Derivatization of ephedrine with *o*-phthaldialdehyde for liquid chromatography after treatment with sodium hypochlorite

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Abstract

The usefulness of the reaction with NaClO followed by derivatization with *o*-phthaldialdehyde (OPA) and *N*-acetyl-L-cysteine (NAC) has been investigated for the chromatographic analysis of ephedrine. The influence of parameters affecting the two-stage reaction has been evaluated, including concentration of NaClO, time of reaction, temperature and pH. On the basis of these studies, conditions for the pre-column and (automated) post-column determination of ephedrine are presented. The described conditions have been applied to the measurement of ephedrine in the concentration intervals 0.2–20.0 µg/ml and 2.0–50.0 µg/ml for the pre-column and post-column methods, respectively. The possibility of applying the NaClO/OPA–NAC method to other primary, secondary and tertiary derivative amphetamines has also been evaluated. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Derivatization, LC; Ephedrine; *o*-Phthaldialdehyde; Sodium hypochlorite

1. Introduction

The rapid and sensitive analysis of amphetamine and amphetamine-analogues is an area of major interest due to the increasing consumption of these drugs of abuse. For this purpose, the tandem gas chromatography (GC)–mass spectrometry (MS) seems to be the most reliable method, as it provides high levels of specificity and sensitivity [1]. However, since GC–MS is not widely available, liquid chromatography (LC) is frequently the technique of choice, particularly for routine analysis. The main drawback of LC is the low sensitivity attainable with common LC detectors as amphetamines exhibit very low UV absorbances and do not show self-fluorescence (except ring substituted amphetamines). For this reason, most LC methods incorporate a derivatization reaction to increase analyte detectability.

The selection of the derivatization reagent depends on a variety of factors: (a) the kind of sample (biofluid, illicit tablets), (b) the chemical structure of the amphetamine (primary, secondary or tertiary), (c) the sensitivity and/or selectivity required, (d) the detection system (UV, fluorescence), (e) the aim of the analysis (for example, screening or separation of optical isomers) and (f) the way in which the derivatization is performed, before (pre-column derivatization), during (on-column derivatization) or after (post-column derivatization) the chromatographic separation. The latter factor determines the possibility of integrating the reaction into the chro-

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matographic process, and thus, the degree of automation possible.

A great number of reagents have been proposed in the literature for the derivatization of primary and secondary amphetamines including *o*-phthaldialdehyde (OPA) in combination with a thiol, 9-fluorenylmethyl chloroformate (FMOC), 1,2-naphthaquinone-4-sulfonate (NQS), phenylisothiocyanate, 3,5-dinitrobenzoyl chloride (DNB-Cl) or dansyl chloride, among others [2,3]. These reagents are typically used in an off-line pre-column mode and, under optimized conditions, they allow the satisfactory determination of amphetamines at µg/ml levels.

Indeed, every reagent has advantages and limitations. For example, reactions with OPA in combination with a thiol such as 2-mercaptoethanol or *N*-acetyl-L-cysteine (NAC) have been extensively used for the analysis of primary amines, including amphetamine, because highly fluorescent products are formed in relatively short times. Another advantage of OPA is that its derivatives exhibit best responses with either UV and fluorescence detectors at different detection conditions compared with the reagent. Therefore, the derivatization process can be integrated in the chromatographic system in a on-column or in a post-column configuration [4,5]. This greatly facilitates the automation of the analysis, which is highly desirable in view of the increasing demand of analysis. Unfortunately, OPA only reacts with primary amino groups. For this reason, its application has been restricted to the analysis of the primary amine amphetamine.

In an attempt to extend the OPA method to secondary amines, various methods have been developed based on the transformation of secondary amino groups to primary amines and their subsequent reaction with OPA. For this purpose, the secondary amines are treated with a source of active halogen, such as *N*-chlorosuccinimide, chloramine-T or NaClO. The resulting imines are then hydrolyzed to primary amines which can be subsequently treated with OPA to form fluorescent derivatives [6]. This methodology has been applied to the analysis of amino acids and some biogenic amines by LC with post-column derivatization [7–9], as well as to the determination of some antibiotics [6,10] by using either LC post-column derivatization or flow injection analysis. However, to our knowledge, the two-stage derivatization with NaClO and OPA–NAC has not been reported for the analysis of secondary amphetamine derivatives.

