Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 798 (2003) 249–255
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Development and validation of a gas chromatography-mass spectrometry assay for hair analysis of amphetamine, methamphetamine and methylenedioxy derivatives

Mitona Pujadas [a,1], Simona Pichini [b,c,1], Sandra Poudevida [a], Ester Menoyo [a], Piergiorgio Zuccaro [b], Magí Farré [a,c], Rafael de la Torre [a,d,*]

[a] Institut Municipal d'Investigació Mèdica (IMIM), Doctor Aiguader 80, E-08003 Barcelona, Spain
[b] Istituto Superiore di Sanità, Rome, Italy
[c] Universitat Autònoma, Barcelona, Spain
[d] Universitat Pompeu Fabra, Barcelona, Spain

Received 23 January 2003; received in revised form 12 September 2003; accepted 24 September 2003

Abstract

A procedure based on gas chromatography-mass spectrometry (GC-MS) is described for the determination of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), 3,4-methylenedioxyethylamphetamine (MDE or MDEA) and *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) in hair. Hair samples were digested with 1 M sodium sulfide at 37 °C (by shaking for 3 h and was kept at room temperature overnight), and extracted with two sequential extraction procedures: liquid-liquid extraction with *tert*-butyl methyl ether and solid-phase extraction with Bond-Elut Certify columns. Extracted analytes were derivatised with *N*-methyl-bis(trifluoroacetamide), separated by a 5% phenylmethylsilicone column and determined by a mass spectrometer detector in selected ion monitoring mode. A good reproducibility (intra-assay R.S.D. = 1.5–15.7%), accuracy (intra-assay error = 2.0–11.7%) and sensitivity (LOD = 0.03–0.08 ng/mg hair) were attained. The method was successfully applied to the analysis of the proximal (1 cm) hair segment to assess recent self-reported use in "ecstasy" consumers. Otherwise, further studies are needed to validate methodology developed in case of amphetamine consumption.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Derivatization, GC; Amphetamine; Methamphetamine

1. Introduction

Recreational use of methylenedioxyphenylderivatives of amphetamine (referred to as designer drugs: 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) 3,4-methylenedioxyethylamphetamine (MDE or MDEA), *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamine (MBDB) either alone or in combination with other drugs, such as alcohol and cannabis, has become increasingly popular among young people [1,2]. In particular, MDMA is the most commonly consumed designer drug [2]. Acute medical complications of drug use include malignant hyperthermia, seizures, cerebral haemorrhage, hepatitis, rhabdomyolisis, disseminated intravascular coagulation, and acute renal failure [3]. There are a number of reports concerning severe intoxication and death after MDMA consumption [4].

Acute intoxication by designer drugs is diagnosed by urine and blood analysis and well documented literature is available [5]. Hair drug testing is an alternative approach to monitor past drug use in settings other than acute intoxication cases (i.e. forensic science) [6].

An individual's past history of drug abuse is usually collected through structured questionnaires. As this information is entirely based on the credibility of subjects which is questioned in many cases, drug testing in hair has been proposed as an objective alternative to self-reported drug

* Corresponding author. Tel.: +34-93-2211009; fax: +34-93-2213237.
E-mail address: rtorre@imim.es (R. de la Torre).
[1] Both authors equally contributed to the conception, design and preparation of the manuscript.

1570-0232/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2003.09.056

abuse histories [6]. Furthermore, when performing analyses of different hair sections corresponding to different periods of time—segmental hair analysis—eventual changes in patterns of consumption can be theoretically defined and associated to eventual alterations observed in cognitive processes or incidence of psychopathology [7]. Hair accumulation of both classical amphetamines and methylenedioxy derivatives have been extensively reported in consumers and in fatal cases, with gas chromatography-mass spectrometry (GC-MS) methods being applied for quantification purposes and to investigate disposition of parent drugs and metabolites in hair [8–12]. The majority of developed methods detected classical amphetamines (amphetamine and methamphetamine) or methylenedioxy derivatives, with few assays simultaneously testing the two classes of drugs [13–16].

The objective of the present report was to develop a sensitive and selective analytical method for hair analysis of amphetamine derivatives that meets the accepted criteria for bioanalytical method validation [17]. Since it has been shown that amphetamines consumed in the dance scene are mainly "ecstasy" pills (containing not only MDMA but also MDA, MDEA, and eventually MBDB), amphetamine and methamphetamine [14], a methodology for hair analysis of both classical amphetamines and methylenedioxy derivatives has been set up, validated and applied in a follow-up study of "ecstasy" abusers. In this paper, preliminary results concerning segmental hair analysis in selected cases will be presented.

