Journal of Chromatography A, 1217 (2010) 6274–6280

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Analysis of phenylpiperazine-like stimulants in human hair as trimethylsilyl derivatives by gas chromatography–mass spectrometry

M. Barroso [a,*], S. Costa [a], M. Dias [a], D.N. Vieira [b], J.A. Queiroz [c], M. López-Rivadulla [d]

[a] Instituto Nacional de Medicina Legal – Delegação do Sul, Rua Manuel Bento de Sousa, 3, 1150-219 Lisboa, Portugal
[b] Instituto Nacional de Medicina Legal, Largo da Sé Nova, 3000-213 Coimbra, Portugal
[c] Centro de Investigação em Ciências da Saúde – Universidade da Beira Interior, Av. Infante D. Henrique, 6201-506 Covilhã, Portugal
[d] Instituto Universitario de Medicina Legal, San Francisco s/n, 15782 Santiago de Compostela, Spain

ARTICLE INFO

Article history:
Received 28 June 2010
Received in revised form 28 July 2010
Accepted 2 August 2010
Available online 11 August 2010

Keywords:
Piperazines
Hair
GC/MS

ABSTRACT

A simple and sensitive procedure, using *p*-tolylpiperazine (pTP) as internal standard (IS), has been developed and validated for the qualitative and quantitative analysis of 1-(3-trifuoromethylphenyl)piperazine (TFMPP), 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP) in hair. Drug extraction was performed by incubation with 1 M sodium hydroxide at 50 °C for 40 min, and the extracts were cleaned up using mixed-mode solid-phase extraction. The analytes were derivatized with N-methyl-N-(trimethylsilyl) trifluoroacetamide with 5% trimethylchlorosilane and analysed by gas chromatography–mass spectrometry in the selected ion monitoring mode. The method was linear from 0.05 (lower limit of quantitation) to 4 ng mg^{-1}, with correlation coefficients higher than 0.99 for all the compounds. Intra- and interday precision and accuracy were in conformity with the criteria normally accepted in bioanalytical method validation, and the sample cleanup step presented a mean efficiency higher than 90% for all the analytes. Due to its simplicity and speed, this method can be successfully applied in the screening and quantitation of these compounds in hair samples, and is suitable for application in forensic toxicology routine analysis.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Piperazine-like compounds are considered as a new group of designer drugs, and have already been found in the illicit drug market as abused drugs [1–3], namely as a constituent or substitute of 3,4-methylenedioxymethamphetamine (MDMA) in suspected MDMA seized pills [3].

These compounds can be structurally divided in two subgroups (benzylpiperazines and phenylpiperazines), and benzylpiperazine (BZP) and 3-trifluoromethylphenylpiperazine (TFMPP) have been circulating among drug consumers since the beginning of the century, readily followed by 1-(3-chlorophenyl)piperazine (mCPP) and 1-(4-methoxyphenyl)piperazine (MeOPP) [4].

These piperazine-like compounds may act as stimulants and can produce euphoria. In addition, TFMPP and mCPP have small hallucinogenic potential as commented on by Maurer et al. [5], and therefore the likelihood for abuse by consumers can be important.

BZP acts mainly by stimulating the release and inhibiting the reuptake of dopamine and serotonin, as indicated by animal studies [6,7], while TFMPP did not produce the same locomotor stimulant effects as BZP in studies in rats [6], despite an observable increase in serotonin. The best pharmacologically characterized piperazine is however mCPP [8], as it is a known metabolite of the atypical antidepressant drugs trazodone and nefazodone [9–12], and acts by increasing the extracellular levels of dopamine, serotonin and noradrenalin, either by inhibiting their reuptake or by stimulating pre-synaptic receptors [13].

Both liquid chromatography [14–20] and gas chromatography [2,15,18,21,22] based methods are available in the literature for the determination of piperazines in biological specimens, all of them utilizing mass spectrometry, except one paper [14], where a UV detector was also used. In these papers, liquid–liquid [2,14,16,18] and solid-phase [15,16,18,19,21,22] extraction procedures have been used for analyte isolation from the matrices prior to chromatography, and urine [2,14–18,20], blood, plasma or serum [14,19,20] samples have been analysed. However, no data is available on hair analysis for these substances, except the inclusion of BZP in a qualitative drug screening by means of two-dimensional gas chromatography/time-of-flight mass spectrometry [22]. However, no specific data regarding limits of detection or quantitation is given, and the analysis of phenylpiperazines was not included, namely mCPP, which has been reported to be detected in MDMA seized pills [3].

* Corresponding author. Tel.: +351 218811800.
E-mail address: mbarroso@dlinml.mj.pt (M. Barroso).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.001

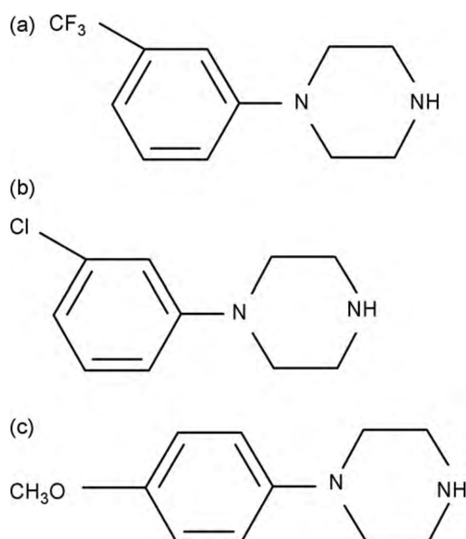

Fig. 1. Structural formulae of the studied phenylpiperazines: TFMPP (a), mCPP (b) and MeOPP (c).

