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Application of solid-phase microextraction combined with derivatization to the enantiomeric determination of amphetamines

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Abstract

The utility of combining chiral derivatization and solid-phase microextraction (SPME) for the enantiomeric analysis of primary amphetamines by liquid chromatography has been investigated. Different derivatization/extraction strategies have been evaluated and compared using the chiral reagent *o*-phthaldialdehyde (OPA)–*N*-acetyl-L-cysteine (NAC) and fibres with a Carbowax-templated resin coating. Amphetamine, norephedrine and 3,4-methylenedioxyamphetamine (MDA) were used as model compounds. On the basis of the results obtained, a new method is presented based on the derivatization of the analytes in solution followed by SPME of the OPA–NAC derivatives formed. The proposed conditions have been applied to determine the compounds of interest at low ppm levels ($\leq$10 μg/ml) in aqueous and urine samples. Data on the linearity, reproducibility, sensitivity and selectivity are given. The utility of the described procedure for the quantification of amphetamine, norephedrine and MDA enantiomers in different kind of samples is also discussed.

Keywords: Solid-phase microextraction; Derivatization; Amphetamines; Liquid chromatography

1. Introduction

The enantioselective analysis of amphetamine and related substances is receiving increasing attention for a number of reasons. These compounds exist as pairs of enantiomers, which may differ in their action with biological specimens. Moreover, unlike clandestine preparations some pharmaceutical preparations contain only one of the enantiomers. Therefore, enantiomeric composition determinations may be useful in differentiating therapeutic from illicit intake of amphetamines. Enantiomeric characterisation and determination can also help to identify the synthetic pathways of clandestine amphetamine preparations.

Among the different methods available for the enantiomeric analysis of amphetamines derivatization with a chiral reagent followed by the separation of the diastereomers formed by liquid chromatography (LC) is the most widely adopted strategy [1,2]. The reason is that derivatization is a common and often obligatory step in most methods (enantioselective or not) owing to the low UV absorbances of amphetamines and also to their very low natural fluorescence. Therefore, derivatization is also aimed at enhancing the sensitivity, which is particularly important in the analysis of biological samples. In this sense, several reagents and derivatization strategies have been described [3–7].

The main disadvantage of indirect LC methods over methods based on the employment of chiral selectors is that they typically involve an extensive sample manipulation. This is because, since chiral determinations could increase the number of peaks to be resolved, intensive matrix elimination is necessary. Moreover, the derivatization process often involves additional steps aimed at eliminating the excess of reagent or side-products. For such purposes liquid–liquid extraction (LLE) or solid-phase extraction (SPE) are the techniques typically used [7]. In some of the reported assays the reaction conditions required to obtain satisfactory conversion yields (i.e. reaction times of several hours) result in procedures unsuitable for routine analysis [8]. Therefore, the development of rapid and simple methods for the enantioselective analysis of amphetamines continues to be an area of major interest.

The employment of a solid-support to perform analyte purification and derivatization (*solid-support assisted derivatization* methodology) appears as a useful alternative to simplify procedures that involve the chemical transformation of the analytes

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[9,10]. In the context of enantiomeric analysis the utility of this approach has been illustrated for the analysis of amphetamine enantiomers using small precolumns packed with a C_{18} phase and the reagent *o*-phthaldialdehyde (OPA)–*N*-acetyl-L-cysteine (NAC) [11]. More recently, the solid-support assisted derivatization approach has been applied to the resolution of some amphetamine-derived designer drugs using C_{18}-packed SPE cartridges and the reagent (−)-1-(9-fluorenyl)ethyl chloroformate (FLEC) [12]. Alternatively, the analytes can be isolated and purified in C_{18} based SPE cartridges, desorbed with the proper solvent, and then derivatized with FLEC in solution [13]. The later option, however, is significantly less sensitive than the solid-support assisted derivatization based method.

The solid-support assisted derivatization concept can be extended to solid-phase microextraction (SPME) with on-fibre derivatization [14]. Besides the inherent advantages of SPME, the combination of SPME and a chemical reaction offers some advantages over conventional derivatization procedures such simplicity or minor solvent consumption [15,16]. As regards amphetamines, SPME with a chemical derivatization has been extensively used in the achiral analysis of some amphetamines by gas chromatography (GC) [17–20]. Derivatization is typically aimed at transforming the analytes into compounds more amenable for GC or at increasing their affinity for the fibre coating. Recently, we have proposed a method for the (achiral) analysis of some amphetamines by LC, which combines SPME and derivatization with 9-fluorenylmethyl chloroformate (FMOC) [21]. To our knowledge, no attempts have been made to combine SPME with chiral reagents for the enantiomeric analysis of amphetamines.

As an attempt to simplify the enantioselective analysis of amphetamines, in the present work we have evaluated for the first time the possibility of coupling SPME and derivatization with a chiral reagent. OPA–NAC has been selected for derivatization because according to previous studies with aliphatic amines this reagent shows excellent compatibility with typical fibre coatings [22]. Moreover, the enantioresolution attainable with OPA–NAC for primary amphetamines is adequate for most purposes [23]. Amphetamine, 3,4-methylenedioxyamphetamine (MDA) and norephedrine have been selected as model compounds, and the fibre coating was Carbowax-templated resin [14]. On the basis of these studies a new method for the enantioselective analysis of the amphetamines is proposed. The analytical performance and possible applications of the proposed conditions are discussed.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade. Racemic amphetamine sulphate, 3,4-methylenedioxyamphetamine hydrochloride and norephedrine were obtained from Sigma (St. Louis, MO, USA). *o*-Phthaldialdehyde and *N*-acetyl-L-cysteine were purchased from Fluka (Buchs, Switzerland). Acetonitrile and methanol were of HPLC grade (Scharlau, Barcelona, Spain). Sodium hydroxide, sodium acetate, boric acid and acetic acid were obtained from Panreac (Barcelona, Spain).