2. Materials and methods

2.1. Chemicals and reagents

Amphetamine (AP), methamphetamine (MA), MDMA, MDA, MDEA, $[^2H_5]$MDMA, $[^2H_5]$MDA, $[^2H_5]$AP and $[^2H_8]$MA were supplied by Cerilliant (Austin, TX, USA). MBDB was provided by Lipomed (Cambridge, MA, USA). Bond Elut Certify® solid-phase extraction (SPE) columns were obtained from Varian Corp. (Harbor City, CA, USA). Gas chromatography grade *N*-methyl-bis(trifluoroacetamide) (MBTFA) was purchased from Macherey-Nagel (Düren, Germany). Analytical grade sodium sulfide nonahydrate was supplied by Aldrich (Steinheim, Germany). All other reagent grade chemicals were supplied by Merck (Darmstadt, Germany).

2.2. Standards and solutions

Separate stock solutions of AP, MA, MDMA, MDA, MDEA and MBDB (1 mg/ml) were prepared in HPLC-grade methanol and stored at −20 °C. From stock solutions, working solutions of 10, 1, and 0.1 μg/ml were made and used for the preparation of calibration curves and quality control (QC) samples. Internal standards (ISTDs) ($[^2H_5]$AP, $[^2H_8]$MA, $[^2H_5]$MDA and $[^2H_5]$MDMA) were diluted in methanol to give a working solution at a concentration of 1 μg/ml and stored at −20 °C.

Daily standard curves were obtained by analyzing hair samples (10 mg blank control samples) fortified with the drugs at 0, 0.5, 5, 10, 15 and 20 ng/mg hair. QC samples (10 mg blank control samples fortified with 0.6, 7.5, 17.5 ng drug/mg hair) were also prepared daily from working solution of stock sources with different lot numbers from the reference materials used to prepare the calibration standards. QC samples were included in each analytical batch to check calibration, accuracy and precision.

2.3. Hair samples collection

Hair samples were collected within the framework of a two-year follow-up study of 40 ecstasy abusers and matched controls. Subjects, with age ranging from 18 to 34 years, completed a structured questionnaire which asked about: initial and final age of consumption, last consumption, and total consumption in the previous month and in the last 6 and 12 months, respectively in their history with drugs. Subjects provided information for several drugs of abuse, among others: MDMA or ecstasy, methamphetamine, other psycho stimulants like amphetamine and cocaine, cannabis, and LSD. Hair analysis was performed for several drugs, but this study describes only findings related to the methodology described: methamphetamine and methylenedioxyderivatives.

Hair samples (as an entire strand) were cut close to the scalp in the vertex region using a stainless steel scissors. From all the samples collected for the study, 17 hair samples were used within the method development to assess association between hair analysis and self-reported use of designer drugs in the last month. The selection was made according to several patterns of consumption representative of the population studied. Consumers recruited for the study were divided in two different categories of drug consumption: occasional users (eventual use, less than once a week) and regular users (weekly to daily use). Hair strands from the 17 subjects (all with natural brown hair colour) were divided in segments. The first was cut at 1 cm from the proximal region, representing hair growth in the last month. Hair segment was finely cut and a duplicate of 10 mg weight was obtained. Results reported in the present study, refer to the analytical findings in this segment supposed to be related with information collected in the questionnaire for the last month consumption.

Hair from 10 ecstasy users was pooled, homogenised and used as positive control to check different digestion and extraction procedures and it was included in each analytical batch as internal QC of the methodology developed. Drug-free human hair samples obtained from 10 non-consumers were reduced in short cuts, analysed during method validation to exclude any source of chromatographic interferences and mixed to obtain a homogeneous pool of blank hair.

2.4. Hair sample preparation and extraction

The pooled drug free hair and hair samples (10 mg) were washed three times (2 min) with 3 ml dichloromethane in an ultrasonic water-bath and allowed to dry at room temperature.

Then, samples, calibrators, QC samples (drug free hair spiked with drugs at concentrations and working standard solutions other than those used for calibrators) and the internal QC positive sample (pooled hair from MDMA users) were fortified with 50 μl of 1 μg/ml [$^2H_5$]AP, [$^2H_8$]MA, [$^2H_5$]MDA and [$^2H_5$]MDMA, as internal standards.

