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Sensitive, rapid and validated gas chromatography/negative ion chemical ionization-mass spectrometry assay including derivatisation with a novel chiral agent for the enantioselective quantification of amphetamine-type stimulants in hair☆

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Abstract

A novel chiral derivatisation agent, (2*S*,4*R*)-*N*-heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride, was used for the indirect resolution of amphetamine (AM), methamphetamine (MA), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDEA) enantiomers using gas chromatography coupled to mass spectrometry operating in the negative-ion chemical ionization mode (GC/MS-NICI). This new chiral derivatisation reagent was readily obtained in optically pure form after a simple two-step synthesis. Optimal derivatisation was accomplished in 15 min at room temperature in a carbonate buffer and the resulting diastereoisomers were base line separated by GC in 12 min only. No racemization was observed during the derivatisation. The method was applied and fully validated for the enantiomeric quantification of amphetamines and methylenedioxylated amphetamines in hair. The analyses of 24 hair specimens from suspected ATS abusers showed that 24 cases were positive for MA and/or AM enantiomers and that in most cases the concentrations of (*S*)-MA and (*S*)-AM exceeded those of the corresponding (*R*)-enantiomers. One hair specimen was tested positive for both enantiomers of MDMA and MDA.

Keywords: Amphetamine; Amphetamine-type stimulants; (2*S*,4*R*)-*N*-Heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride; Enantiomeric ratios; Hair analyses

1. Introduction

Amphetamine (AM), methamphetamine (MA) and the amphetamine-type stimulants (ATS) such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and 3,4-methylenedioxyethylamphetamine (MDEA) are central nervous system (CNS) stimulants. All these compounds contain a chiral center and their enantiomers show different pharmacological properties. Thus, the (*S*)-enantiomers of AM, MA and MDMA, have more CNS stimulant activity than the (*R*)-enantiomers [1–3]. They are consumed either as drugs of abuse, as doping agents or, to a minor extent, appear as metabolites after legal medication. MDA, MDMA and MDEA are usually used as racemic mixtures [4–6] and no corresponding legal precursor drugs exist. However, it is known that several therapeutic drugs contain or are metabolized to AM and MA. For example, selegiline used for the treatment of Parkinson's disease, has been shown to metabolized to (*R*)-MA and (*R*)-AM, while benzphetamine prescribed as an anoretic, is metabolized to (*S*)-MA and (*S*)-AM, respectively [7–11]. Furthermore, in some countries, Vicks Inhaler, a nasal decongestant, contains (*R*)-MA [12,13]. Therefore, the enantioselective separation of ATS has been found to be useful for the correct interpretation of ATS positive results in clinical and forensic toxicology. Indeed, as the metabolism of these enantiomers is stereospecific, the proportions of the enantiomers of ATS found in a sample are powerful tools for the interpretation of the form of the drug administered [8,13].

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In recent years, hair analysis for drug abuse has rapidly emerged as a useful tool for detecting and monitoring drugs over a long time period [13–15]. Only a few publications have described methods for the separation of ATS enantiomers extracted from the human hair matrix [16–23]. Analytical procedures including capillary electrophoresis [16,17], liquid chromatography (HPLC) [18–22] or gas chromatography coupled to mass spectrometry [24] have already been used for the enantioselective analysis of ATS extracted from hair. However, the published methods have shown some important drawbacks including long acquisition time and especially, poor sensitivity. Recently, a negative chemical ionization gas chromatography-mass spectrometry (GC/MS-NICI) assay using (*S*)-heptafluorobutyrylprolyl chloride ((*S*)-HFBPCl) as chiral derivatisation reagent has been developed in our laboratory for the determination of the enantiomeric ratios of ATS in hair specimens [23]. Compared to the above-mentioned studies, a great advantage offered by this GC/MS-NICI method was at least a 20-fold decrease of the limits of detection (LODs) compared to previous studies. Nevertheless, the drawbacks of this procedure included overlapping of the diastereomeric peaks of MDMA and MDEA and higher LODs for MDMA and MDEA enantiomers compared to those of MDA, AM and MA.

As the separation of diastereoisomers on a non-chiral phase is based on the differences of the physico-chemical properties of the diastereoisomers, the choice of a chiral derivatisation reagent is of great importance for the indirect chromatographic resolution of chiral molecules [25,26].

The main purpose of this study was to develop and validate an indirect sensitive GC/MS-NICI method using a new in-house synthesized pure chiral tagging molecule as derivatisation reagent for the indirect and simultaneous separation of the ATS enantiomers in hair. In addition the procedure was applied to the quantitative analysis of the selected target enantiomers in 24 authentic hair specimens from suspected ATS abusers.

