propanol/ammonia (80:20:2 by volume). After the addition of 20 μL of 1% (volume fraction) HCl in methanol, the eluant was removed under nitrogen at 37 °C and the enantiomers were derivatised with (*S*)-HFBPCl according to a procedure described in the literature [15].

2.4. Derivatization reagent

The derivatization reagent (*S*)-HFBPCl was synthesized according to the literature [15,19]. The optically purity of the reagent was tested by derivatization of the optically pure enantiomers of (*R*)-PEA and (*S*)-PEA, which were of certified high optical purity. The reagent was considered of high optical purity, as the impurity peak area was ≤0.1% of the total peak area [15].

2.5. Instrumentation

An Agilent GC–MS instrument was used; 7673A automatic sampler, 6890 series II gas chromatograph, 5973 mass selective detector (Agilent Technologies, Brussels, Belgium). The gas chromatograph was equipped with an HP-Ultra 2 capillary column (12 m × 0.2 mm × 0.33 μm film thickness). The injector temperature was 260 °C, the GC/MS interface temperature 280 °C; the helium carrier gas flow rate was 0.6 mL/min. A 4 μL of the sample solution was injected. Initial temperature was 100 °C for 2 min, followed by an increase of 30 °C/min to 180 °C, 5 °C/min from 180 to 230 °C, 30 °C/min to 310 °C.

The mass spectrometer was operated in negative chemical ionization mode with methane as reagent gas (flow of 40%). The parameters for AM, MA, MDA, MDMA and MDEA analysis in selected-ion monitoring (SIM) mode are given in Table 1. Ions used for the quantification. The most abundant ions and/or ions without apparent cross-contribution for the most analytes were chosen as target ions for the quantification [21].

2.6. Quantification procedure

For calibration, 20 mg of drug-free hair were spiked with 14 concentration levels between 0.007 and 120 ng/mg for each enantiomer of the authentic drugs of AM, MA, MDA, MDMA and MDEA. Assuming a 1:1 ratio between the enantiomers of each analyte, calibration curves were done for each enantiomer by plotting the peak-area ratios of the spiked

Table 1
SIM parameters for GC–MS analysis of AM, MA, MDA, MDMA and MDEA after derivatization by (*S*)-HFBPCl

Time window (min)	Analyte	Monitored ions (*m*/*z*)
9–10	AM-d_5	373, 393*, 433
	AM	368, 388*, 428
10–12	MA-d_5	387, 407*, 447
	MA	382, 402*, 442
13–15	MDA-d_5	417*, 437, 477
	MDA	412*, 432, 472
15–16	MDMA-d_5	431, 451*, 491
	MDMA	426*, 446, 486
	MDEA-d_5	445, 465*, 485
	MDEA	440, 460*, 480

* Ions selected for quantification.

calibrations standards versus their concentrations. The enantiomers of AM, MA and methylendioxylated amphetamines in abusers' hair were quantitated by comparison of their peak-area ratios (enantiomer of analyte versus corresponding enantiomers of IS) to calibration curves.

2.7. Method validation

Analytical recoveries of each enantiomer were calculated by comparison between peak areas of the calibration specimen analyzed with the normal specimen procedure and those obtained after adding the same amounts of reference substances and IS to blank hair after extraction. Recoveries were analyzed at the concentration of 0.25, 25 and 50 ng/mg hair, using two replicates for the concentration of each enantiomer.

Two replicates of blank hair sample spiked with 0.25 ng/mg hair were used for the estimation of the limits of detection (LOD) and the lowest limits of quantification (LLOQ) of each enantiomer. LOD and LLOQ were determined as the 3- and 10-fold deviation of the base line noise, respectively [22].

Nine replicates drug-free hair spiked with 2 ng/mg of each enantiomer were used for the determination of intra-assay precision (relative standard deviation expressed as percentage) and accuracy (expressed as percentage error of concentration found compared to target concentrations). Inter-day