This paper reports the development and validation of an analytical method for the determination of phenylpiperazines (TFMPP, mCPP and MeOPP; Fig. 1) as optimized trimethylsilyl derivatives in human hair after a short analyte extraction of 40 min and sample cleanup using mixed-mode solid-phase extraction. This short incubation time provides a drastic analysis time reduction if compared to the 16 h extraction published elsewhere [21] for the determination of BZP in hair samples.

2. Experimental

2.1. Reagents and standards

The analytical standard of TFMPP was kindly provided by the Portuguese Antidoping Laboratory (Laboratório de Análises de Dopagem e Bioquímica, Lisboa, Portugal), mCPP hydrochloride and MeOPP dihydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). The internal standard (pTP dihydrochloride) was obtained from Lancaster Synthesis (Lancashire, UK). Methanol (HPLC grade), dichloromethane, n-hexane, 2-propanol, ammonium hydroxide, hydrochloric acid, and potassium dihydrogenphosphate (analytical grade) were obtained from Merck Co. (Darmstadt, Germany).

N-Methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) and trimethylchlorosylane (TMS) were purchased from Macherey-Nagel, Düren, Germany.

Oasis® MCX (3 mL, 60 mg) extraction cartridges were obtained from Waters (Milford, MA, USA).

Stock solutions for each analyte were prepared at 1 mg mL^{-1} by weighing 10 mg of the compound to a 10 mL volumetric flask, and filling up to volume with methanol. Working solutions at 1 and 0.02 $\mu g\, mL^{-1}$ of all the compounds were prepared by proper dilution of the stock solutions with methanol. A working solution of the internal standard at 2 $\mu g\, mL^{-1}$ was prepared also in methanol. All these solutions were stored light protected between 2 and 8 °C.

To prepare the potassium dihydrogenphosphate 0.1 M solution, 13.61 g of potassium dihydrogenphosphate was weighed into a volumetric flask, obtaining a final volume of 1 L with deionised water.

2.2. Biological samples

Blank hair used in the optimization experiments and validation was obtained from laboratory staff. Authentic samples belonging to drug addicts were collected at the beginning of autopsies performed at the Forensic Pathology Service of the National Institute of Legal Medicine – South Branch, Lisbon, Portugal. Hair samples were also collected from living subjects under psychiatric evaluation for child custody issues at the same institution, and also from persons undergoing treatment with trazodone.

2.3. Gas chromatographic–mass spectrometric conditions

Chromatographic analysis was performed using an HP 6890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany), equipped with a model 5972 mass selective detector (Hewlett-Packard, Waldbronn, Germany). A capillary column (30 m × 0.25 mm I.D., 0.25-μm film thickness) with 5% phenylmethylsiloxane (HP-5 MS), supplied by J & W Scientific (Folsom, CA, USA), was used.

Chromatographic conditions were as follows: initial oven temperature was 90 °C for 2 min, which was increased by 15 °C min^{-1} to 300 °C. The temperatures of the injection port and detector were set at 220 and 280 °C, respectively. The split injection mode was used (split ratio of 1:5), and helium with a flow rate of 0.8 mL min^{-1} was used as the carrier gas. The mass spectrometer was operated with a filament current of 300 μA and electron energy of 70 eV in the electron ionization (EI) mode. Quantitation was done in the selected ion monitoring (SIM) mode, and the ions were monitored at m/z $\underline{302}$, 287 and 172 for TFMPP; at m/z $\underline{268}$, 139 and 226 for mCPP; and at m/z $\underline{264}$, 249 and 135 for MeOPP (quantitation ions are underlined). For the IS, only one ion was monitored, at m/z 248.

2.4. Sample preparation

To avoid drug detection arising from environmental contamination, hair was washed sequentially with dichloromethane, deionised water and methanol. The last wash was stored for further analysis.

Twenty milligrams of hair cut into small pieces (of less than 1 mm) was weighed into 10 mL glass tubes, and 1 mL of 1 M sodium hydroxide was added. The tubes were slightly agitated in a vortex-mixer and incubated for 40 min at 50 °C.

The extracts were neutralized with an equimolar amount of hydrochloric acid, transferred to clean polypropylene tubes and 5 mL of KH_2PO_4 0.1 M was added. After addition of 50 μL of the internal standard solution (2 $\mu g\, mL^{-1}$) to compensate for analyte losses during solid-phase extraction (SPE), the samples were homogenised for 15 min by rotation/inversion movements.