Stock standard solutions of amphetamine, MDA and norephedrine (50 μg/ml, each enantiomer) were prepared in water. Working solutions of these compounds were prepared by dilution of the stock solutions with water. Water was deionized and filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain). All solutions were stored in the dark at 2 °C.

The derivatization reagent was a mixture of OPA and NAC at a concentration of 100 mM in each compound. It was prepared by dissolving the pure compounds in methanol–water (15:85, v/v). Since derivatizations with OPA–NAC require a basic pH, a 0.05 M borate buffer of pH 10.0 was also added to the reaction medium.

2.2. Apparatus and chromatographic conditions

The chromatographic system consisted of a quaternary pump (Hewlett-Packard 1050 Series, Palo Alto, CA, USA), a SPME–HPLC interface (Supelco, Bellefonte, PA, USA), and a fluorescence detector (Hewlett-Packard, 1050 Series). The detector was coupled to a data system (Hewlett-Packard, HPLC ChemStation) for data acquisition and calculation. The excitation and emission wavelengths were 231 and 425 nm, respectively. The SPME fibres, coated with Carbowax-templated resin (CW-TPR, 50 μm) were also obtained from Supelco.

A LiChrospher 100 RP_{18}, 125 mm × 4 mm i.d. column (Merck, Darmstadt, Germany) was the analytical column. A precolumn and a high-pressure six-port valve (Hewlett-Packard) were inserted between the SPME–HPLC interface and the analytical column to effect peak compression [14]. The precolumn (20 mm × 2.1 mm i.d.) was dry-packed with a Hypersil C_{18}, 30 μm, stationary phase. Before each assay the precolumn and the analytical column were flushed with water at a flow rate of 1.0 ml/min. At the beginning of the run the SPME–HPLC interface was activated and the switching valve was rotated. In such a way, the mobile phase emerging from the interface (water) was sent to the precolumn and then to waste. At 0.5 min the six-port valve was again rotated so the precolumn and the analytical column were connected, and the mobile phase composition was changed to acetate buffer–methanol–acetonitrile (46:48:6, v/v). The mobile phase flow rate was kept at 1 ml/min. The acetate buffer (0.05 M, pH 7.5) was prepared by dissolving sodium acetate in water; then the pH was adjusted to the required value by adding 0.5 M sodium hydroxide.

All solvents were filtered through 0.45 μm nylon membranes (Teknokroma, Barcelona, Spain) and degassed with helium before use.

3. SPME and derivatization procedures

3.1. SPME of the analytes followed by on-fibre derivatization

The fibres were first immersed into the samples (25 ml) for 30 min, and then into the derivatization solution for other 5 min. The derivatization solution consisted of 1.0 ml of OPA–NAC and 0.5 ml borate buffer. The samples and the derivatization solution were stirred during extraction. Finally, the fibres were removed

from the vials and placed into the SPME–HPLC interface. The derivatives formed were desorbed from the fibres under static mode: fibres were soaked with 200 μl of methanol for 10 min. Next, the valve of the SPME–HPLC interface was rotated, so the analytes were sent to the precolumn and to the analytical column. At the end of each run the fibres were cleaned by immersing them into a vial containing 15 ml of water for 2 min.

3.2. On-fibre derivatization with coated fibres

Fibres were first immersed into the reaction solution (1.0 ml of OPA–NAC plus 0.5 ml of borate buffer) for 5 min, and then into the samples (25 ml) for other 30 min. The extraction processes were effected under magnetic stirring. Finally, the fibres were removed from the samples and placed into the SPME interface for desorption and for chromatography. Conditions used to transfer the derivatives formed as well as to re-equilibrate the fibres were those of the above section.

3.3. Solution derivatization followed by SPME

The experimental conditions used to effect solution derivatization were selected according to previous studies [23]: 1.25 ml of the samples were placed in 2 ml glass vials and mixed with 0.50 ml of borate buffer and with 0.25 ml of the OPA–NAC solution. After a reaction time of 3 min, the fibres were immersed in the resulting mixture for 5–45 min while being stirred. Finally, the fibres were removed from the samples and placed into the SPME interface for desorption and for chromatography. The other conditions were those indicated above.

Each sample was derivatized in triplicate, and all assays were carried out at ambient temperature.