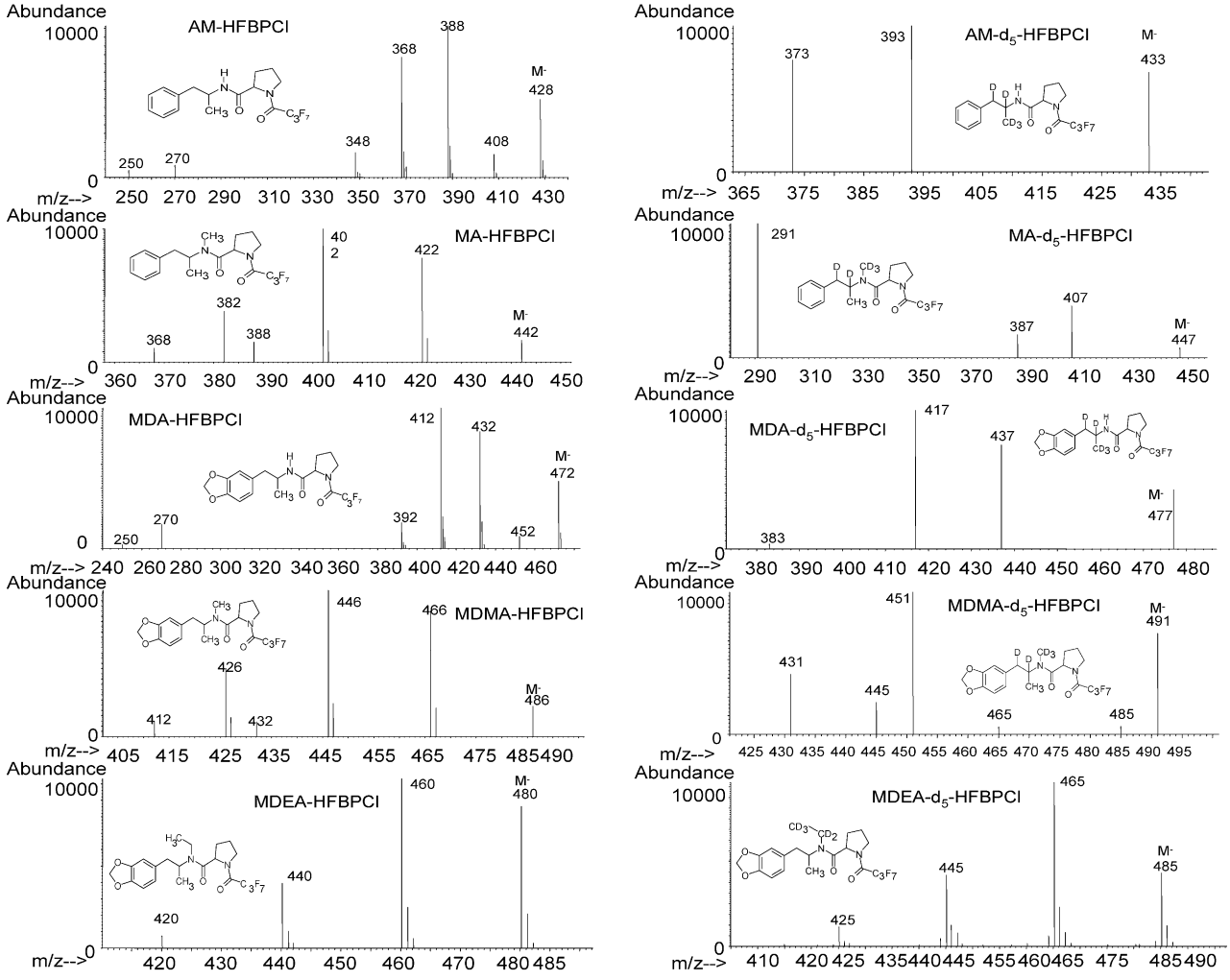

Fig. 1. NCI mass spectra of (*S*)-HFBPCl derivates of AM, AM-d_5, MA, MA-d_5, MDA, MDA-d_5, MDMA, MDMA-d_5, MDEA and MDEA-d_5 enantiomers.

and accuracy were determined at the same concentration for 5 days.

3. Results and discussion

3.1. GC/NCI–MS analysis

Fig. 1 shows the NCI mass spectra of (*S*)-HFBPCl derivates of all authentic drugs and the internal standards enantiomers. Fig. 2 represents a typical mass chromatogram of a extract of blank hair spiked with racemic standards of AM, MA, MDA, MDMA and MDEA at the concentration of 30 ng/mg hair an derivatized by (*S*)-HFBPCl. The (*R*)-isomers of the five analyzed substances were distinctly separated from their respective (*S*)-isomers and primary peaks corresponded in each case to the (*R*)-enantiomers. Although, the peak corresponding to (*S*)-MDMA and (*R*)-MDEA were not fully separated, the quantification could be done correctly, because the target ions used for the quantification of MDMA and MDEA had different *m*/*z* values. The enantiomeric ratios (*R*/*S*) of racemic standards of AM, MA, MDA, MDMA and MDEA were close to their theoretical values (*R*/*S* = 1.00). The average enantiomeric ratios (*R*/*S*) were 1.00 ± 0.01 for AM, 1.02 ± 0.01 for MA, 1.02 ± 0.02 for MDA, 0.98 ± 0.02 for MDMA and 0.99 ± 0.01 (*n* = 4).