This homogenate was added to mixed mode extraction cartridges, previously conditioned with 2 mL of methanol and 2 mL of deionised water. After the sample had passed through, the cartridges were washed sequentially with 2 mL of each of the following: deionised water, hydrochloric acid 0.1 M, dichloromethane:methanol (70:30), and n-hexane. After drying under full vacuum for 1 min, the analytes were eluted with 2 mL of a mixture of dichloromethane:isopropanol (80:20, v/v) with 2% of ammonium hydroxide.

The extracts were evaporated to dryness at 45 °C under a gentle N_2 stream. After this step, 65 μL of MSTFA with 5% of TMS was added, the tubes were vortex mixed for about 30 s and incubated at 80 °C for 30 min. The extracts were transferred to autosampler vials, and a 2 μL aliquot was injected onto the chromatographic system.

2.5. Validation procedure

The procedure was validated in terms of selectivity, linearity, intra- and interday precision and accuracy, and cleanup

efficiency. Selectivity was evaluated by analyzing blank hair samples of 6 different origins (laboratory staff), and it was checked for interferences at the retention times and monitored ions for each analyte. In addition, the interference of several other compounds [ecgonine methyl ester, benzoylecgonine, cocaine, morphine, 6-acetylmorphine, tramadol, codeine, 6-acetylcodeine, fentanyl, norfentanyl, Δ^9-tetrahydrocannabinol, 11-nor-Δ^9-tetrahydrocannabinol-9carboxylic acid, 11-hydroxy-Δ^9-tetrahydrocannabinol, amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), MDMA, 3,4-methylenedioxyethamphetamine (MDEA) and N-methyl-1-(3,4-methylenedioxyphenyl)-2-butanamide (MBDB)] that might be also present in the samples was studied as well.

This parameter was evaluated as follows. A pool of blank hair samples from 6 different origins was prepared, and 20 aliquots (20 mg) of this pool were weighed into 10 mL glass tubes. These 20 blank samples were spiked with 5 ng mg^{-1} of all the interfering compounds, while only 10 out of them were further spiked with 2 ng mg^{-1} of the analytes of interest. Thus, 10 positive and 10 negative samples for phenylpiperazines were obtained, which were afterwards spiked with the IS and analysed using the above-mentioned procedure.

The criteria for compound identification were as follows [23]. Concerning chromatography, the relative retention time of the analyte had to be within a 1% window from that of the same compound in a quality control sample analysed contemporaneously. As regards mass spectrometric identification in the SIM mode, at least three diagnostic ions need to be acquired, and their relative intensities should not differ by more than a tolerated amount from those generated by the same compound in a quality control sample analysed contemporaneously (on one hand, if the relative intensity of the ion is within a 25–50% interval of the base peak in the control sample, a maximum relative tolerance of ±20% will be allowed for the same ion in the sample; on the other hand, if this intensity is less than 25% or higher than 50% in the control sample, then absolute tolerances of respectively ±5% and ±10% will be allowed for the ion in the sample).

Calibration data were generated by spiking blank hair digests, and the calibration curve was established between 0.05 and 4 ng mg^{-1} (seven calibrators evenly distributed) for all the analytes. Five calibration curves have been prepared, and the criteria for acceptance included a R^2 value of at least 0.99, and the calibrators' accuracy within a ±15% interval, except at the lower limit of quantitation (LLOQ), for which ±20% was accepted.

The limit of quantitation was defined as the lowest amount of analyte that presented a discrete (and clearly distinguishable from the blank) peak and could be measured with adequate precision and accuracy (coefficient of variation of less than 20% and an inaccuracy of ±20%).

Intra-day precision was characterized in terms of relative standard deviation (RSD, %) by analyzing sets of 7 spiked hair digests at 4 different concentrations (0.05, 0.1, 0.2 and 2 ng mg^{-1}) in the same day. Interday precision was assessed at all the calibrators' concentrations over a 5-day period. Accuracy was evaluated in terms of mean relative error (bias, %) between the measured and the spiked concentrations for the calibrators and in the intra- and interday precision assays; the limits of acceptable variability were set at 15% for all the concentrations, except at the lower limit of quantitation (LLOQ), for which 20% was accepted. The efficiency of the sample cleanup step was determined by replicate analysis ($n = 6$) of samples spiked at three concentrations (0.05, 0.5 and 2 ng mg^{-1}), in which the internal standards were only added after that procedure. The obtained peak area ratios were compared to those obtained by spiking blank extracts with the same amounts of all the compounds; the latter were used as neat standards.

3. Results and discussion

3.1. Choice of derivatizing reagent

When GC/MS is used for the analysis of those compounds, a derivatization step is usually required, despite the fact that de Boer et al. [1] succeeded without derivatization, although not using biological specimens. The published procedures usually employ acetylation reactions, namely using trifluoroacetyl anhydride [19,24] or N-methyl-bis-trifluoroacetamide (MBTFA) [13]; or by reaction with heptafluorobutyric anhydride [21]. However, the derivatization of phenylpiperazines by means of trimethylsilylation is not yet described in the literature. This fact looks somewhat odd, particularly if one takes into account that TMS-derivatization is one of the most used derivatizing reactions for GC/MS; this may be explained by the fact that piperazines present a kind of amine group, and therefore acetylation reactions are more often used for their detection. In addition, using this derivatization reaction good-shaped chromatographic peaks were obtained (Fig. 2) for all the selected ions, which allowed achieving quite low limits of quantitation.