In the present work we have evaluated the possibility of using the two-stage derivatization with NaClO and OPA–NAC for the analysis of the secondary amphetamine derivative ephedrine for both the pre-column and post-column approaches. Firstly, we have investigated the influence of parameters affecting the reactions by using a (off-line) pre-column approach. Next, conditions have been optimized for a on-line post-column configuration by incorporating a post-column reactor into the chromatographic system. The possibility of applying the NaClO/OPA–NAC method to other primary, secondary and tertiary amphetamines has also been evaluated, using a variety of amphetamine-related compounds of interest in the pharmaceutical and biomedical fields (see Fig. 1).

2. Experimental

2.1. Apparatus

The chromatographic system used consisted of a

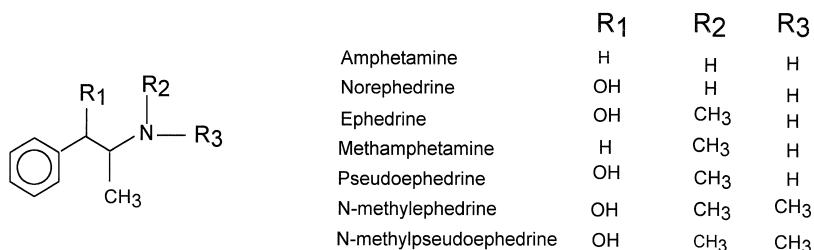|	R$_1$	R$_2$	R$_3$
Amphetamine	H	H	H
Norephedrine	OH	H	H
Ephedrine	OH	CH$_3$	H
Methamphetamine	H	CH$_3$	H
Pseudoephedrine	OH	CH$_3$	H
N-methylephedrine	OH	CH$_3$	CH$_3$
N-methylpseudoephedrine	OH	CH$_3$	CH$_3$

Fig. 1. Chemical structures of the amphetamines assayed.

quaternary pump (Hewlett-Packard, 1050 Series, Palo Alto, CA, USA), a high-pressure six-port injection valve (Rheodyne Model 7000) with a sample loop injector of 20 μl, and a fluorescence detector (Hewlett-Packard, 1046 Series). The detector, which operated at 231 nm for excitation and at 425 nm for emission, was linked to a data system (Hewlett-Packard HPLC Chem Station) for data acquisition and storage.

For the post-column derivatization assays, a post-column reactor (PCX 5100 Pickering Labs., Mountain View, CA, USA) was used. Different PTFE tubing (0.5 mm I.D.) was used as reactor coils.

2.2. Reagents

All the reagents were of analytical grade. HPLC-grade acetonitrile and ethanol were purchased from Scharlau (Barcelona, Spain). OPA was obtained from Fluka (Buchs, Switzerland), sodium hypochlorite (15% Cl active) was obtained from Probus (Barcelona, Spain), sodium dihydrogenphosphate monohydrate was obtained from Merck (Darmstadt, Germany), boric acid and sodium hydroxide were obtained from Panreac (Barcelona, Spain), and *N*-acetyl-L-cysteine, triethylamine (TEA) and norephedrine hydrochloride were purchased from Aldrich (Steinheim, Germany). Amphetamine sulfate, ephedrine hydrochloride, *N*-methylephedrine, *N*-methylpseudoephedrine, pseudoephedrine hydrochloride and methamphetamine hydrochloride were obtained from Sigma (St. Louis, MO, USA).