2. Materials and methods

2.1. Reagents and equipment

Standard solutions of 1 mg mL^{-1} of racemic ATS, (*R*)-AM, (*S*)-AM, (*R*)-MA 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). *Trans*-4-Hydroxy-L-(−)-proline, *R*-(+)-1-phenylethylamine ((*R*)-PEA, *R*:*S* ≥ 99.5:0.5), *S*-(−)-1-phenylethylamine ((*S*)-PEA, *S*:*R* ≥ 99.5:0.5), anhydrous toluene (99.8%), anhydrous dichloromethane (CH_2Cl_2, 99.8%), thionyl chloride ($SOCl_2$, >99%), phosphorus pentachloride (PCl_5, 95%) and cyclohexane were purchased from Sigma–Aldrich (Bornem, Belgium). Sodium bicarbonate (Na_2CO_3, 99.5%), sodium hydrogenocarbonate ($NaHCO_3$, 95.5%) were obtained from Merck (Overijse, Belgium). Heptafluorobutyric anhydride (HFBA) was purchased from Macherey-Nagel (Hoerdt, France). The ball mill used was purchased from Retsch (Haan, Germany). All synthetic reactions were carried out under inert atmosphere (dry N_2).

2.2. Synthesis of (2S,4R)-N-heptafluorobutyryl-4-heptafluorobutoyloxy-prolyl chloride

To a 50 mL round-bottom flask containing 250 mg (1.9 mmol) *trans*-4-hydroxy-L-(−)-proline, and 5 mL of anhydrous CH_2Cl_2, 1.9 mL (7.8 mmol) of HFBA were added under stirring at 0 °C. After 10 min, the mixture was allowed to stir at room temperature (RT) during 3 h. The unreacted HFBA and CH_2Cl_2 were removed under vacuum at room temperature. Anhydrous toluene (5 mL) and 395 mg (1.9 mmol) of PCl_5 were added to the viscous residue at 0 °C. After 10 min, the mixture was allowed to warm-up to RT and stirring continued for a further 90 min. The solvent was removed under vacuum. The residue was washed twice with anhydrous CH_2Cl_2 (1 mL). Finally, the slightly viscous residue was diluted in 9 mL of anhydrous CH_2Cl_2 to give a theoretical concentration of 0.2 mol/L of (*S*,*R*)-HFBOPCl. Aliquots of 1 mL were sealed and stored at −20 °C.

The chiral purity of the freshly prepared derivatisation reagent was determined by GC separation of the diastereoisomers formed from the reaction with optically pure (*R*)-PEA and (*S*)-PEA (20 μg mL^{-1} solution). The derivatisation was carried out as described in Section 2.5.

2.3. Hair specimens

Drug free hair specimens were collected from healthy subjects in the authors' laboratory. As in Luxembourg ATS positive hair specimens were rare, 24 suspected amphetamines abusers' hair specimens from Korea were analysed.

2.4. Hair specimen digestion and extraction

The digestion and extraction of hair specimen were realized according to the procedure previously described by Martins et al. [23]. 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 by volume). After the addition of 20 μL of 1% (volume fraction) HCl in methanol, the eluant was removed under nitrogen at 37 °C.

2.5. Optimized derivatisation procedure

The derivatisation conditions were determined after the investigation of various experimental parameters including the reaction time, pH and amount of (*S*,*R*)-HFBOPCl added, on the analytical responses of the diastereoisomers. For more details see Section 3.2.

Drug enantiomers were converted to their diastereomeric derivates 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) pH 9.5 to which 20 μL of a 0.2 M solution of (*S*,*R*)-

Table 1
SIM parameters for GC/MS-NICI analysis of PEA, AM, MA, MDA, MDMA and MDEA after derivatisation with (*S*,*R*)-HFBOPCl

Time window (min)	Analyte	Monitored ions (*m*/*z*)
6.5–7	PEA	606, 626, 586
7.5–8.1	AM-d_5	625[a], 645, 456
	AM	620[a], 640, 451
8.1–8.8	MA-d_5	639[a], 659, 619
	MA	634[a], 654, 614
10.5–11.1	MDA-d_5	669[a], 649, 689
	MDA	664[a], 644, 684
11.1–12.2	MDMA-d_5	683[a], 703, 663
	MDMA	678[a], 698, 658
	MDEA-d_5	697[a], 717, 677
	MDEA	692[a], 712, 672

[a] Ions used for quantitation.

HFBOPCl was added. 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 GC analysis.