Furthermore, and in comparison to the reaction with MBTFA (Fig. 3), mCPP was more efficiently separated from MeOPP when TMS was used, and the shape of the chromatographic peaks was better.

3.2. Method validation

The described analytical method was validated according to the guiding principles of the FDA [25] and ICH [26]. The studied parameters were selectivity, linearity, limits of quantitation, intra- and interday precision and accuracy, and cleanup efficiency.

3.2.1. Selectivity

This parameter has been assessed as previously described. The samples were extracted by a 40 min incubation in 1 mL of 1 M sodium hydroxide at 50 °C. The hair extracts were afterwards neutralized with an equimolar amount of hydrochloric acid, transferred to clean test tubes and 5 mL of KH_2PO_4 0.1 M was added. After addition of 50 µL of the IS solution to each tube, the samples were homogenized and subjected to the above mentioned cleanup procedure; the obtained chromatograms were compared.

All the analytes were successfully identified in all the spiked hair samples (both in terms of ion ratios and relative retention time). In addition, the analysis of the negative samples revealed no interfering peaks at the retention times and selected ions of the studied compounds, meaning that neither hair constituents nor any of the tested substances do interfere significantly in the analysis. Therefore, the described method was considered selective for the determination of the studied piperazines in hair samples. Figs. 2 and 4 show representative ion chromatograms of a spiked and a blank sample.

3.2.2. Calibration curves and limits

To evaluate the method's linearity, hair digests spiked at final concentrations ranging from 0.05 to 4 ng mg^{-1} were prepared and analysed by the described procedure (seven calibrators, five replicates). Along with each calibration curve, a zero sample (blank sample with internal standard) and a quality control sample at a medium level (0.75 ng mg^{-1}; in triplicate) were also analysed.

Calibration curves were obtained by plotting the peak–area ratio between each analyte and the internal standard against concentration. Due to the wide calibration range and to compensate for heterocedasticity, weighted least squares regressions had to be adopted. Six weighting factors were evaluated for each analyte ($1/\sqrt{x}$, $1/x$, $1/x^2$, $1/\sqrt{y}$, $1/y$, $1/y^2$), and the one which originated the