Four different digestion and extraction procedures were tested with the pool of blank hair fortified with analytes under investigation and in a pool of hair samples from consumers. Two digestions were performed in alkaline conditions and consisted of 1 ml of 1 M sodium sulfide (Na_2S) or 1 M NaOH placed in silanized glass tubes with 10 mg hair samples with periodic shaking for 3 h and then kept at room temperature overnight [18]. After digestion, hair samples were ultracentrifuged at 12000 rpm for 10 min and the aqueous layer was transferred in a new silanized glass tube. Analytes were extracted from the aqueous layer with two subsequent portions of 3 ml of *tert*-butyl methyl ether by rocking mixing for 30 min and centrifuged at 3500 rpm for 5 min. The organic phase was separated and evaporated to dryness under nitrogen stream at 23 °C with a c.a.10 psi pressure. Sample extracts were reconstituted with 1 ml of 0.1 M sodium phosphate buffer (pH 6). Reconstituted extracts underwent a solid–liquid extraction (SPE) with Bond Elut Certify columns according to a previously reported method [19]. Two acid digestions, were assayed as follows: hair samples were placed in silanized glass tubes with 1 ml methanol/5 M HCl (v/v 20:1) or methanol/trifluoroacetic acid (v/v 9:1) [20,21] and allowed to stand 1 h under ultrasonication. Then the solution was left to stand at room temperature overnight. Afterwards, the organic phase was separated from hair samples and evaporated to dryness under nitrogen stream at 23 °C (c.a.10 psi pressure). Sample extracts were reconstituted with 1 ml of 0.1 M sodium phosphate buffer (pH 6). Reconstituted extracts underwent a solid–liquid extraction (SPE) with Bond Elut Certify columns according to a previously reported method [20].

Eluates from SPE after both basic and acid digestion, were added with 20 μl of MBTFA to prevent amphetamines losses, were evaporated to dryness under nitrogen stream at 40 °C (c.a.10 psi pressure). Trifluoroacyl derivatives were formed by reaction with 50 μl of MBTFA as derivatization agent in a dry bath at 70 °C during 45 min.

2.5. GC-MS analysis

GC-MS analysis was performed in a Hewlett Packard 6890 gas chromatograph coupled to an HP 5973 quadrupole mass spectrometer detector (Palo Alto, CA). The gas chromatograph was fitted with an HP 7683 auto sampler injector.

Samples were injected in splitless mode into a 12 m × 0.2 mm i.d., 0.33 μm film thickness 5% phenylmethylsilicone column (Ultra 2-Hewlett Packard).

The oven temperature was initially maintained at 70 °C during 2 min and programmed to 160 °C at 30 °C per min, then to 170 °C at 5 °C per min, to 200 °C at 15 °C, and finally to 290 °C at 30 °C per min.

The injector and the interface were operated at 280 °C. Helium was used as carrier gas at a flow rate of 1.2 ml/min.

The mass spectrometer was operated in electron impact ionization mode at 70 eV. Qualifying ions selected for analytes under investigation were: *m/z* 91, 118, 140 for AP-*N*-TFA, *m/z* 91, 118, 154 for MA-*N*-TFA, *m/z* 154, 162, 289 for MDMA-*N*-TFA, *m/z* 135, 162, 275 for MDA-*N*-TFA, *m/z* 162, 168, 303 for MDEA-*N*-TFA, *m/z* 168, 176, 303 for MBDB-*N*-TFA, *m/z* 96, 123, 140 for [$^2H_5$]AP-*N*-TFA, *m/z* 92, 113, 161 for [$^2H_8$]MA-*N*-TFA, *m/z* 136, 167, 280 for [$^2H_5$]MDA-*N*-TFA, *m/z* 158, 164, 294 *m/z* for [$^2H_5$] MDMA-*N*-TFA. Ion ratio acceptance criterion was a deviation ≤20% of the average of ion ratios of all the calibrators. The ions: *m/z* 118 for AP-*N*-TFA, *m/z* 154 for MA-*N*-TFA and MDMA-*N*-TFA, *m/z* 162 for MDA-*N*-TFA and MDEA-*N*-TFA, *m/z* 168 for MBDB-*N*-TFA, *m/z* 123 for [$^2H_5$]AP-*N*-TFA, *m/z* 161 for [$^2H_8$]MA-*N*-TFA, *m/z* 136 for [$^2H_5$]MDA-*N*-TFA, *m/z* 158 for [$^2H_5$] MDMA-*N*-TFA were used for quantification.