2.6. Instrumentation

An Agilent 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% phenylmethylpolysiloxane) capillary column (30 m × 0.25 mm × 0.25 μm film thickness). The injector temperature was 260 °C, the GC/MS interface temperature 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 operated in the NICI mode with methane as reagent gas (flow of 40%). The retention times and the characteristic fragments of the NICI mass spectra were determined by total ion monitoring (SCAN). The most abundant ions and/or ions without apparent cross-contribution and interferences were chosen as target ions for the quantification [27]. For quantitative analysis, the chosen diagnostic mass fragments of PEA, AM, MA, MDA, MDMA and MDEA monitored in the selected-ion monitoring (SIM) mode are given in Table 1.

2.7. Quantitation procedure

For calibration, 10 mg of drug-free hair were spiked with ATS, each enantiomer covering the range from 0.002 to 60 ng/mg. The internal standards (IS) ATS-d_5 were added at a fixed concentration of 5 ng/mg. The peak area ratios between each enantiomer and the corresponding IS were used for calculations. The regression line was calculated using a weighted ($1/x$) least-square regression model.

2.8. Validation procedure

Calibration curve linearity was tested over the range 0.002–60 ng/mg for each enantiomer of the selected ATS.

In order to evaluate the efficiency of the solid-phase extraction, the recoveries of blank hair specimen spiked with the target substances and the IS at the concentrations of 0.5, 10 and 50 ng/mg were evaluated, each in five replicates. The recoveries of the enantiomers were then calculated by comparing the peak areas of the extracted hair specimens with those obtained by adding the same amounts of reference substances and IS after extraction.

Three replicates of blank hair specimen spiked with 0.1 ng/mg of ATS 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 standard deviation of the base line noise, respectively [28].

Intra- and inter-assay precision (relative standard deviation expressed as percentage) and accuracy (expressed as percentage error of concentration found compared to target concentrations) were determined by using control hair ($n = 10$) spiked for each enantiomer at a low concentration (0.25 ng/mg) and a higher concentration (5 ng/mg). Inter-day precision and accuracy were determined at the same concentrations during 5 days.

3. Results and discussion

3.1. Synthesis and purity of (S,R)-HFBOPCl

The rationale for the synthesis of (*S*,*R*)-HFBOPCl was to obtain a rigid and stable derivatisation reagent in order to influence the physico-chemical properties such as polarity, lipophilicity, adsorbability or solubility to enhance the resolution of two diastereomeric peaks. The presence of the two stereogenic centers separated by two bonds in the tagging molecule may enhance the relative differences in the chromatographic properties of their diasteromeric derivatives [25,26]. In addition, the use of heptafluorobutyryl-chains as functional groups at the chiral centers may provide a certain structural stability and conformational rigidity [25,26]. Finally, the attachment of a great number of electronegative atoms on the molecule may enhance the volatility of these derivatives and elicit a strong detector response [26,29].

The synthesis of (*S*,*R*)-HFBOPCl was partly based on a two step procedure proposed by Mori et al. [30] and Uozumi et al. [31,32], who used trifluoro-derivatives of 4-hydroxyl-proline as intermediates in the synthesis of optically pure antibiotics. The synthesis of (*S*,*R*)-HFBOPCl is summarized in Fig. 1. We used HFBA instead of the trifluoroacetic anhydride for the reasons already mentioned above. The formation of the acyl chloride during the second step could be accomplished either using PCl_5

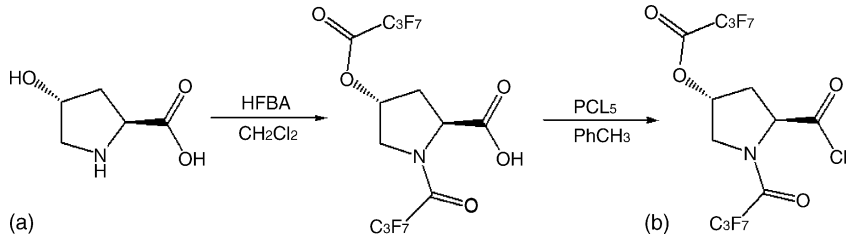

Fig. 1. (a) *trans*-4-Hydroxy-L-proline and (b) (*S*,*R*)-HFBOPCl.

or $SOCl_2$, reagent used by Lim et al. [29] for the halo-dehydroxylation of *N*-heptafluorobutyryl-proline. In both cases, (*S*,*R*)-HFBOPCl was obtained, but the reaction time was longer with $SOCl_2$ than with PCl_5. Therefore, PCl_5 was used as reagent in our optimized synthetic procedure.