2.3. Preparation of solutions

Stock standard solutions of the amphetamines (1000 μg/ml) were prepared in water. Working solutions of the amphetamines were prepared by dilution of the stock solutions with water. The OPA–NAC reagent was prepared daily by dissolving the pure compounds (at the same concentration) in the minimum amount of ethanol (0.5–1.0 ml, depending on the concentration of OPA–NAC, per 10 ml of reagent solution). This solution was further diluted with 0.1 *M* borate buffer (pH 10.5). The NaClO solution was prepared by diluting the commercial product with 0.1 *M* borate buffer. The borate buffer was prepared by dissolving boric acid in water; the

pH was adjusted to appropriate value with 10% (w/v) NaOH. All solutions were stored in the dark at 2°C.

Water was distilled, deionized and filtered through 0.45-μm nylon membranes (Teknokroma, Barcelona, Spain).

2.4. Columns and mobile phases

The chromatographic column was a LiChrospher 100 RP$_{18}$, 5 μm, 125 mm×4 mm I.D. (Merck) column. For the pre-column derivatization studies the mobile phase was acetonitrile–water (15:85, v/v) at a flow-rate of 0.75 ml/min. For the post-column derivatization studies the mobile phase was acetonitrile–0.01 *M* phosphate buffer (pH 3.0) (15:85, v/v), at a flow-rate of 0.50 ml/min; the buffer contained 2% (v/v) TEA. The phosphate buffer was prepared by dissolving sodium dihydrogenphosphate monohydrate in water. Next, TEA was added and the pH was adjusted to 3.0 with concentrated phosphoric acid. The mobile phases were filtered with nylon membranes, 0.45 μm (Teknokroma) and degassed with helium before use.

2.5. Derivatization procedures

In the pre-column approach, the two-stage derivatization was performed in 2-ml glass vials, by mixing 125 μl of the samples and 75 μl of the NaClO solution. The vials were introduced in the heater of the post-column reactor system for controlling the temperature of the reaction. After the reaction time (variable), the mixture was further diluted to 1 ml with the OPA–NAC solution. The resulting solution was led to react at ambient temperature, and after the reaction time, aliquots of 20 μl of the solutions were injected into the chromatographic column for separation and detection. Each sample was assayed in triplicate.

In the post-column derivatization method, the analyte was derivatized by means of a post-column reactor. An schematic diagram of the system used is presented in Fig. 2. The post-column reactor delivered the NaClO and the OPA–NAC solutions at flow-rates of about 0.3 ml/min. For the linearity, reproducibility and sensitivity studies, samples were introduced in the system in position A (see Fig. 2),
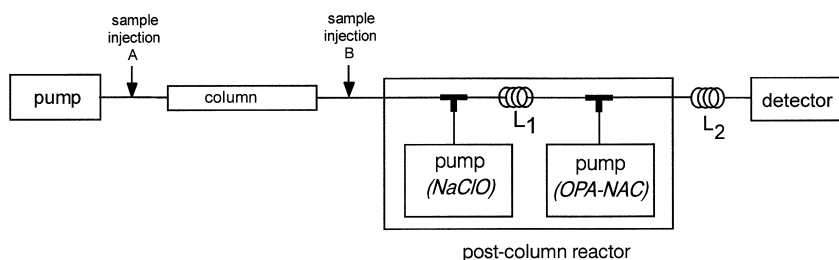
Fig. 2. Schematic set-up of the chromatographic system used for the post-column derivatization method.

so they were chromatographed and subsequently derivatized. For the optimization studies, the injection valve was positioned after the chromatographic column (position B in Fig. 2). Each sample was assayed in triplicate.

3. Results and discussion

3.1. Pre-column derivatization

Firstly, we studied the NaClO/OPA–NAC derivatization in pre-column mode because in such a method no additional instrumentation (post-column reactor) is needed over a simple chromatographic system. Moreover, if more than one product is formed, a pre-column approach may provide additional information about the course of the reaction as the reaction products may be separated. In previous works we optimized the reaction between the OPA–NAC and primary amphetamines [4,11]. Consequently, the present study has been mainly focused on the reaction with NaClO. The parameters investigated were the time of reaction, the concentration of NaClO, the temperature and the pH. The effect of the time of reaction with the OPA–NAC reagent was also investigated. In accordance with previous results [4,11], the concentration of OPA–NAC was 10 mM (unless otherwise stated), and the derivatization with OPA–NAC was performed at ambient temperature.