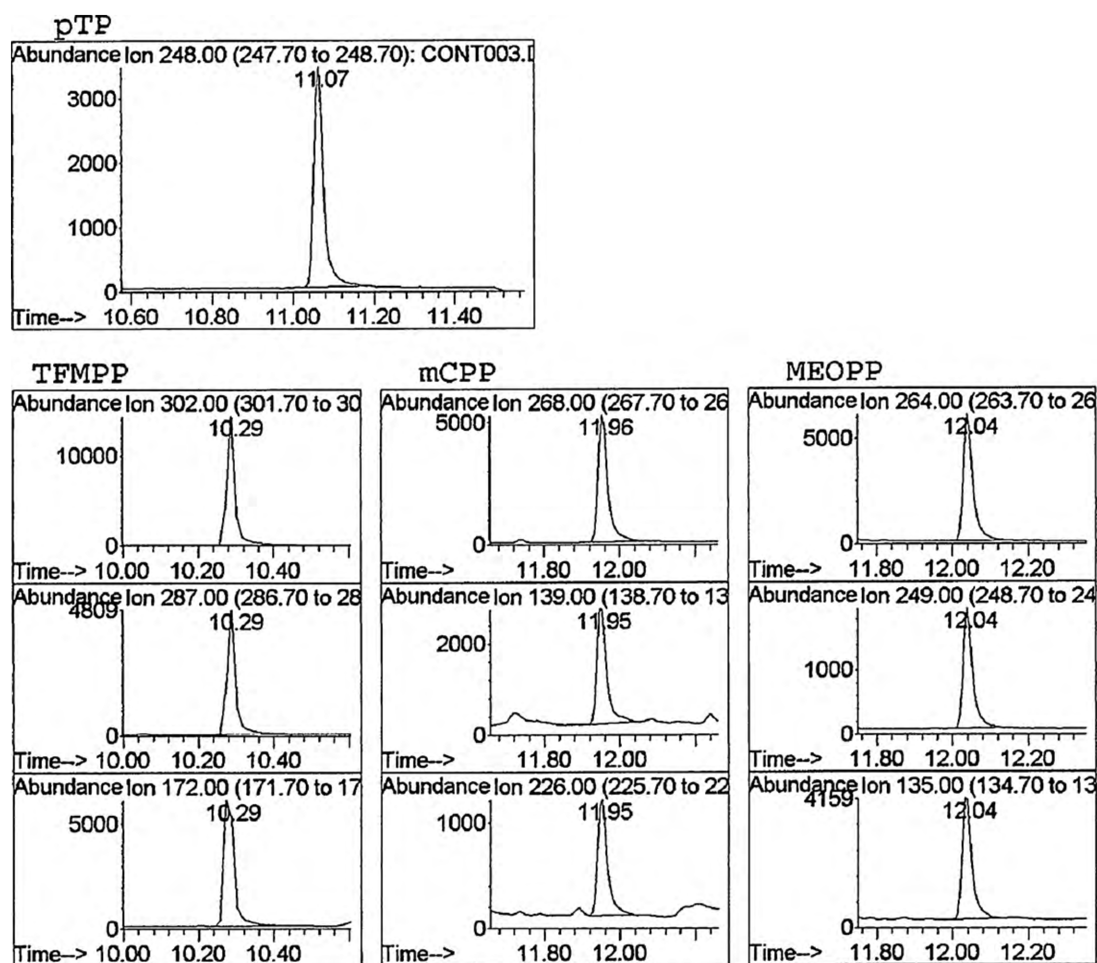

Fig. 2. GC/MS SIM ion chromatogram of a TMS-derivatized extract of a spiked hair sample (2 ng mg^{-1}), after incubation with 1 M sodium hydroxide for 40 min at 50 °C and cleanup by mixed-mode SPE.

best results was selected taking into account the data obtained during the assessment of the interday precision and accuracy [27]. Using each of those factors, the mean relative errors of each calibrator were calculated and their absolute value was summed. The weighting factor for which the sum of errors was smaller (presenting simultaneously a mean R^2 value of at least 0.99) was selected for each analyte. These factors were $1/x^2$ for TFMPP and mCPP; and $1/x$ for MeOPP.

By means of these weighted least squares regressions, linear relationships were obtained, and the calibrators' accuracy [mean relative error (bias) between measured and spiked concentrations] was in accordance with the above-mentioned criteria (±15% for all the concentrations, except at the LLOQ, for which ±20% was accepted). Calibration data is shown in Table 1.

Limits of quantitation (LLOQ) were defined as the lowest concentration of analyte that could be measured reproducibly and accurately (CV < 20% and bias within a ±20% interval), and were determined by analysing six replicates of spiked hair digests independent from those of the calibration curve. These limits have been found to be 0.05 ng mg^{-1} for all the analytes. It is not possible to compare those limits to those obtained by other authors because hair analysis for these compounds has not been published yet. It should be stressed that these low limits of quantitation were obtained using a sample amount as low as 20 mg, and this assumes relevance in those situations where there is little sample available, and/or segmental analysis is required, as often occurs in forensic toxicology. The method's limits of detection were not systematically evaluated because all the samples fulfilling the above-mentioned positivity criteria are quantitated, and values below the LLOQ are not reported.

3.2.3. Intra- and interday precision and accuracy

Intra-day precision was evaluated at 4 concentration levels (0.05, 0.1, 0.2 and 2 ng mg^{-1}) using spiked samples prepared and

Table 1
Linearity data.

	Weight	Linear range (ng mg^{-1})	Linearity		R^2	LLOQ (ng mg^{-1})
			Slope	Intercept		
TFMPP	$1/x^2$	0.05–4	0.9656 ± 0.056	0.0013 ± 0.00294	0.9956 ± 0.0024	0.05
mCPP	$1/x^2$	0.05–4	0.3932 ± 0.074	−0.0017 ± 0.00822	0.9918 ± 0.0087	0.05
MeOPP	$1/x$	0.05–4	0.3395 ± 0.042	−0.0057 ± 0.00736	0.9955 ± 0.0032	0.05

Mean values ± standard deviation; LLOQ, lower limit of quantitation.