The optical purity of the freshly synthesized (*S*,*R*)-HFBOPCl was tested by derivatising optically pure *R*-(+)-PEA and *S*-(+)-PEA and analyzing the resulting diasteroisomers by calculating the diastereomeric excess [de (%) = 100 × (main peak − impurity peak)/(main peak + impurity peak)].

No racemization was observed during derivatisation and the diastereomeric excesses were >99.8% for both diastereoisomers of HFBOP-PEA. The area corresponding to the minor diastereosisomer was in each case <0.1% of the total peak area (main peak + impurity peak). (*S*,*R*)-HFBOPCl has shown to be optically pure and stable for at least 6 months (de > 99.7%) when stored at −20 °C in anhydrous CH_2Cl_2.

3.2. Optimization of the derivatisation conditions of ATS by (S,R)-HFBOPCl

Conditions for the derivatisation of ATS by (*S*,*R*)-HFBOPCl were investigated for each compound separately including the reaction time, the amount of derivatisation reagent, the pH and the stability of (*S*,*R*)-HFBOPCl derivatives. The derivatisations of the ATS were carried out under aqueous alkaline conditions [29]. Each set of the reaction conditions was studied in five replicates using a solution of racemic ATS at the concentration of 40 μg/mL. The results of this study are resumed for the MDMA enantiomers in Fig. 2.

3.2.1. Influence of the amount of (S,R)-HFBOPCl on the analytical responses

The influence of the amount of the derivatisation reagent on the reactivity with the five racemic ATS enantiomers was investigated by adding different volumes of a 0.2 M solution of (*S*,*R*)-HFBOPCl in the range of 5–25 μl to the samples. The acylation of the amines reached a plateau at 20 μl of (*S*,*R*)-HFBOPCl for both enantiomers of MDMA [Fig. 2(a)]. Similar results were obtained for the enantiomers of AM, MA, MDA and MDEA.

3.2.2. Influence of the pH on the derivatisation

As the acylation of amines is usually completed under aqueous alkaline conditions [29], the objective of this study was to evaluate if there were any influences on the conversion yields between different alkaline pH values. As supposed, only small differences in the analytical responses were observed at pH values ranging from 8 to 10 [Fig. 2(b)]. Roughly, the best conversions yields of AM, MA, MDMA and MDEA enantiomers into their respective diasteroisomers were observed at pH 9.5. Thus no special adjusting of the pH is needed after the preparation of the carbonate buffer which is already alkaline.

3.2.3. Reaction time optimization and stability of the derivatives

Fig. 2(c) indicates that the time necessary to achieve the maximum analytical response for the diastereoisomers of MDMA was 10 min. The acylation reactions of the enantiomers of the other ATS were also maximal in the range 10–15 min. As expected, no significant differences between responses obtained

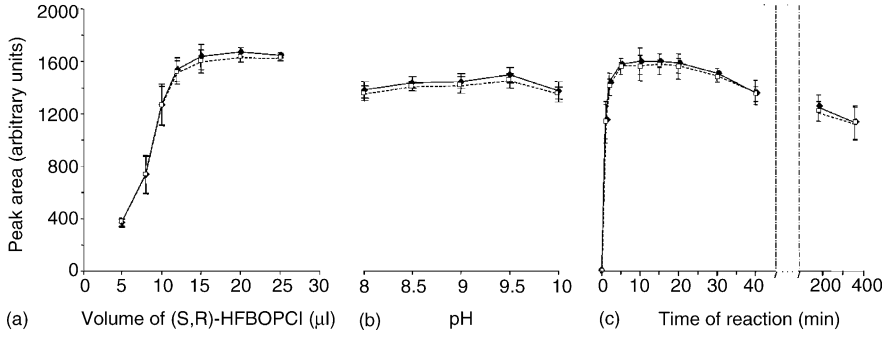

Fig. 2. Effect of experimental parameters on the analytical responses of (*S*)-MDMA (□) and (R)-MDMA (♦) for solution derivatisation: (a) effect of the volume of (*S*,*R*)-HFBOPCl 0.2 M (pH 9.5 and reaction time of 5 min); (b) effect of pH (20 μl of (*S*,*R*)-HFBOPCl 0.2 M, time of reaction of 5 min); (c) effect of the time of reaction (20 μl of (*S*,*R*)-HFBPCl 0.2 M, pH 9.5). For other experimental details, see text.