3.4. Analysis of real samples

The proposed conditions were applied to urine samples. Samples were prepared by spiking untreated urine with standard solu-

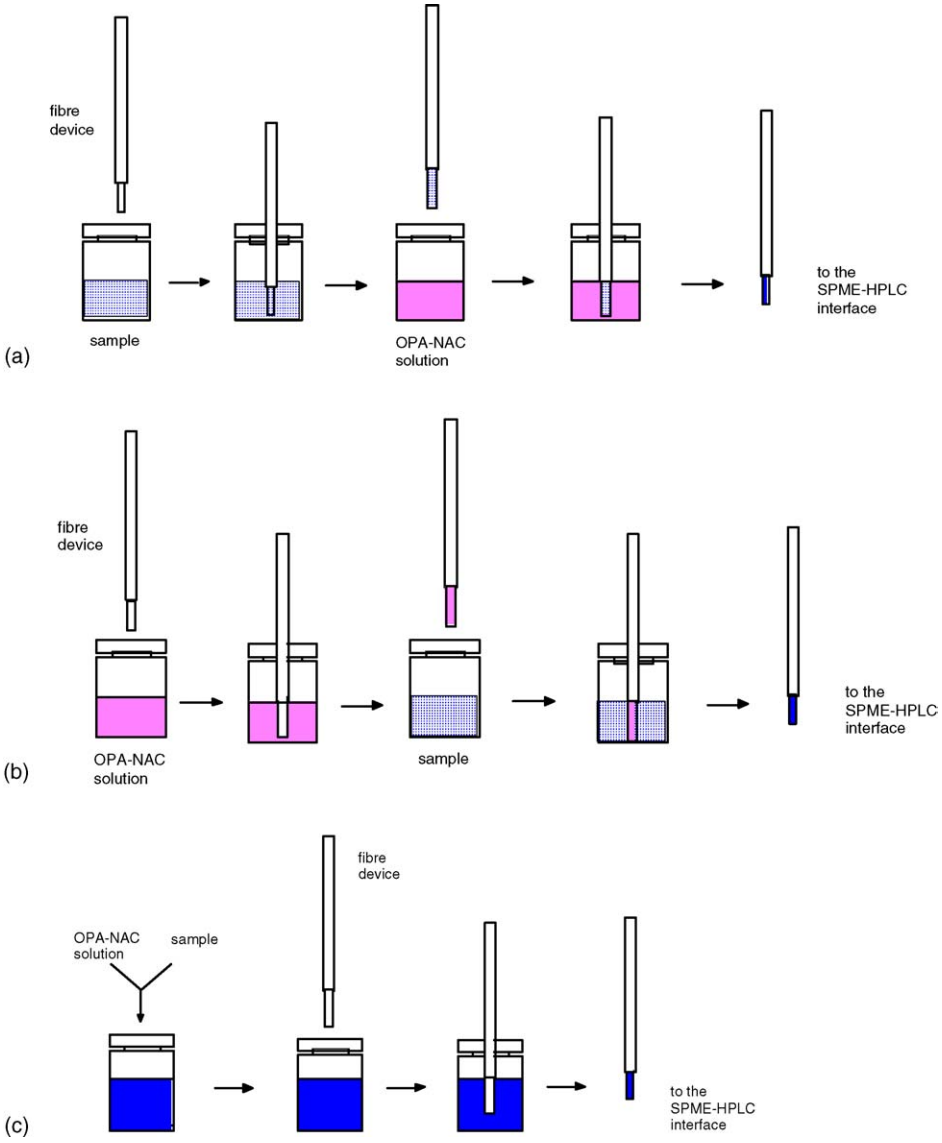

Fig. 1. Schemes of the extraction/derivatization procedures tested: (a) SPME of the analyte followed by on-fibre derivatization, (b) extraction/derivatization of the amphetamine with fibres coated with OPA–NAC and (c) solution derivatization followed by SPME of the derivatives formed. For other details see text.

tions of the analytes producing different concentrations within the tested interval. The proposed method was also applied to the analysis of *Senioral* tablets (Laboratories Belmac, Zaragoza, Spain) labelled to contain 30 mg of norephedrine hydrochloride. Three tablets were weighed, powered and homogenized, and the required amount was suspended in 250 ml of water. This suspension was filtered and the filtrate was further diluted with distilled water. The resulting solution was processed by the derivatization/SPME proposed method. Urine samples obtained after the administration of one of those tablets were also analyzed.

Each sample was analyzed in triplicate and all assays were carried out at ambient temperature.

4. Results and discussion

4.1. Optimization of the derivatization/SPME procedure

Derivatization can be effected before, during or after SPME of the target compounds. In the first option the derivatives are formed in solution and then extracted by immersing the fibres in the reaction medium. In the second approach the fibres, previously coated with the reagent, are immersed into the samples (or exposed to the headspace of a vial containing the sample), so the analytes are extracted and derivatized simultaneously. In the last option the fibres are successively immersed into the samples and into the reagent solution. Only the last two approaches can be considered *on-fibre* derivatization procedures.

In the present study, the three possibilities have been tested and compared using standard solutions of each amphetamine (see Fig. 1). Equivalent SPME conditions were used for the *on-fibre* derivatization methods: adsorption times for the analytes and the reagent of 30 and 5 min, respectively, and a desorption time for the OPA–NAC derivatives of 10 min. Conditions for the solution derivatization were selected according to the results presented in [23]; then, the derivatives formed were subjected to SPME using adsorption and desorption conditions equivalent of those of the *on-fibre* derivatization procedures.

Under the three approaches tested equivalent peak areas (within experimental fluctuations) were observed for the two diastereomers obtained from the racemic solutions of each amphetamine. This indicates that as in solution, and provided that SPME was effected under non-equilibrium conditions, the reaction yields obtained for each pair of enantiomers were identical. In other words, unlike other reactions taking place on polymer resins [24], no racemization occurred in on-fibre mediated derivatizations with CW-TR fibres and OPA–NAC. Best efficiencies were observed when the derivatization was carried out in solution and then the derivatives were subjected to SPME. This is illustrated for amphetamine in Fig. 2. The peak areas obtained for the three amphetamines tested by the solution derivatization/SPME approach were of about 6–15 times greater than those obtained with the other methods. These results indicate that the affinity of the OPA–NAC derivatives for the fibre coating is significantly greater than those of the underivatized amphetamines and/or OPA–NAC, which can be explained by the relatively high polarities of amphetamines and OPA–NAC compared with the polarity of their respective isoindole derivatives.