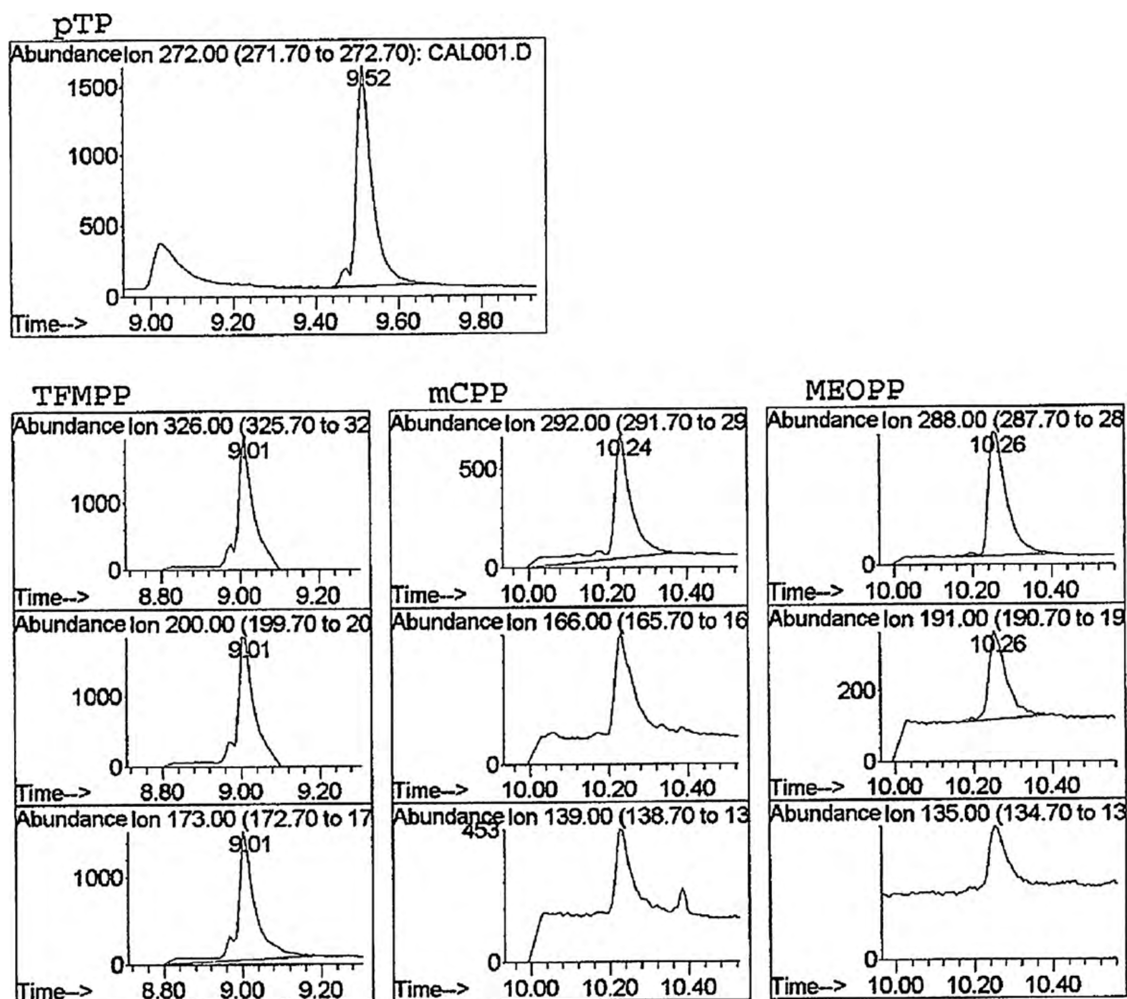

Fig. 3. GC/MS SIM ion chromatogram of a MBTFA-derivatized extract of a spiked hair sample (2 ng mg^{-1}), after incubation with 1 M sodium hydroxide for 40 min at 50 °C and cleanup by mixed-mode SPE.

analyzed as mentioned above (seven replicates for each concentration). The obtained coefficients of variation (CVs) were typically lower than 15% for all the compounds at all the concentrations (except at the LLOQ), presenting a mean relative error within a ±13% interval. These results are presented in Table 2.

Interday precision and accuracy were evaluated at seven concentrations within a 5-day period. The calculated CVs were lower than 14% for all the compounds at all the concentration levels, while accuracy (in terms of mean relative error) was within a ±11% interval (except for MeOPP at the LLOQ, presenting a value of 17%). These data are presented in Table 3. In addition, combined intra- and interday intermediate precision was assessed by analysis of a quality control sample (0.75 ng mg^{-1}) analysed in triplicate over the same 5-day period (15 measurements). The obtained CVs and bias were less 11% and within a ±9% interval.

Table 2
Intra-day precision and accuracy ($n = 7$).

Compound	Spiked	Measured	CV (%)	RE (%)
TFMPP	0.05	0.05 ± 0.003	5.49	5.79
	0.1	0.11 ± 0.013	12.12	10.07
	0.2	0.19 ± 0.015	7.62	−5.57
	2	1.83 ± 0.089	4.70	−8.45
mCPP	0.05	0.05 ± 0.009	16.17	9.67
	0.1	0.11 ± 0.008	7.70	13.21
	0.2	0.20 ± 0.016	8.67	−0.80
	2	2.15 ± 0.225	10.59	7.62
MeOPP	0.05	0.06 ± 0.003	4.46	18.53
	0.1	0.10 ± 0.013	12.77	0.83
	0.2	0.18 ± 0.016	8.82	−8.45
	2	2.19 ± 0.146	6.67	9.69

All the concentrations in ng mg^{-1} of hair; mean values ± standard deviation; CV, coefficient of variation; RE, relative error [(measured concentration − spiked concentration) × 100/spiked concentration].

3.2.4. Cleanup efficiency

This parameter was determined by replicate analysis ($n = 6$) of samples spiked at three concentrations (0.05, 0.5 and 2 ng mg^{-1}), in which the internal standard was only added after that procedure. The obtained peak area ratios were compared with those obtained by spiking blank extracts with the same amounts of all the compounds and the internal standard (100% recovery). These recovery values were higher than 90% for all the analytes at all the tested concentrations, and are presented in Table 4.