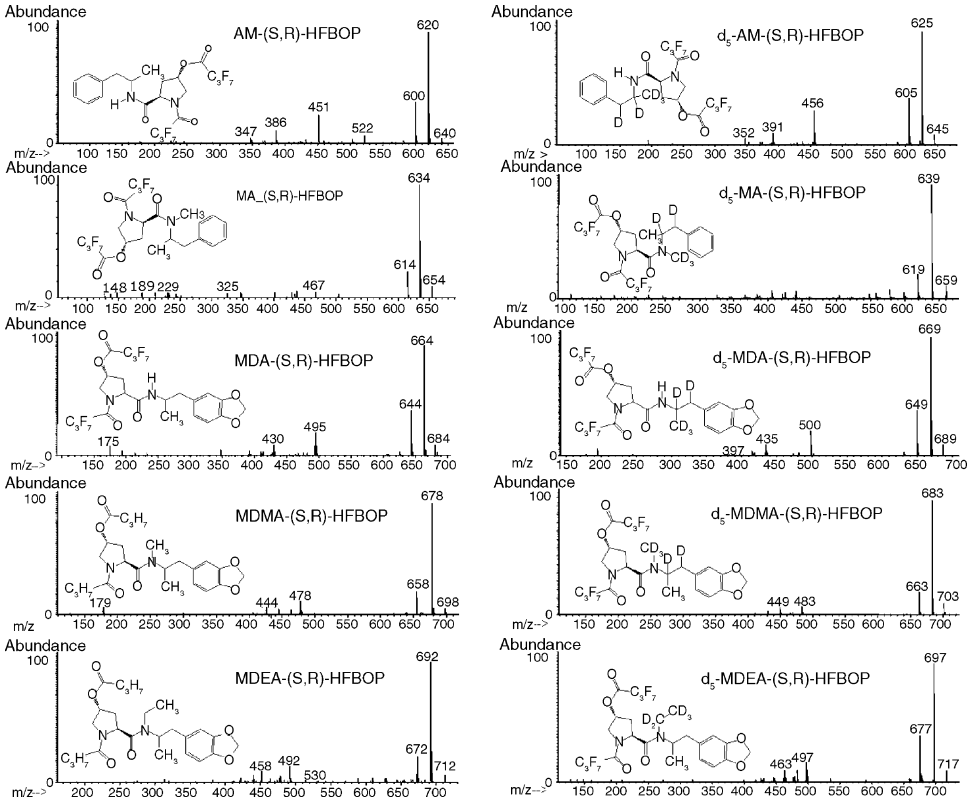

Fig. 3. NICI full SCAN mass spectra of (*S*,*R*)-HFBOPCl derivatives of AM, AM-d_5, MA, d_5-MA, MDA, d_5-MDA, MDMA, d_5-MDMA, MDEA and d_5-MDEA.

for the (*S*)- and (*R*)-isomers were observed, which means that the enantiomers exhibit the same kinetic behaviour during their derivatisation.

However, after 20 min, the analytical signals of the ATS derivatives tend to decline slightly, which may be due to the slow hydrolysis of the derivatives and/or of the (*S*,*R*)-HFBOPCl in the alkaline conditions [29]. Thus to get a maximum analytical response, the reaction has to be stopped by extraction with cyclohexane. Indeed, the evaluation of the degradration of the (*S*,*R*)-HFBOPCl derivates in the cyclohexane showed that these derivatives were stable for at least 72 h (longest time range tested) when stored in the organic phase at three different temperatures (20, 4 and −20 °C). The mean range of the amounts of ATS derivatives (expressed as percentage) detected by GC/MS-NICI after 72 h varied between 40 and 69% when stored at 20 °C, between 48 and 70% when stored at 4 °C and from 54 to 75% when stored at −20 °C. Furthermore, the ratios between the standard drugs and their respective IS enantiomers were equal at any time and at any storage temperature.

Finally, these results let us to conclude that the ATS-(*S*,*R*)-HFBOP derivatives should be extracted after 15 min with cyclohexane and, after their analysis the sealed vials should be stored at −20 °C in cyclohexane to allow their re-analysis.

3.2.4. Optimal derivatisation conditions of ATS

Based on the above results, it can be assumed that analytical responses of the ATS derivatives were maximum using 20 μl of a 0.2 M (*S*,*R*)-HFBOPCl solution, a carbonate buffer solution at pH 9.5 and a maximum reaction time of 15 min.

3.3. GC/MS-NICI analysis of spiked hair specimen

The NICI full SCAN mass spectra of the ATS-(*S*,*R*)-HFBOP, are shown in Fig. 3. The spectra are dominated by the sequential neutral loss of HF and the fragmentation pattern of the (*R*)- and (*S*)-isomers were identical. The characteristic ions, one target ion and two qualifier ions chosen without apparent cross-contribution for SIM conditions are summarized in Table 1.

Fig. 4 shows a typical SIM chromatogram obtained by analysing an extract of blank hair spiked with a standard mixture of racemic ATS (40 ng/mg) derivatised with (*S*,*R*)-HFBOPCl.