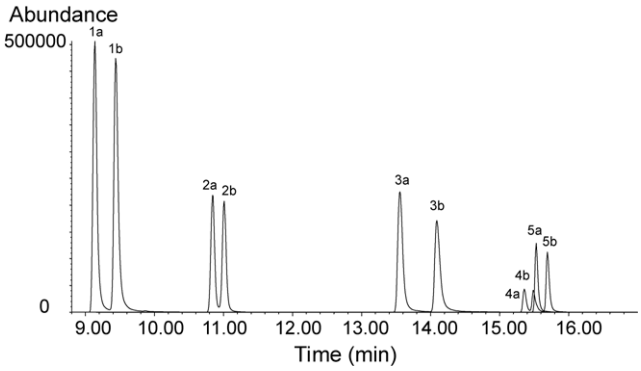

Fig. 2. Typical SIM chromatogram of a (*S*)-HFBPCl-derivatised extract of blank hair spiked with amphetamines at the concentration of 30 ng/mg per hair. Peaks: (1a) (*R*)-AM, *m*/*z* 388; (1b) (*S*)-AM; (2a) (*R*)-MA *m*/*z* 402; (2b) (*S*)-MA; (3a) (*R*)-MDA, *m*/*z* 412; (3b) (*S*)-MDA; (4a) (*R*)-MDMA, *m*/*z* 426; (4b) (*S*)-MDMA; (5a) (*R*)-MDEA, *m*/*z* 460; (5b) (*S*)-MDEA.

3.2. Validation results

Data on method validation are reported in Tables 2 and 3. Standard curve plots for each enantiomer were linear in the range of tested concentrations with a coefficient of correlation (r^2) higher than 0.992. LOD and LLOQ values varied between 0.002 and 0.046 ng/mg and between 0.007 and 0.151 ng/mg, respectively. Precision and accuracy were al-

Table 2
Validation data for enantioselective analysis of AM, MA, MDA, MDMA and MDEA in hair

	LOD (ng/mg)	LLOQ (ng/mg)	Linearity (ng/mg)	Intra-day (*n* = 9)		Inter-day (*n* = 5)	
				Precision (R.S.D., %)*	Accuracy (bias, %)	Precision (R.S.D., %)*	Accuracy (bias, %)
(*R*)-AM	0.002	0.007	0.007–120	2.4	7.9	1.7	9.4
(*S*)-AM	0.002	0.007	0.007–120	2.8	4.0	2.8	4.6
(*R*)-MA	0.011	0.035	0.035–120	7.4	4.0	6.6	10.8
(*S*)-MA	0.011	0.035	0.035–120	6.3	3.2	5.0	4.6
(*R*)-MDA	0.005	0.017	0.017–120	4.4	1.1	2.4	1.6
(*S*)-MDA	0.004	0.014	0.014–120	5.0	0.2	2.7	2.2
(*R*)-MDMA	0.024	0.078	0.078–120	6.6	0.4	5.8	1.6
(*S*)-MDMA	0.046	0.151	0.151–120	8.9	1.2	6.2	1.1
(*R*)-MDEA	0.016	0.054	0.054–120	6.9	3.7	4.2	2.7
(*S*)-MDEA	0.013	0.042	0.042–120	6.4	0.1	5.0	0.2

* R.S.D., relative standard deviation.

Table 3
Analytical recoveries at three different concentrations (*n* = 2)

Concentration (ng/mg)		Analytical recoveries (%)									
		(*R*)-AM	(*S*)-AM	(*R*)-MA	(*S*)-MA	(*R*)-MDA	(*S*)-MDA	(*R*)-MDMA	(*S*)-MDMA	(*R*)-MDEA	(*S*)-MDEA
0.25	Mean	95.3	97.9	84.3	88.5	91.5	87.5	83.5	80.4	73.2	73.0
	S.D.*	7.6	7.1	9.4	10.0	8.9	9.6	7.2	7.9	5.2	4.8
25	Mean	100.2	99.9	96.9	101.4	96.0	96.1	91.5	95.3	98.3	95.8
	S.D.*	6.1	6.9	8.4	7.5	5.6	5.8	5.6	6.1	2.1	6.8
50	Mean	101.8	103.0	101.3	100.5	101.6	101.9	100.0	100.8	101.1	101.0
	S.D.*	3.7	3.4	4.3	4.7	2.9	3.6	3.1	4.4	5.4	3.3

* S.D., standard deviation.