3.3. Method applicability (authentic hair samples)

After validation, the herein described procedure was applied to authentic samples obtained from autopsies performed at the National Institute of Legal Medicine – South Branch, Lisbon, Portugal, and also to samples collected from living subjects under psychiatric evaluation at the same institution. In addition, samples

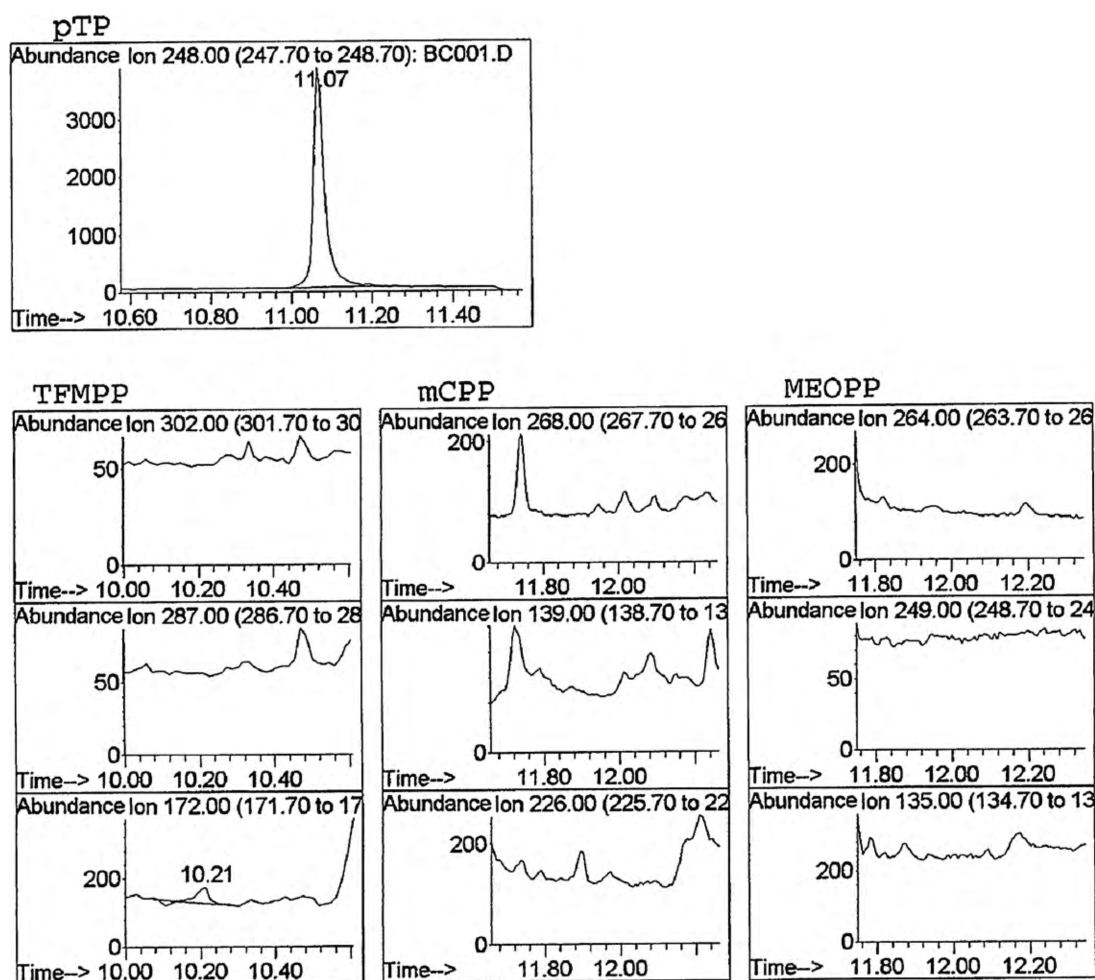

Fig. 4. GC/MS SIM ion chromatogram of a TMS-derivatized extract of a blank hair sample, after incubation with 1 M sodium hydroxide for 40 min at 50 °C and cleanup by mixed-mode SPE.

Table 3
Interday precision and accuracy (n = 5).

Compound	Spiked	Measured	CV (%)	RE (%)
TFMPP	0.05	0.05 ± 0.001	2.15	−3.23
	0.1	0.10 ± 0.003	2.76	4.10
	0.2	0.21 ± 0.010	4.70	4.30
	0.5	0.51 ± 0.034	6.55	2.98
	1	0.98 ± 0.032	3.27	−1.87
	2	1.97 ± 0.054	2.72	−1.71
	4	3.82 ± 0.213	5.57	−4.57
mCPP	0.05	0.05 ± 0.001	2.01	3.24
	0.1	0.10 ± 0.005	5.55	−3.43
	0.2	0.19 ± 0.006	3.39	−5.03
	0.5	0.48 ± 0.039	8.06	−3.51
	1	0.98 ± 0.067	6.85	−2.16
	2	2.09 ± 0.093	4.46	4.53
	4	4.25 ± 0.410	9.64	6.36
MeOPP	0.05	0.06 ± 0.008	13.70	17.23
	0.1	0.10 ± 0.002	2.14	3.65
	0.2	0.18 ± 0.014	8.04	−10.18
	0.5	0.46 ± 0.045	9.69	−7.66
	1	0.98 ± 0.070	7.13	−2.37
	2	1.90 ± 0.052	2.75	−4.84
	4	4.17 ± 0.072	1.72	4.17

All the concentrations in ng mg^{-1} of hair; mean values ± standard deviation; CV, coefficient of variation; RE, relative error [(measured concentration − spiked concentration) × 100/spiked concentration].

Table 4
Cleanup efficiency (n = 6).