2.6. Method validation

Analytical recoveries were calculated by comparison between peak areas of the calibration samples analyzed with the normal procedure and those obtained after adding the same amounts of reference substances and ISTDs to blank hair after extraction. Recoveries were analyzed at three different concentrations, 0.5, 10 and 20 ng/mg hair, using four replicates for each evaluated concentration. Linearity was determined by checking different calibration curves ($n = 10$ in four consecutive days) at five different concentrations (limit of quantification, 5, 10, 15 and 20 ng/mg for all compounds). Peak area ratios between compounds and internal standards were used for calculations.

[$^2H_5$]AP was used as internal standard for AP, [$^2H_8$]MA for MA, [$^2H_5$]MDA for MDA and [$^2H_5$]MDMA for MDMA, MDEA and MBDB. A weighted least-square regression analysis was used (SPSS for Windows 9.0.1).

Five replicates of blank samples added with 5 ng/mg of ISTDs were used for calculating the limits of detection and quantification. Standard deviation (S.D.) of the analytical background response was used to determine the detection limit (LOD = 3.3 S.D.) and the quantification limit (LOQ = 10 S.D.).

Five replicates at three different concentrations of the analytes (0.6, 7.5 and 17.5 ng/mg hair) spiked in blank hair were used for the determination of intra-assay precision (expressed as coefficient of variation for specific added target concentrations) and accuracy (expressed as percentage error

of concentration found as compared with target added concentrations). Inter-day precision and accuracy were determined in three different experimental days.

Mid-term stability test was performed for hair samples stored at ambient temperature. Hair pool from 10 ecstasy consumers, used as internal QC, was included in each analytical batch during a 3 months period. The stability was expressed as a percentage of the initial concentration (first analyzed batch) of the analytes in pooled hair.

Selectivity tests were performed with 10 hair samples from non consumers extracted and analysed for assessment of potential interferences from endogenous substances. The apparent response at the retention times of the analytes under investigation was compared to the response of analytes at the limit of quantification. Furthermore, potential interferences from principal drugs of abuse (opiates, cocaine and main metabolites, cannabinoids-Δ-9-tetrahydrocannabinol and 11-nor-9-carboxy-tetrahydrocannabinol-), were also evaluated spiking 10 mg of blank hair spiked with 10 ng of the aforementioned substances and carried through the entire procedure.

3. Results and discussion

3.1. Analytical method

Recently, it was suggested [18] that hair digestion with 1 M sodium sulfide increased drug recovery from keratin matrix in case of nicotine and flunitrazepam. Hence, this digestion agent was tested during method development and compared with other digestion approaches commonly used to extract drugs of abuse and in particular amphetamines from hair samples, such as sodium hydroxide, methanol/HCl and methanol/trifluoroacetic acid [18–21].

Table 1 shows the recovered amounts of AP, MA and methylendioxy derivatives in blank hair fortified with 2.5 ng/mg of each analyte and in a pool of hair samples from consumers following different digestions and extraction procedures.

Complete dissolution of hair samples, as obtained with sodium sulfide or sodium hydroxide, required a subsequent two-step extraction procedure in order to isolate analytes under investigation from substances released from keratin matrix. In contrast, acid digestions are usually described associated with a single SPE extraction step. However, extracts obtained following acid digestion and SPE resulted dirty and hence presented problems during GC injection giving rise to many chromatographic interferences in sample analysis by GC-MS. On the other hand, if a liquid–liquid extraction was applied before SPE to acid digestion in order to improve sample clean-up, analyte recoveries resulted sensibly lowered (intramural data not shown).

Digestion with 1 M sodium sulfide, followed by a liquid–liquid extraction and a SPE with Bond-Elut Certify resulted to be the best compromise between recovery of analytes from hair matrix, clean-up of extracts and absence of chromatographic interferences.

Consequently, this procedure was used to examine hair from ecstasy consumers. A comparison of some digestion and extraction procedures for amphetamines in hair has been reported by Kintz and Cirimele [22]. The authors concluded that best recoveries were observed after alkaline hydrolysis with NaOH 1 N. This result is not in contrast with our findings. Indeed, apparent best recoveries here obtained after acid extractions were due to the fact that in this case only a SPE extraction was used after digestion and when a liquid–liquid step was introduced before SPE, recoveries were lower than those obtained by alkaline hydrolysis with NaOH. Nonetheless, sodium sulfide, which was not evaluated by aforementioned authors gave better results.