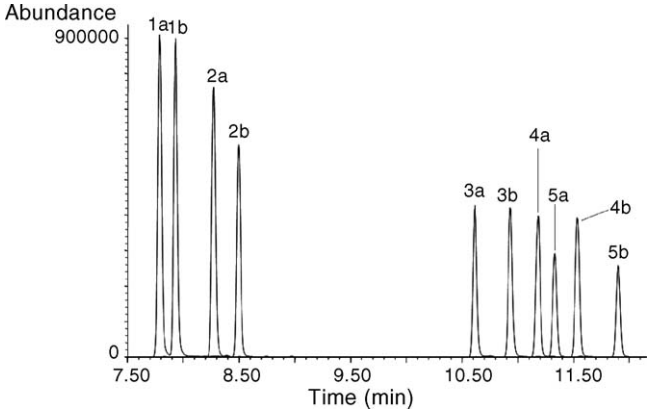

Fig. 4. Typical SIM chromatogram of an extract of blank hair spiked with 10 ng/mg of ATS. Peaks: (1a) (*R*)-AM, *m*/*z* 600; (1b) (*S*)-AM; (2a) (*R*)-MA *m*/*z* 634; (2b) (*S*)-MA; (3a) (*R*)-MDA, *m*/*z* 664; (3b) (*S*)-MDA; (4a) (*R*)-MDMA, *m*/*z* 678; (4b) (*S*)-MDMA; (5a) (*R*)-MDEA, *m*/*z* 692; (5b) (*S*)-MDEA.

Table 2
Method calibration

Compound	Linearity (ng/mg)	Regression line		Correlation coefficient (r^2)	LOD (pg/mg)	LLOQ (pg/mg)
		Slope	Intercept			
(*R*)-AM	0.003–60	0.985 ± 0.012	3.498 ± 2.235	0.998	0.8	2.7
(*S*)-AM	0.002–60	0.979 ± 0.010	2.396 ± 2.180	0.997	0.7	2.4
(*R*)-MA	0.007–60	0.967 ± 0.013	3.955 ± 2.644	0.996	2.1	6.9
(*S*)-MA	0.005–60	0.969 ± 0.014	5.885 ± 2.916	0.996	1.5	5.0
(*R*)-MDA	0.005–60	1.017 ± 0.013	−1.408 ± 2.789	0.998	1.6	5.3
(*S*)-MDA	0.004–60	1.011 ± 0.010	−1.600 ± 2.164	0.999	1.3	4.3
(*R*)-MDMA	0.006–60	1.001 ± 0.015	1.899 ± 1.249	0.998	1.7	5.6
(*S*)-MDMA	0.005–60	0.995 ± 0.009	1.838 ± 1.787	0.998	1.5	5.1
(*R*)-MDEA	0.009–60	0.984 ± 0.012	3.203 ± 2.497	0.997	2.7	8.9
(*S*)-MDEA	0.008–60	0.995 ± 0.013	2.607 ± 2.057	0.997	2.3	7.7

Table 3
Extraction recoveries of the ATS enantiomers added at three different concentrations to human blank hair ($n = 5$)

Concentration (ng/mg)	(*R*)-AM	(*S*)-AM	(*R*)-MA	(*S*)-MA	(*R*)-MDA	(*S*)-MDA	(*R*)-MDMA	(*S*)-MDMA	(*R*)-MDEA	(*S*)-MDEA
0.5										
Mean (%)	97.1	96.3	93.8	90.0	97.3	96.2	94.2	93.4	96.0	92.7
S.D.[a]	4.3	5.7	4.1	4.2	5.9	5.1	4.2	4.7	3.6	4.7
10										
Mean (%)	94.8	94.8	91.2	91.0	96.3	97.9	91.1	91.7	92.5	93.6
S.D.[a]	2.6	2.8	4.9	2.7	3.0	3.4	2.6	3.0	3.2	2.9
50										
Mean (%)	97.7	96.8	90.0	92.6	98.1	96.6	94.5	94.1	96.8	94.9
S.D.[a]	3.7	2.9	3.1	3.9	5.9	4.6	5.4	5.2	7.1	6.5

[a] S.D.: standard deviation.