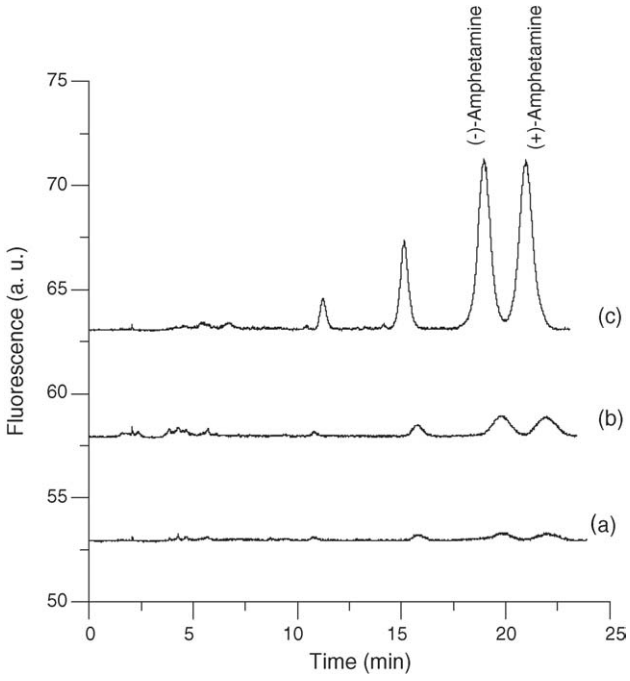

Fig. 2. Chromatograms obtained for racemic amphetamine (7.5 μg/ml, each isomer) by the different extraction/derivatization approaches tested: (a) SPME of the analyte followed by on-fibre derivatization, (b) extraction/derivatization of the amphetamine with fibres coated with OPA–NAC and (c) solution derivatization followed by SPME of the derivatives formed. Elution order for norephedrine and amphetamine enantiomers taken from Ref [23]. For other details see text.

In accordance with the above results, solution derivatization followed by SPME of the derivatives formed was the strategy adopted for the enantiomeric analysis of amphetamines. The main parameters affecting the SPME process were optimized. Different adsorption times in the 5–45 min interval were applied for standard solutions of the amphetamines. The desorption time was 5 min. Maximum analytes responses were observed for adsorption times in the 30–45 min interval. Next, different desorption times were assayed in the 2–15 interval (for an adsorption time of 30 min). Maximum peak areas were reached when using a desorption time of 10 min. Different sample volumes in the 0.25–1.25 ml interval were also assayed. Increasing the sample volume had a positive effect on analyte responses within the tested interval. However, higher sample volumes were not tested as they are rarely used in the analysis of biological samples. On the basis of the above results the conditions selected for the enantiomeric analysis of amphetamines were as follows: solution derivatization (1.25 ml of samples + 0.25 ml of OPA–NAC + 0.50 ml of borate and a reaction time of 3 min) followed by adsorption of the derivatives for 30 min, and a desorption time of 10 min.

Typical chromatograms obtained under the optimized conditions for a blank (water) and for standard solutions of the analytes are depicted in Fig. 3. Unlike previously reported assays for amphetamines using the conventional solution derivatization approach no peaks due to the excess OPA–NAC products were observed in the chromatogram obtained for a blank [23]. This is consistent with the low affinity of OPA–NAC for the fibre coating previously observed. No other peaks corresponding to

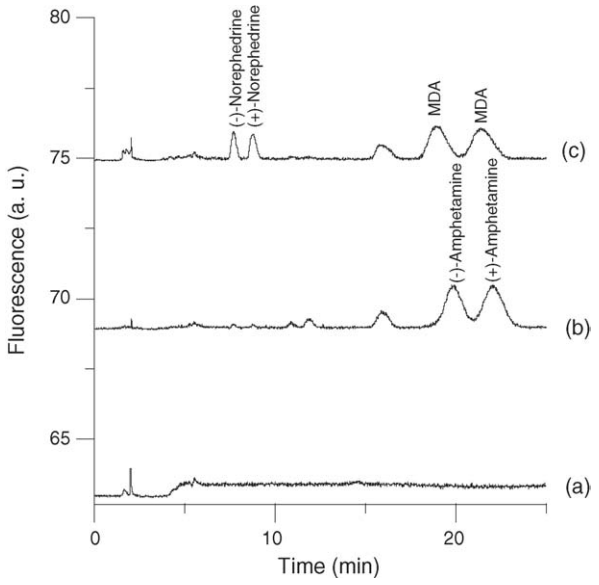

Fig. 3. Chromatograms obtained under the proposed solution derivatization/SPME procedure for: (a) a blank (water), (b) amphetamine in water and (c) a mixture of norephedrine and MDA in water. Concentration of each enantiomer, 2.5 μg/ml. For other details see text.

side-products were observed. The presence of unwanted peaks in the chromatograms obtained by the solid-support assisted derivatization methodology is also general due not only to the reagent but also to the impurities of the solid-support [9–11]. Therefore, compared with other derivatization/extraction procedures, the present method provides improved chromatograms. This is particularly important in the context of chiral analysis because, as stated below, chiral separations may increase drastically the number of peaks in the final chromatograms.