Fig. 1 shows the fragmentograms from an extract of a blank hair (A), an extract of a blank hair fortified with 1 ng of each analyte per mg hair (B), and an extract of hair from a " ecstasy" consumer containing 0.63 ng/mg of MDA, 4.53 ng/mg of MDMA and 0.70 ng/mg of MDEA. Hair from non consumers, analyzed as separate and pooled samples, did not interfere with the assay.

Data on method validation are reported in Tables 2–4. Standard curve plots for the analytes were linear in the range of tested concentrations with a coefficient of correlation (r^2) higher than 0.99. Intra- and inter-assay accuracy and precision results satisfactorily met current acceptance criteria in the validation of bioanalytical methods [18]. Analytical recoveries and calculated limits of detection and quantification were considered adequate for the purpose of the study.

Table 1
Recovered amount of amphetamine, methamphetamine and methylendioxyderivatives in blank hair fortified with 2.5 ng/mg analytes and pool of consumers following different digestions and extraction procedures

Analyte	Fortified hair (ng/mg)				Pool of consumers (ng/mg)			
	Na_2S 1 M	MeOH–HCl	MeOH–TFA	NaOH 1 M	Na_2S 1 M	MeOH–HCl	MeOH–TFA	NaOH 1 M
AP	0.91 ± 0.10	1.15 ± 0.87	1.28 ± 0.09	0.99 ± 0.29	N.D.	N.D.	N.D.	N.D.
MA	1.80 ± 1.11	0.57 ± 0.26	1.94 ± 0.49	0.46 ± 0.11	N.D.	N.D.	N.D.	N.D.
MDA	2.38 ± 0.11	2.77 ± 0.25	0.69 ± 0.23	0.51 ± 0.06	0.33 ± 0.08	0.18 ± 0.05	0.11 ± 0.02	N.D.
MDMA	2.22 ± 0.08	2.50 ± 0.44	1.25 ± 0.82	0.70 ± 0.20	3.71 ± 0.27	2.96 ± 0.25	3.55 ± 0.12	1.67 ± 0.40
MDEA	1.85 ± 0.19	2.56 ± 0.26	1.22 ± 0.24	0.48 ± 0.10	N.D.	N.D.	N.D.	N.D.
MBDB	1.98 ± 0.12	2.54 ± 0.30	1.68 ± 0.32	0.54 ± 0.16	N.D.	N.D.	N.D.	N.D.

Results are expressed as mean ± S.D. ($n = 4$). N.D.: not detected.