Table 4
Intra- and inter-assay precision and accuracy data

Compound	Concentration (ng/mg)	Intra-day ($n = 10$)		Inter-day ($n = 5$)	
		Precision (R.S.D., %)[a]	Accuracy (bias, %)	Precision (R.S.D., %)[a]	Accuracy (bias, %)
(*R*)-AM	0.25	5.0	3.0	7.2	4.4
	5	3.4	2.2	3.0	8.1
(*S*)-AM	0.25	2.7	8.6	7.5	7.1
	5	2.8	4.8	2.8	5.8
(*R*)-MA	0.25	5.4	0.4	6.7	4.2
	5	5.8	7.5	4.1	6.3
(*S*)-MA	0.25	3.6	1.7	8.7	4.3
	5	3.1	2.6	2.9	2.3
(*R*)-MDA	0.25	7.3	3.5	7.5	3.7
	5	3.0	9.0	3.1	7.3
(*S*)-MDA	0.25	2.9	9.5	5.7	5.1
	5	2.8	9.3	2.9	6.9
(*R*)-MDMA	0.25	3.0	1.5	4.4	3.4
	5	2.8	6.8	2.9	5.7
(*S*)-MDMA	0.25	3.6	1.4	5.6	3.8
	5	3.2	6.7	3.2	4.5
(*R*)-MDEA	0.25	3.1	3.8	6.1	2.9
	5	3.1	2.6	3.0	2.7
(*S*)-MDEA	0.25	4.4	0.5	6.5	2.7
	5	3.0	5.4	3.0	2.5

[a] R.S.D.: relative standard deviation.

Table 5
Concentrations and enantiomeric ratios of AM and MA in abusers' hair specimens determined by GC/NCI-MS after derivatisation with (*S*,*R*)-HFBOPCl

Specimen	(*R*)-AM (ng/mg)	(*S*)-AM (ng/mg)	*R* vs. *S* ratio	(*R*)-MA (ng/mg)	(*S*)-MA (ng/mg)	*R* vs. *S* ratio
1	ND[a]	0.2	–	ND	3.8	–
2	1.0	7.5	0.13	10.8	43.6	0.25
3	0.2	2.0	0.07	1.5	12.3	0.12
4	0.2	0.9	0.21	0.4	21.9	0.02
5	ND	0.1	–	ND	3.1	–
6	0.1	1.9	0.03	0.2	21.6	0.01
7	0.2	1.4	0.16	0.3	28.0	0.01
8	0.1	1.2	0.06	1.0	14.5	0.07
9	0.2	1.3	0.15	1.4	27.3	0.05
10	1.0	3.2	0.30	9.7	23.6	0.41
11	ND	1.5	–	0.6	20.4	0.03
12	ND	0.8	–	0.6	9.0	0.07
13	0.2	0.3	0.79	1.6	2.0	0.82
14	0.4	1.0	0.40	4.3	18.0	0.24
15	ND	1.4	–	ND	26.1	–
16	1.2	1.3	0.92	43.2	20.5	2.11
17	0.1	0.4	0.23	0.1	6.5	0.11
18	0.2	0.2	0.95	0.2	2.4	0.07
19	ND	2.8	–	1.7	44.5	0.04
20	0.1	0.7	0.14	0.8	13.9	0.05
21	ND	2.4	–	ND	31.8	–
22	0.2	1.3	0.17	0.2	40.1	0.01
23	0.4	1.8	0.20	0.3	39.5	0.01

[a]Not detectable.

The diastereoisomers were all separated to base line by GC within less than 12 min without overlapping of peaks, which represented a very good separation for a short acquisition time compared to former procedures [16–23]. Furthermore, the derivatisation procedure is simple and fast and the background of the chromatograms was always extremely low. Optically pure (*R*)- and (*S*)-AM and (*R*)- and (*S*)-MA were used for the identification of the corresponding diastereoisomers' peaks. The determination of their respective retention times permitted to conclude that the (*R*)-enantiomers of AM and MA eluted in front of their corresponding (*S*)-isomers. As no optically pure MDA, MDMA and MDEA were available, according to the literature [33–35], we assumed that these compounds would follow an elution pattern similar to the amphetamine and the methamphetamine.

3.4. Validation of the analytical method for hair analysis

Data on method validation are summarized in Tables 2–4. Standard curve plots for each enantiomer were linear in the range of 0.002–60 ng/mg with a coefficient of correlation (r^2) higher than LOD and LLOQ values varied between 0.7 and 2.7 pg/mg and between 2.4 and 8.9 pg/mg, respectively. Compared to former HPLC or EC methods [16–22], the sensitivity has been improved by a mean factor of 30 and compared to our previous study [23], the LOD respectively the LLOQ values of AM, MA, MDA have been reduced by a factor of 3, while those of MDMA and MDEA have been reduced by a factor of 10.

Table 3 shows the data of the analytical recoveries investigated using blank hair spiked with the ATS at three different concentrations (0.5, 5 and 50 ng/mg). The extraction recoveries varied in the range of 90–98.1%.