4.2. Analytical performance

The utility of the optimized derivatization/SPME method for the quantification of amphetamine, MDA and norephedrine in water and spiked urine samples was evaluated. The calibration equations obtained for the analytes are presented in Table 1. The results of this table indicate that the proposed method provided adequate linearity within the tested concentration intervals. In all instances the slopes of the calibration graphs obtained for each pair of enantiomers were statistically equivalent (at a confidence level of 95%). The slopes obtained for norephedrine enantiomers were much lower than those of the other amphetamine derivatives. The higher polarity of norephedrine, and thus of norephedrine–OPA–NAC, compared to those of amphetamine and MDA derivatives may be the explanation. This is in agreement with the much lower retention times observed for norephedrine–OPA–NAC isomers under reversed phase conditions (see Fig. 3).

The mean recovery percentages in urine calculated from the slopes of calibration equations are also listed in Table 1. The efficiency of the derivatization/SPME process obtained for urine samples was significantly lower than that of aqueous samples,

Table 1
Analytical data for the determination of amphetamine, MDA and norephedrine in water and urine by the solution derivatiaztion/SPME method

Sample type	Compound	LOD (μg/ml)	LOQ (μg/ml)	Concentration interval (μg/ml)	Linearity, $y = a + bx$ ($n = 8$)			Mean recovery in urine (%) ($n = 16$)	Reproducibility			
					$a \pm s_a$	$b \pm s_b$	R^2		Intra-day precision CV (%) ($n = 3$)		Inter-day precision CV (%) ($n = 3$)	
									2.5 μg/ml	7.5 μg/ml	2.5 μg/ml	7.5 μg/ml
Aqueous standards	(−)-Amphetamine	0.1	0.4	0.375–10	16 ± 3	24.4 ± 0.7	0.99	–	2	13	9	20
	(+)-Amphetamine	0.1	0.4		13 ± 4	25.8 ± 0.9	0.99	–	6	18	11	20
	(−)-Norephedrine	0.1	0.4	1–10	2 ± 2	6 ± 0.3	0.98	–	17	10	16	7
	(+)-Norephedrine	0.1	0.4		3 ± 2	6 ± 0.3	0.98	–	17	9	17	6
	MDA first eluting isomer	0.1	0.4	1–10	8 ± 6	26 ± 1	0.98	–	4	11	19	15
	MDA last eluting isomer	0.1	0.4		7 ± 6	26 ± 1	0.98	–	17	11	20	16
Urine	(−)-Amphetamine	0.25	0.75	1–10	16 ± 4	12.2 ± 0.8	0.97	50	11	0.5	9	20
	(+)-Amphetamine	0.25	0.75		14 ± 5	13.6 ± 0.7	0.98	53	20	5	20	18
	(−)-Norephedrine	0.25	1	1–10	5.4 ± 1.2	3.4 ± 0.2	0.98	57	2	5	10	7
	(+)-Norephedrine	0.25	1		6.5 ± 1.2	3.9 ± 0.2	0.98	65	3	8	10	11
	MDA first eluting isomer	0.25	1	1–10	−18 ± 6	26 ± 1	0.98	100	16	5	15	8
	MDA last eluting isomer	0.25	1		−7 ± 8	25 ± 1	0.97	104	7	14	9	14

especially for norephedrine and amphetamine enantiomers. This suggest that some urinary compounds were also extracted to the fibres thus limiting the amount of OPA–NAC derivatives that could be extracted.

The intra- and inter-day coefficients of variation were calculated at two concentration levels of each amphetamine. As shown in Table 1, the obtained values were ≤20%, and no significant differences were found between aqueous and urine samples. The LODs and LOQs found in aqueous standards and in urine samples are also listed in Table 1.

The selectivity was evaluated by processing other amphetamines (racemates) as well as different compounds commonly found with amphetamine and MDA in clandestine preparations. The compounds evaluated were: 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy), methamphetamine, ephedrine, pseudoephedrine, *p*-metoxyamphetamine, paracetamol, caffeine, acetylsalicylic acid, saccharine, sodium chloride and lactose. None of these compounds modified the derivatization/extraction of the analytes. Only *p*-metoxyamphetamine was observed in the resulting chromatograms, as it was the only amphetamine with a derivatizable primary amino group. However, the derivatives formed eluted at 22.5 and 26.1 min, respectively, and thus they did not interfere with the analytes. Of particular interest is the absence of reaction for methamphetamine and MDMA, as these compounds are mainly metabolised to amphetamine and MDA, respectively. The selectivity observed for urine samples was also adequate. As an illustrative example, the chromatograms obtained for blank urine and for urine spiked with norephedrine and MDA enantiomers are shown in Fig. 4.