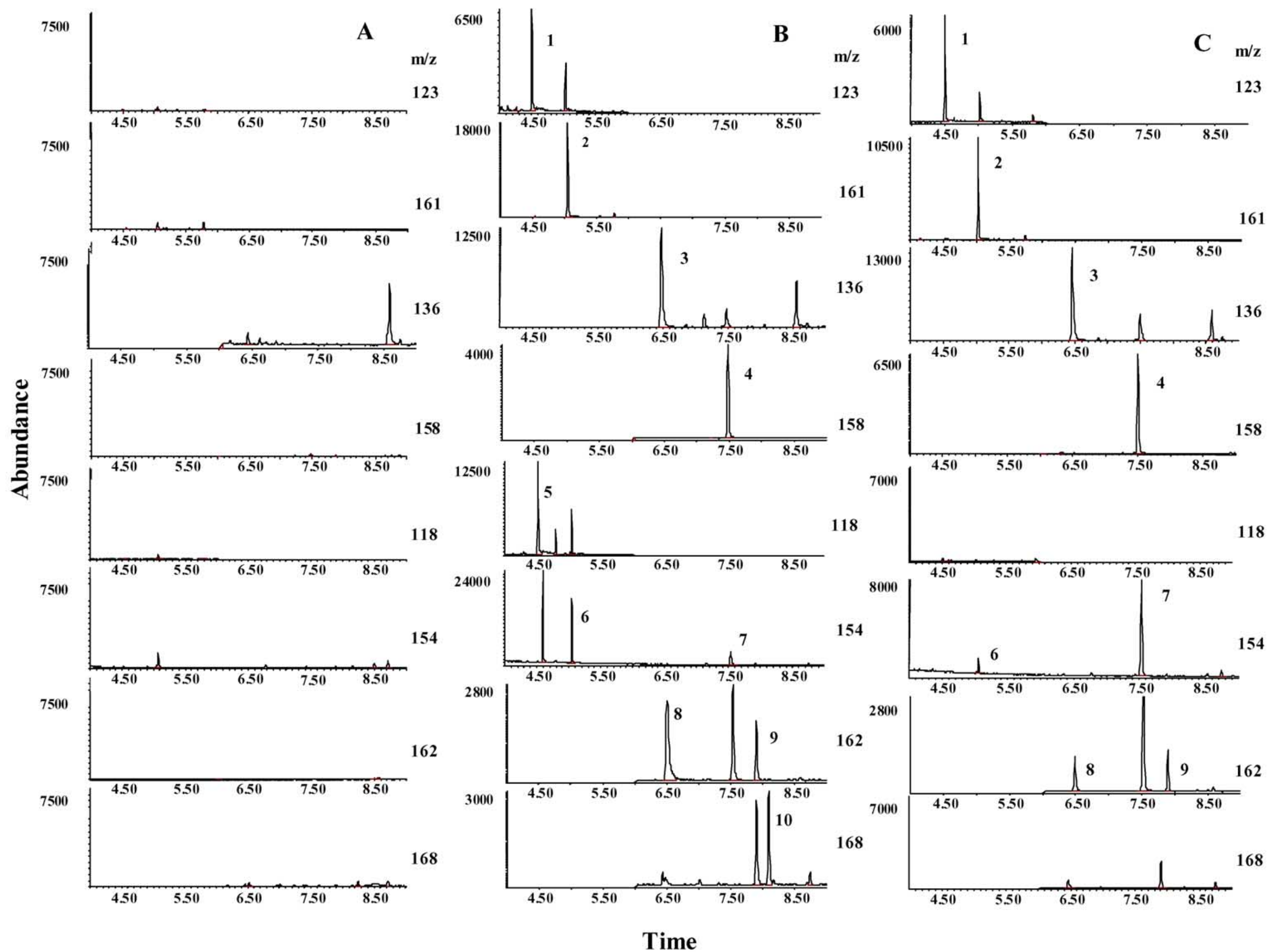

Fig. 1. GC-MS-SIM Fragmentograms of TFA-derivatised extracts from (A) human blank hair, (B) human blank hair spiked with 1 ng/mg analytes under investigation and (C) subject scalp hair. (1) [$^{2}H_5$]amphetamine, 123 *m/z*; (2) [$^{2}H_8$]methamphetamine, 161 *m/z*; (3) [$^{2}H_5$]MDA, 136 *m/z*; (4) [$^{2}H_5$]MDMA, 158 *m/z*; (5) amphetamine, 118 *m/z*; (6) methamphetamine, 154 *m/z*; (7) MDMA, 154 *m/z*; (8) MDA, 162 *m/z*; (9) MDEA, 162 *m/z*; (10) MBDB, 168 *m/z*.

Table 2
Method calibration

Analyte	Calibration slope	Calibration intercept	Correlation coefficient (r^2)	Limit of detection (LOD) (ng/mg)	Limit of quantification (LOQ) (ng/mg)
AP	0.3704 ± 0.0282	0.0362 ± 0.0091	0.9958 ± 0.0056	0.08	0.25
MA	0.1361 ± 0.0075	0.0151 ± 0.0112	0.9932 ± 0.0029	0.05	0.15
MDA	0.0712 ± 0.0083	0.0086 ± 0.0042	0.9912 ± 0.0009	0.03	0.10
MDMA	0.2131 ± 0.0034	0.0021 ± 0.0012	0.9974 ± 0.0009	0.05	0.15
MDEA	0.0582 ± 0.0110	0.0048 ± 0.0020	0.9940 ± 0.0031	0.05	0.15
MBDB	0.2792 ± 0.0125	−0.0168 ± 0.0082	0.9984 ± 0.0016	0.08	0.25

Table 3
Intra-assay ($n = 5$) and inter-assay ($n = 15$) precision and accuracy calculated for the determination of MDMA, AP, MA, MDA, MDEA and MBDB in hair

Analyte	Concentration (ng/mg)	Intra-assay		Inter-assay	
		Precision (R.S.D.%)	Accuracy (error%)	Precision (R.S.D.%)	Accuracy (error%)
AP	0.6	4.5	9.1	7.5	13.5
	7.5	13.9	10.4	11.9	10.6
	17.5	9.3	9.0	12.7	10.1
MA	0.6	7.2	6.6	9.0	7.9
	7.5	13.9	9.8	12.7	14.1
	17.5	2.0	2.0	5.3	4.3
MDA	0.6	14.3	10.5	11.7	10.9
	7.5	5.4	7.0	11.8	8.1
	17.5	5.8	4.0	10.2	7.9
MDMA	0.6	15.7	11.7	15.0	9.9
	7.5	3.1	2.2	5.3	3.9
	17.5	4.5	3.0	4.8	4.2
MDEA	0.6	2.8	2.6	19.5	19.2
	7.5	5.5	9.6	13.3	10.3
	17.5	5.1	3.6	5.9	5.4
MBDB	0.6	8.4	6.6	16.3	12.4
	7.5	14.6	11.0	11.6	8.1
	17.5	1.5	3.9	7.8	5.9