Concentration	Cleanup efficiency (%)		
	TFMPP	mCPP	MeOPP
0.05	101.32 ± 6.26	100.03 ± 4.38	101.32 ± 6.29
0.5	96.89 ± 3.75	98.75 ± 5.61	99.07 ± 6.22
2	91.30 ± 2.72	92.71 ± 5.71	92.01 ± 0.55

All the concentrations in ng mg^{-1} of hair; mean values ± standard deviation.

belonging to persons undergoing treatment with trazodone were also analysed, for the detection of mCPP.

4. Conclusions

A simple and fully validated procedure is described for the qualitative and quantitative determination of piperazine-like stimulants in human hair samples, using mixed-mode solid-phase extraction and single quadrupole GC/MS. This method has shown to be linear within the adopted ranges for all the analytes, and presented adequate precision and accuracy. Furthermore, the procedure can be useful for those laboratories performing routine hair analysis, as it is sensitive and specific enough as to detect small amounts of the compounds using only 20 mg of sample and a single quadrupole MS, which is an accessible tool in most laboratories nowadays.

References

[1] D. de Boer, I.J. Bosman, E. Hidvégi, C. Manzoni, A.A. Benkö, L.J.A.L. dos Reys, R.A.A. Maes, Forensic Sci. Int. 121 (2001) 47.
[2] R.F. Staack, G. Fritschi, H.H. Maurer, J. Chromatogr. B 773 (2002) 35.
[3] European Monitoring Centre for Drugs and Drug Addiction, 2009 Annual Report on the State of Drugs Problem in Europe, 2009, URL: http://www.emcdda.europa.eu/publications/annual-report/2009.
[4] A. Wohlfarth, W. Weinmann, Bioanalysis 2 (2010) 965.
[5] H.H. Maurer, T. Kraemer, D. Springer, R.F. Staack, Ther. Drug Monit. 26 (2004) 127.
[6] M.H. Baumann, R.D. Clarck, A.G. Budzynski, J.S. Partilla, B.E. Blough, R.B. Rothman, Neuropsychopharmacology 30 (2005) 550.
[7] R.F. Staack, Lancet (2007) 1411.
[8] S. Caccia, S. Garattini, Acta Pharmacol. Jugosl. 40 (1990) 441.
[9] S. Rotzinger, J. Fang, G.B. Baker, Drug Metab. Dispos. 26 (1998) 572.
[10] M.L. Holland, E.T. Heebner, J. Chromatogr. 567 (1991) 433.
[11] M.H. Fong, S. Garattini, S. Caccia, J. Pharm. Pharmacol. 34 (1982) 674.
[12] R.F. Staack, H.H. Maurer, J. Anal. Toxicol. 27 (2003) 560.
[13] S. Costa, Master Thesis, Aveiro, 2007.
[14] S. Elliott, C. Smith, J. Anal. Toxicol. 32 (2008) 172.
[15] S.P. Vorce, J.M. Holler, B. Levine, M.R. Past, J. Anal. Toxicol. 32 (2008) 444.
[16] H. Tsutsumi, M. Katagi, A. Miki, N. Shima, T. Kamata, K. Nakajima, H. Inoue, T. Kishi, H. Tsuchihashi, J. Anal. Toxicol. 30 (2006) 38.
[17] H.K. Nordgren, P. Holmgren, P. Liljeberg, N. Eriksson, O. Beck, J. Anal. Toxicol. 29 (2005) 234.
[18] H. Tsutsumi, M. Katagi, A. Miki, N. Shima, T. Kamata, M. Nishikawa, K. Nakajima, H. Tsuchihashi, J. Chromatogr. B 819 (2005) 315.
[19] A. Wohlfarth, W. Weinmann, S. Dresen, Anal. Bioanal. Chem. 396 (2010) 2403.
[20] H.K. Nordgren, O. Beck, Ther. Drug Monit. 26 (2004) 90.
[21] F.T. Peters, S. Schaefer, R.F. Staack, T. Kraemer, H.H. Maurer, J. Mass Spectrom. 38 (2003) 659.
[22] B. Guthery, T. Bassindale, A. Bassindale, C.T. Pillinger, G.H. Morgan, J. Chromatogr. A (2010), doi:10.1016/j.chroma.2010.04.020.
[23] WADA – World Anti-Doping Agency, Identification Criteria for Qualitative Assays Incorporating Chromatography and Mass Spectrometry, International Standard for Laboratories, 2004, http://www.wada-ama.org/rtecontent/document/criteria_1_2.pdf.
[24] M. Wikström, P. Holmgren, J. Ahlner, J. Anal. Toxicol. 28 (2004) 67.
[25] Food and Drug Administration: U.S. Department of Health and Human Services. Guidance for Industry, Bioanalytical Method Validation, FDA, 2001, URL: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf.
[26] International Conference on Harmonization (ICH). Validation of Analytical Procedures: Methodology ICH Q2 B. ICH, 2005, URL: http://www.ich.org/LOB/media/MEDIA417.pdf.
[27] A.M. Almeida, M.M. Castel-Branco, A.C. Falcão, J. Chromatogr. B 774 (2002) 215.