The same conclusion can be derived from Fig. 5, which shows the chromatograms obtained from two subjects at different times after the administration of norephedrine. It should be noted that

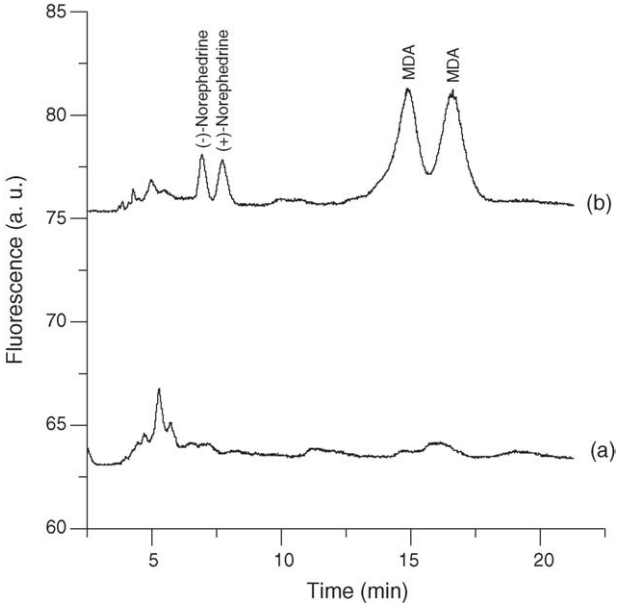

Fig. 4. Chromatograms obtained under the proposed solution derivatization/SPME procedure for: (a) blank urine and (b) urine spiked with norephedrine and MDA (7.5 μg/ml, each isomer). For other details see text.

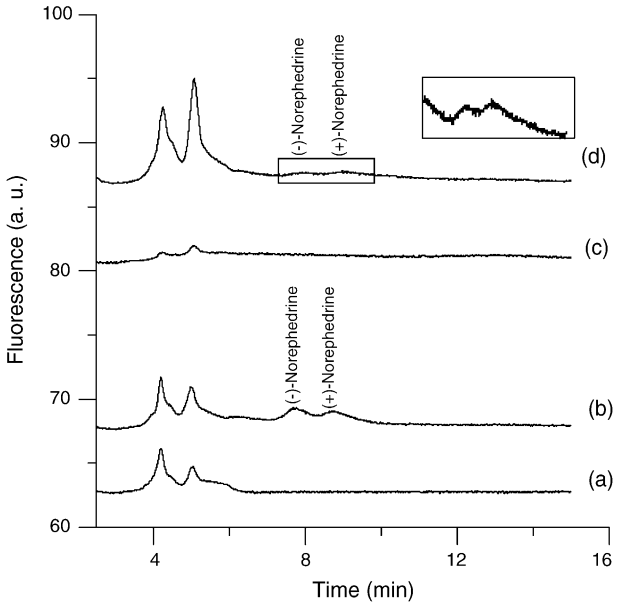

Fig. 5. Chromatograms obtained after a single dose administration of 30 mg of norephedrine hydrochloride: subject 1 (a) immediately and (b) 4.5 h after drug administration; subject 2 (c) immediately and (d) 11 h after drug administration. For other details see text.

in all urine samples assayed endogenous compounds eluted at retention times of 4–6 min.

Finally, the accuracy was investigated by determining amphetamines in spiked urine. Samples were spiked with the analytes at two concentration levels within the tested concentration interval, and processed under the proposed conditions. The concentrations of the analytes in these samples were established from the calibration equations of Table 1. In Table 2 are listed the results obtained. In all cases, the concentrations calculated were close to the concentrations present. The proposed conditions were also applied to the quantification of norephedrine in a pharmaceutical preparation. The only peaks detected in the resulting chromatograms were those corresponding to norephedrine enantiomers. The concentrations of these

Table 2
Accuracy for the determination of amphetamines in spiked urine

Analyte	Added concentration (μg/ml)	Determined concentration (μg/ml) ($n = 3$)	Relative error (%)
(−)-Amphetamine	2.5	2.6 ± 0.2	+5
	7.5	9 ± 2	+20
(+)-Amphetamine	2.5	2.4 ± 0.6	−3
	7.5	8 ± 2	+7
(−)-Norephedrine	2.5	2.3 ± 0.3	−8
	7.5	8.0 ± 0.5	+7
(+)-Norephedrine	2.5	2.22 ± 0.12	−11
	7.5	8.7 ± 0.8	+16
MDA first eluting isomer	2.5	2.5 ± 0.3	0
	7.5	7.3 ± 0.6	+3
MDA last eluting isomer	2.5	2.48 ± 0.04	−0.6
	7.5	7.7 ± 0.6	+3

compounds were calculated from the calibration graphs corresponding to aqueous standard solutions in Table 1. The amounts of (−)- and (+)-norephedrine found in the samples (expressed as hydrochlorides) were (13 ± 2) and (15 ± 3) mg, respectively (n = 3). Therefore, the tablets contained a racemic mixture of norephedrine (at a confidence level of 95%). These values were consistent with the total amount of norephedrine hydrochloride declared by the manufacturer (30 mg per tablet). Urine samples collected from two subjects after a single dose administration of one of those tablets were also processed (Fig. 5). The concentrations of (−)- and (+)-norephedrine were calculated from the calibration graphs corresponding to spiked urine in Table 1. The values obtained in sample corresponding to Fig. 5b were (5.5 ± 0.6) and (4.9 ± 0.3) μg/ml for the (−)- and (+)-enantiomers, respectively (n = 3). It can be deduced that urine also contained equal proportion of the two enantiomers (at a confidence level of 95%). The chromatogram of Fig. 5d, which was obtained from urine collected 11 h after the administration of the tablet, showed analytical signals for norephedrine enantiomers close to the LODs.

4.3. Comparison with other derivatization and sample treatment approaches

The features of the present method have been compared with those of other approaches proposed for the chiral analysis of amphetamines through the formation of their respective OPA–NAC derivatives: the conventional solution derivatization method [23], and the solid-support assisted derivatization method using a precolumn connected on-line to the analytical column [11]. In the former method, the analysis of urine samples entailed analyte purification with C_{18} based SPE cartridges before derivatization. In Table 3 are compared relevant analytical data of these methods. This table also shows data obtained by a method previously reported for the achiral determination of some amphetamines following a solution derivatization/SPME scheme with the fluorogenic reagent FMOC [21].