None of the drugs of abuse other than analytes under investigation carried through the entire procedure interfered with the assay. In reference to mid-term stability test, no relevant degradation was observed in the pooled positive control analysed in a 3 months period, with differences when compared to the initial concentration lower than 5%.

Table 5 presents the results obtained after applying the developed analytical methodology to proximal 1 cm hair segments of individuals claiming use of “ecstasy” and methamphetamine.

Concordance between the self-reported data on last month “ecstasy” consumption and MDMA concentration was good ($r^2 = 0.76$) even in the limited number of individuals examined. Recent findings show that drug incorporation in hair depends from melanin concentration in hair [23]. Indeed, the hair color of the subjects recruited for this study was quite homogeneous, being all the subjects brown-haired, as is the majority of Spanish population. Furthermore, in those hair samples in which MDA could be also determined and other amphetamines were absent, the metabolite/parent drug (MDA/MDMA) ratio ranged between 0.04 and 0.06. When comparing the self-report data for the use of methamphetamine with AP and MA hair concentration, results were disappointing in case of AP. AP could never be detected in hair samples of subjects claiming methamphetamine consumption while it was found, together with MA, in a hair

Table 4
Analytical recoveries at three different concentrations ($n = 4$)

Concentration (ng/mg)	AP (mean ± S.D.)	MA (mean ± S.D.)	MDA (mean ± S.D.)	MDMA (mean ± S.D.)	MDEA (mean ± S.D.)	MBDB (mean ± S.D.)
0.5	92.8 ± 12.1	81.0 ± 2.4	75.1 ± 6.3	91.3 ± 16.4	84.6 ± 15.9	85.0 ± 9.5
10	97.8 ± 23.9	77.3 ± 2.8	98.0 ± 3.8	97.9 ± 2.9	98.7 ± 17.4	96.2 ± 5.3
20	92.5 ± 7.7	85.0 ± 7.8	96.2 ± 13.9	93.9 ± 4.7	80.5 ± 12.9	90.4 ± 3.3

Table 5
Amphetamine-type stimulant consumed and results of hair for study volunteers

Vol.	Consumed substance	Declared consumption last 30 days	Analyte found in the proximal 1 cm hair (ng/mg)				
			MDMA	MDA	MA	AP	MDEA
002	Ecstasy, methamphetamine	2 tablets	3.68	N.D.	N.D.	N.D.	N.D.
003	Ecstasy, methamphetamine	2 tablets, 500 mg	3.08	0.15	N.D.	N.D.	N.D.
007	Ecstasy, methamphetamine	2 tablets, 250 mg	3.14	0.16	N.D.	N.D.	N.D.
011	Ecstasy, methamphetamine	4 tablets	6.13	N.D.	N.D.	N.D.	N.D.
015	Ecstasy, methamphetamine	12 tablets, 1000 mg	12.60	9.00	2.18	N.D.	N.D.
016	Ecstasy, methamphetamine	1 tablet	2.35	0.10	N.D.	N.D.	N.D.
018	Ecstasy, methamphetamine	3 tablets	4.34	0.25	N.D.	N.D.	N.D.
020	Ecstasy, methamphetamine	2 tablets	1.20	N.D.	N.D.	N.D.	N.D.
023	Ecstasy, methamphetamine	1.5 tablets	1.70	N.D.	N.D.	N.D.	N.D.
037	Ecstasy, methamphetamine	2.5 tablets	6.98	0.59	N.D.	N.D.	N.D.
038	Ecstasy, methamphetamine	3 tablets, 150 mg	2.98	2.02	0.54	N.D.	N.D.
040	Ecstasy, methamphetamine	–	N.D.	N.D.	1.04	0.74	N.D.
051	Ecstasy, methamphetamine	4 tablets, 750 mg	4.15	2.10	0.57	N.D.	N.D.
075	Ecstasy, methamphetamine	0.75 tablets, 250 mg	4.53	0.63	0.22	N.D.	0.70
103	Ecstasy, methamphetamine	3 tablets, 150 mg	4.68	0.30	N.D.	N.D.	N.D.