Accuracy and precision results obtained for the concentrations of 0.25 and 5 ng/mg of the five ATS in hair are presented in Table 4. As reported in the table intra- and inter-day assay data and accuracies were lower than 9.5%, which were acceptable for the purpose of this study.

3.5. Application to forensic hair analysis

The applicability of the developed method was demonstrated by analyzing hair specimens from 24 suspected ATS abusers. AM, MA, MDA and MDMA enantiomers were detected, whereas no hair specimen was tested positive for MDEA. The results are reported in Tables 5 and 6.

Even with very small amounts of hair (ranging from 1 to 15 mg), it was possible to detect MA and/or AM in 24 cases (Tables 5 and 6). For these 24 cases, MA was always found in higher concentrations than AM, which suggested that MA was principally used by all the abusers [18,36,37]. Both enantiomers of AM and/or MA were detected in 19 specimens with a predominance of their (*S*)-form. The enan-

Table 6
Case no. 24: Hair specimen tested positive for MA, MDA and MDMA enantiomers in hair by the present (*S*,*R*)-HFPOPCl procedure

Analyte	Concentration (ng/mg)	*R* vs. *S* ratio
(*R*)-MA	0.1	0.25
(*S*)-MA	0.4	
(*R*)-MDA	0.1	0.50
(*S*)-MDA	0.2	
(*R*)-MDMA	5.2	2.00
(*S*)-MDMA	2.6	

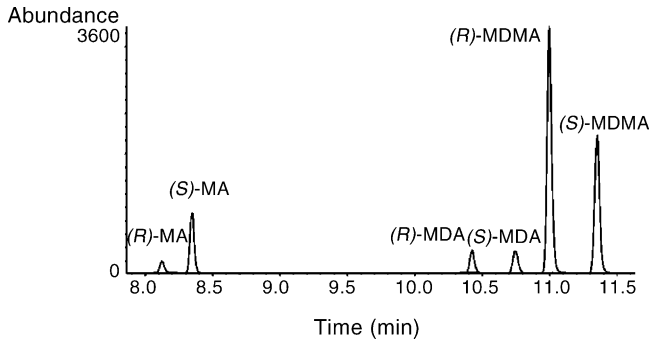

Fig. 5. Chromatogram of an hair extract from a suspected ATS abuser tested positive for MA, MDA and MDMA enantiomers. Single ion monitoring at $m/z = 634$ for MA, $m/z = 664$ for MDA and $m/z = 678$ for MDMA.

tiomeric ratios (*R* versus *S*) of MA ranged from 0.01 to 0.82 and from 0.03 to 0.95 for AM. Moreover, only (*S*)-MA and (*S*)-AM were found in four hair specimens, which indicates an ingestion of optically pure (*S*)-MA. In addition, one hair specimen showed the presence of both enantiomers of AM and MA with the concentration of the (*R*)-MA exceeding that of (*S*)-MA, while the isomers of its metabolite were almost at equal *R*/*S* ratio. This result may be explained by a concomitant ingestion of racemic MA and optically pure (*R*)-MA [38].

Finally, the specimen of case no. 24 was tested positive for both enantiomers of MA, MDA and MDMA (Fig. 5). The corresponding quantification results are summarized in Table 6. (*R*)-MDMA was predominant, which confirms the results of Tagliaro et al. [17] after analysis of a single real case "ecstasy user" by EC/UV. Furthermore the *R*/*S* ratio >1 for MDMA and *R*/*S* ratio <1 for its metabolite MDA were also in accordance with those observed in plasma, saliva or urine [4,39–41]. As MDMA is usually consumed as racemic mixture [4–6], the accumulation of the (*R*)- or the (*S*)-enantiomers of MDMA and MDA in hair may be the consequence of the stereoselective metabolism [17]. However, further studies are needed for the understanding of the stereoselective incorporation mechanisms of the ATS in hair.

4. Conclusion

A GC/MS-NICI method using an in-house synthesized chiral derivatisation reagent, (*S*,*R*)-HFBOPCl has been developed for the enantiomeric quantification of ATS extracted from human hair matrix. Stereoselectivity and sensitivity of ATS for GC/MS-NICI analysis has been improved through this novel chiral derivatisation reagent. The optimized and fully validated assay was applied for the determination of the enantiomeric ratios of MA, AM, MDA and MDMA in abusers' hair. Due to the good sensitivity of the (*S*,*R*)-HFBOPCl derivatives in the MS-NICI detection, it was also possible to quantify ATS in small amounts of hair.

This rapid and sensitive method may also be applied to enantioselective quantification of ATS in other biological matrices like plasma, urine or oral fluid.

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