The three derivatization methods using OPA–NAC in Table 3 are suitable to quantify amphetamine enantiomers at low ppm levels either in water and in urine. However, the solution derivatization and the solid-support assisted derivatization methods provided better reproducibility than the proposed solution derivatization/SPME procedure. Nevertheless, the precision of the proposed method can be considered adequate for the analysis of amphetamines in biosamples [7].

The LODs and LOQs achieved by the proposed solution derivatization/SPME were about were 5–50 times greater than those achieved by other procedures. Therefore, the proposed procedure seem to be less sensitive. Nevertheless, the sensitivity attained by present method is suitable for the enantiomeric analysis of amphetamines in real samples (e.g. in urine of drug abusers) [25].

The application of the solution derivatization method to urine required the purification of the analytes. In the procedure in Table 3 samples were loaded in the previously conditioned SPE cartridges, which were then flushed with water to eliminate matrix compounds. Next, the cartridges were dried with air, and

Table 3
Analytical data of different approaches for determining amphetamines in water and urine

Methodology	Reagent	Compounds tested	Values for the first eluting and last eluting isomer, respectively				Sample manipulation degree[b]	Selectivity[c]	Reference
			Reproducibility[a]		LOD (ng/ml)	LOQ (ng/ml)			
			Intra-day precision CV (%)	Inter-day precision CV (%)					
1. Solution derivatization[c]	OPA–NAC	Amphetamine	1, 1[d]	9, 7[d]	50[d,e]	250[d,e]	*	*	[23]
		Norephedrine	1, 2[d]	9, 6[d]	25[d,e]	100[d,e]			
		MDA	2, 4[d]	4, 7[d]	50[d,e]	250[d,e]			
2. Solid-support assisted derivatization into a precolumn connected to the analytical column[c]	OPA–NAC	Amphetamine	5, 4[e]	5, 7[e]	50, 50[e]	–	***	**	[11]
3. Solution derivatization followed by SPME	OPA–NAC	Amphetamine	13, 18[d]; 0.5, 5[e]	20, 20[d]; 20, 18[e]	100, 100[d]; 250, 250[e]	400, 400[d]; 750, 750[e]	**	***	This work
		Norephedrine	10, 9[d]; 5, 8[e]	7, 6[d]; 7, 11[e]	100, 100[d]; 250, 250[e]	400, 400[d]; 1000,1000[e]			
		MDA	11, 11[d]; 5, 14[e]	15, 16[d]; 8, 14[e]	100, 100[d]; 250, 250[e]	400, 400[d]; 1000,1000[e]			
4. Solution derivatization followed by SPME	FMOC	Amphetamine	8[d]; 16[e]	14[d]; 15[e]	50[d]; 100[e]	250[d]; 500[e]	**	***	[21]

[a] Determined at 5.0 μg/ml in methods 2 and 4, and at 7.5 μg/ml in methods 1 and 3.
[b] The more asterisks, the better procedure.
[c] SPE into C_{18} packed cartridges was used in the analysis of urine samples.
[d] Water.
[e] Urine.

the analytes were desorbed with a methanol/phosphate buffer mixture. Finally, the collected extract was derivatized in solution and chromatographed. With the proposed derivatization/SPME method the treatment of the samples is drastically simplified: addition of the reagents to the samples followed by the immersion of the fibres into the resulting solution. The solid-support assisted derivatization based method in Table 3 is an on-line procedure developed for the analysis of amphetamine enantiomers. Analyte purification and derivatization were effected into the precolumn through the successive injection of the samples and the reagent, and therefore, minimum sample manipulation was involved.

As regards the selectivity, the proposed solution derivatization/SPME method is superior to the other methods. This is illustrated in Fig. 6, which shows the chromatograms obtained for urine under the different derivatization/extraction approaches. The number and intensity of the peaks in the final chromatograms is lower when applying the solution derivatization/SPME approach. Therefore, the latter approach is clearly superior for chiral analysis.