*N.D.: not detected.

sample of an individual, which did not declare consumption. This finding is in agreement with what reported by other authors [11] which affirmed that LOD and LOQ of AP higher than those for the other compounds could decrease the number of potentially positive results. MBDB was never detected in the examined subjects, as this amphetamine derivative seemed not to be present in Spain, nor as at the moment in any other European country. Conversely, in a single case out of 17, MDEA could be detected.

4. Conclusions

The GC-MS method reported in this paper to simultaneously analyze amphetamine, methamphetamine and methylendioxy derivatives in hair was validated according to internationally accepted criteria [14]. The method consists of sample digestion in sodium sulfide followed by a liquid–liquid and solid phase extraction, chromatographic separation on a 5% phenylmethylsilicone column and detection in SIM mode by GC/MS. The method showed adequate range of linearity, intra and inter-assay accuracy and precision for its application in hair analysis of MDMA and MDA for assessment of recent self-reported "ecstasy" use. Assessment of methamphetamine use requires further investigation to improve sensitivity to detect hair AP and analysis of a larger number of samples.

Acknowledgements

This study was financially supported by 'Area Progetto Droga' from Istituto Superiore di Sanità, Roma (Italy) and FIS grant 00/077.

References

[1] J.D. Wright, L. Pearl, BMJ 310 (1995) 20.
[2] H. Zhao, R. Brenneisen, A. Scholer, A. McNally, M.A. Elsohly, T. Murphy, S.J. Salamone, J. Anal. Toxicol. 25 (2001) 258.
[3] U. McCann, S.O. Slate, G.A. Ricaurte, Drug Saf. 15 (1996) 107.
[4] J.A. Henry, K.J. Jeffreys, S. Dawling, Lancet 340 (1992) 384.
[5] T. Kraemer, H.H. Maurer, J. Chromatogr. Biomed. Appl. 713 (1998) 163.
[6] S. Pichini, I. Altieri, P. Zuccaro, R. Pacifici, Clin. Pharmacokinet. 30 (1996) 211.
[7] D.J. McHenna, S.J. Peroutka, J. Neurochem. 54 (1990) 14.
[8] P. Kintz, V. Cirimele, A. Tracqui, P. Mangin, J. Chromatogr. Biomed. Appl. 670 (1995) 162.
[9] Y. Nakahara, Forensic Sci. Int. 70 (1995) 135.
[10] M. Rothe, F. Pragst, K. Spiegel, T. Harrach, K. Fischer, J. Kunkel, Forensic Sci. Int. 89 (1997) 111.
[11] R. Kronstrand, R. Grundin, J. Jonsson, Forensic Sci. Int. 92 (1998) 29.
[12] G.A.A. Cooper, D.L. Allen, K.S. Scott, J.S. Oliver, J. Ditton, I.D. Smith, J. Forensic Sci. 45 (2000) 400.
[13] H. Sachs, P. Kintz, J. Chromatogr. B. 713 (1998) 147.
[14] J. Röhrich, G. Kauert, Forensic Sci. Int. 84 (1997) 179.
[15] P. Kintz, N. Samyn, J. Chromatogr. B 733 (1999) 137.
[16] J. Liu, K. Hara, S. Kashimura, M. Kashiwagi, M. Kageura, J. Chromatogr. B 758 (2001) 95.
[17] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, May 2001 (http://www.fda.gov/cder/guidance/4252fnl.htm).
[18] D.J. Claffey, P.R. Stout, J.A. Ruth, J. Anal. Toxicol. 24 (2000) 54.
[19] J. Ortuño, N. Pizarro, M. Farré, M. Mas, J. Segura, J. Camí, R. Brenneisen, R. de la Torre, J. Chromatogr. B 723 (1999) 221.
[20] R. Kikura, Y. Nakahara, Biol. Pharm. Bull. 18 (1995) 1694.
[21] J. Segura, C. Stramesi, A. Redón, M. Ventura, C.J. Sánchez, G. González, L. San, M. Montagna, J. Chromatogr. B 724 (1999) 9.
[22] P. Kintz, V. Cirimele, Forensic Sci. Int. 84 (1997) 151.
[23] R. Kronstrand, S. Förstberg-Peterson, B. Kågedal, J. Ahlner, G. Larson, Clin. Chem. 45 (1999) 1485.