Table 4
Concentrations and enantiomeric ratios of AM and MA in abusers' hair specimens determined by GC/NCI–MS after derivatization by (*S*)-HFBPCl

Specimen	(*R*)-AM (ng/mg)	(*S*)-AM (ng/mg)	Enantiomer ratio *R* vs. *S*	(*R*)-MA (ng/mg)	(*S*)-MA (ng/mg)	Enantiomer ratio *R* vs. *S*
1	ND[a]	1.8	–	ND	25.2	–
2	0.5	4.1	0.1	15.6	64.4	0.2
3	0.1	0.6	0.2	4.1	24.5	0.2
4	0.4	0.3	1.2	92.1	9.1	10.1
5	0.5	1.4	0.3	7.0	22.7	0.3
6	0.4	3.4	0.1	18.3	81.6	0.2
7	0.2	1.7	0.1	12.9	21.7	0.6
8	ND	1.1	–	ND	44.8	–
9	0.3	2.1	0.2	6.0	27.3	0.2
10	1.0	3.2	0.3	9.7	23.6	0.4
11	0.6	1.8	0.3	8.3	18.6	0.4

[a] Not detectable.

ways lower than 10.8%. Table 3 shows the analytical recoveries determined at three different concentrations using two replicates for each evaluated concentration. They were considered adequate for the purpose of the study.

3.3. Enantioselective determination in abusers' hair specimens

Hair specimens from 11 suspected amphetamine abusers were analyzed and the results are shown in Table 4. Only MA and its demethylated metabolite AM were detected. Moreover, MA was found in higher concentrations in hair than AM, which suggests that MA was used by all the abusers [12,23,24]. Fig. 3 illustrates chromatograms obtained after analysis of three abusers' hair. Eight specimens contained both enantiomers of AM and MA with a prevalence of their (*S*)-forms. The enantiomeric ratios (*R*/*S*) of MA ranged from 0.17 to 0.59 and from 0.12 to 0.31 for AM (Fig. 3A). In addition, only (*S*)-MA and (*S*)-AM were found in two hair specimens (Fig. 3B), which might be explained by ingestion of optically pure (*S*)-MA. Finally, in one hair specimen, both enantiomers of AM and MA were quantified and the enantiomeric ratios were 10.1 for MA and 1.2 for AM (Fig. 3C). This might be due to the ingestion of both racemic MA and optically pure (*R*)-MA [16]. (*R*)-MA might be originating from medication for example (e.g., Vicks Inhaler) [7].

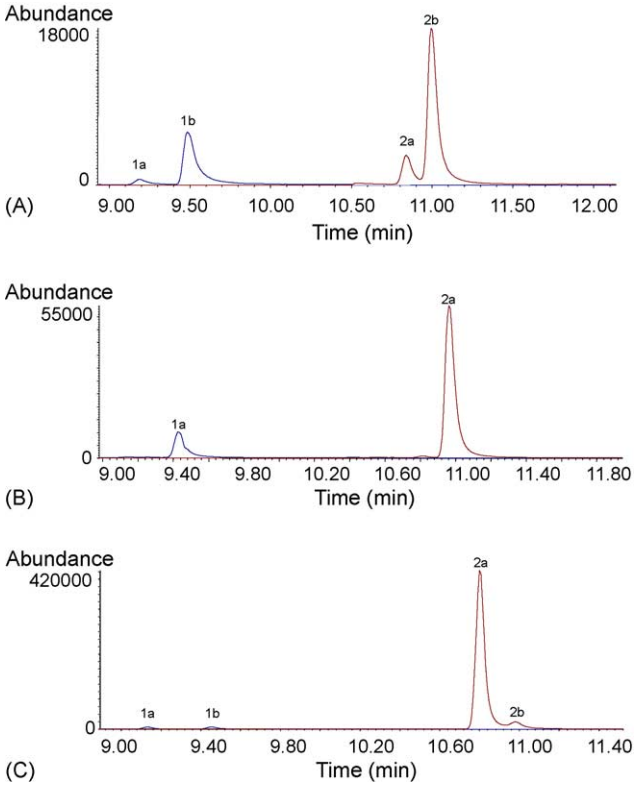

Fig. 3. SIM chromatograms of hair extracts from suspected amphetamine abusers presenting enantimeric ratios $R/S < 1$ (A), only (*S*)-enantiomers (B) and enantiomeric ratios $R/S > 1$ (C). Peaks: (1a) (*R*)-AM, (1b) (*S*)-AM, *m*/*z* 388; (2a) (*R*)-MA, (2b) (*S*)-MA, *m*/*z* 40.

4. Conclusions

This paper describes a rapid method for the simultaneous determination of the enantiomeric ratio of AM, MA, MDA, MDMA and MDEA in hair. Advantages offered by the GC/NCI–MS method were high separation efficiency, short time analysis and typically high sensitivity. The method has successfully been applied to the determination of enantiomeric composition of MA and AM in 11 abusers' hair.

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