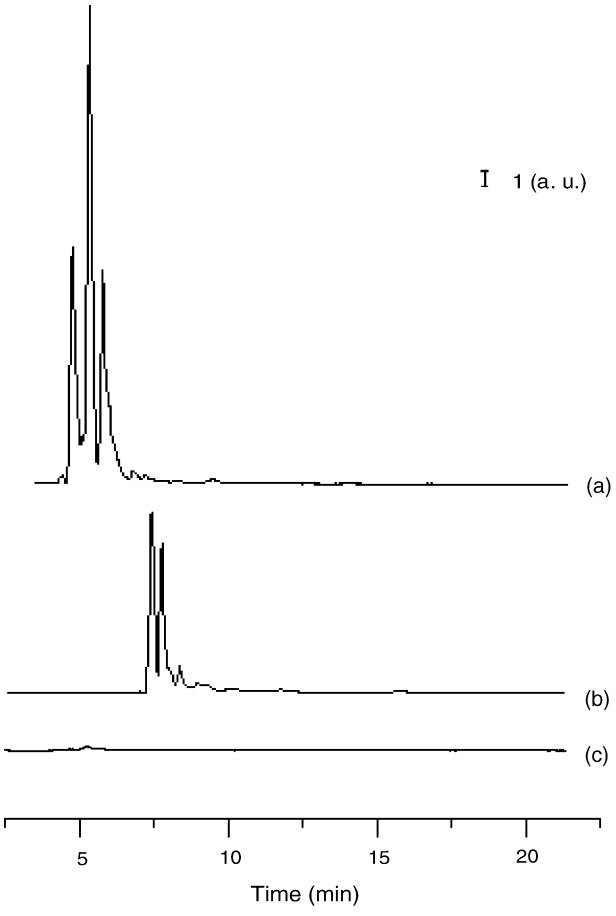

Fig. 6. Chromatograms obtained for urine under different extraction/derivatization approaches: (a) SPE with a C_{18} packing followed by solution derivatization, (b) solid-support assisted derivatization into a precolumn containing a C_{18} packing and (c) the proposed solution derivatization SPME method. The time shift in figure (b) is due to the purification and derivatization stages, which are effected in the same chromatographic system before transfering the derivatives to the analytical column. For other experimental details see text.

On the other hand, the solution derivatization/SPME approaches either with chiral and achiral reagents have shown the same tendency in terms of reproducibility, sensitivity and selectivity.

5. Conclusions

This study shows for the first time the possibility of combining derivatizations with a chiral reagent, such as OPA–NAC and SPME with CW-TPR fibres for the enatiomeric analysis of amphetamines by LC. On-fibre mediated derivatizations do not modify the reaction rates between enantiomers, which is highly desirable if the method has to be used for quantitative purposes. However, solution derivatization followed by SPME is the best option as it provides better reaction/extraction efficiencies.

The proposed methodology permits the enantiomeric analysis of primary amphetamines at low ppm levels in aqueous and urine samples with adequate linearity, reproducibility and accuracy. The proposed derivatization/SPME strategy seems to be less sensitive and reproducible than other indirect methods using OPA–NAC, such as those using solution derivatization or solid-support assisted derivatization. However, the proposed method is clearly superior in terms of selectivity, which is particularly important in chiral analysis when processing biological samples. In addition, compared with conventional chiral methods for amphetamines the proposed procedure is very simple and rapid, as in most of such procedures intensive sample treatments by LLE or SPE are necessary to achieve the required selectivity. Therefore, the proposed method can be considered a simple and rapid alternative to conventional methods for the enantiomeric analysis of primary amphetamines in different kind of samples. If high precision and/or sensitivity are required, other sample treatments must be considered.

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References

[1] J.T. Liu, R.H. Liu, J. Biochem. Biophys. Methods 54 (2002) 115–146.
[2] R. Herráez, P. Campíns, J. Verdú, J. Biochem. Biophys. Methods 54 (2002) 147–167.
[3] K.S. Boos, B. Wilmers, R. Sauerbrey, E. Schlimme, Chromatographia 25 (1988) 199–204.
[4] I.S. Krull, Z. Deyl, H. Lingeman, J. Chromatogr. B 659 (1994) 1–17.
[5] J.C.M. Waterval, H. Lingeman, A. Bult, W.J.M. Underberg, Electrophoresis 21 (2000) 4029–4045.
[6] S. Görög, M. Gazdag, J. Chromatogr. B 659 (1994) 51–84.
[7] T. Kraemer, H.H. Maurer, J. Chromatogr. B 713 (1998) 163–187.
[8] C. Gunaratna, P.T. Kissinger, J. Chromatogr. A 828 (1998) 95–103.
[9] R. Herráez, P. Campíns, A. Sevillano, Anal. Chem. 68 (1996) 734–739.
[10] R. Herráez, P. Campíns, A. Sevillano, J. Chromatogr. Sci. 35 (1997) 169–175.

[11] M.D. Pastor, R. Porras, R. Herráez, P. Campíns, Analyst 123 (1998) 319–324.
[12] J. Verdú, P. Campíns, R. Herráez, Chromatographia 60 (2004) 537–544.
[13] P. Campíns, J. Verdú, R. Herráez, Chromatographia 57 (2003) 309–316.
[14] R. Herráez, C. Chafer, P. Campíns, Anal. Chim. Acta 513 (2004) 425–433.
[15] L. Pan, J. Pawliszyn, Anal. Chem. 69 (1997) 196–205.
[16] E.E. Stashenko, J.R. Martinez, Trends Anal. Chem. 23 (2004) 533–561.
[17] E.H.M. Koster, C.H.P. Bruins, G.J. de Jong, Analyst 127 (2002) 598–602.
[18] M.-K. Huang, C. Liu, S.-D. Huang, Analyst 127 (2002) 1203–1206.
[19] M. Yonamine, N. Tawil, R.L. de Moraes Moreau, O.A. Silva, J. Chromatogr. B 789 (2003) 73–78.
[20] M. Pujadas, S. Pichini, S. Poudevida, E. Menoyo, P. Zuccaro, M. Frré, R. de laTorre, J Chromatogr. B 798 (2003) 249–255.
[21] C. Chafer, R. Herráez, P. Campíns, Anal. Biochem. 333 (2004) 328–335.
[22] C. Chafer, R. Herráez, P. Campíns, Talanta 66 (2005) 1139–1145.
[23] R. Herráez, P. Campíns, J. Verdu, Chromatographia 56 (2002) 559–566.
[24] M. Pumera, M. Flegel, L. Lepsa, I. Jelinek, Electrophoresis 23 (2002) 2449–2456.
[25] N. Samyn, G. De Boeck, M. Wood, C. Lamers, Forensic Sci. Int. 128 (2002) 90–97.