Preliminary assays demonstrated that the NaClO/OPA–NAC method led to two main products of reaction. The chromatographic conditions were then adjusted to achieve suitable resolution of the derivatives originated in the minimum time of analysis. Fig. 3 shows the chromatograms obtained under the

selected conditions for blank (water) and a solution of ephedrine.

3.1.1. Effect of the time of reaction with NaClO

The effect of the time of reaction with NaClO was investigated within the 1–10 min interval, for solutions of ephedrine at a concentration of 20 μg/ml (the highest concentration assayed). Next, the resulting mixtures were led to react with the OPA–NAC reagent. According with previous findings the reaction time with the OPA–NAC reagent was 2 min [4]; both reactions were carried out at ambient temperature. The results of this study are depicted in
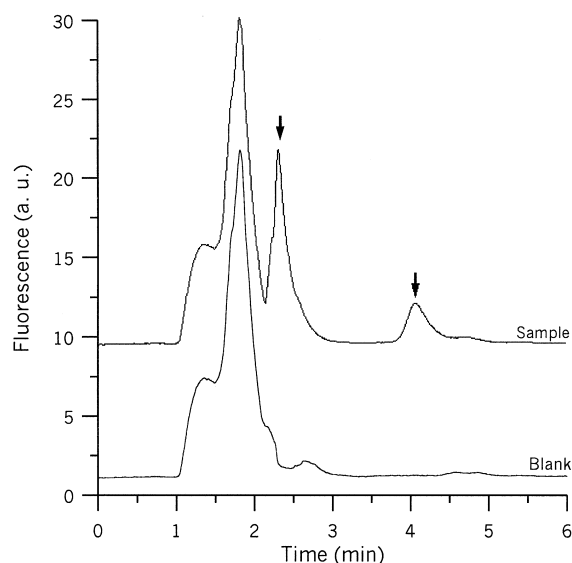

Fig. 3. Chromatograms obtained for a blank (water) and a solution of ephedrine (20.0 μg/ml) derivatized in the pre-column mode. Concentration of NaClO, 6 mM; concentration of OPA–NAC, 10 mM; time of reaction with NaClO, 2 min; time of reaction with OPA–NAC, 2 min; both reactions were performed at ambient temperature. For other experimental details, see text.

Fig. 4a. As can be deduced from this figure, the analytical response obtained for the two derivatives increased as the time of reaction was increased within the 1–5 min time interval; extending the time of reaction resulted in little further improvement.

3.1.2. Effect of time of reaction with OPA–NAC

In this instance, the time of reaction with the OPA–NAC reagent was varied from 2 to 20 min, the time of reaction with NaClO being 5 min. The other conditions were those indicated in the above section. The results obtained within the 2–10 min interval (Fig. 4b) indicated that the peak of the first-eluting derivative decreased with increasing time, whereas the peak of the last-eluting derivative increased. However, the addition of the two signals remained approximately constant. Although the mechanism of reaction was not investigated and the products of the derivatization were not identified, the above results suggest that the first-eluting derivative transforms into the other one in the course of reaction. For times of reaction higher than 10 min, the signal originated for the last-eluting derivative also decreased. Consequently, a time of reaction of 10 min was used in further experiments.

3.1.3. Concentration of NaClO

Fig. 4c illustrates the influence of the concentration of NaClO (within the 4–25 mM range) on peak areas; the other experimental conditions were those indicated in previous sections. This figure demonstrates that a large excess of NaClO decreased the intensity of the analytical signal, most probably due to the destruction of the OPA–NAC reagent. This is in agreement with the fact that an increase in the OPA–NAC concentration led to higher analytical responses (data not shown). To overcome problems associated to the excess of NaClO, some authors recommended the elimination of the reagent by adding 2,2′-thiodiethanol before performing the reaction with OPA–NAC [9,12]. However, the addition of 2,2′-thiodiethanol is not a general procedure to improve sensitivity [10]. Moreover, this method introduces extra-sample handing in the off-line approach, and it is difficult to perform in post-column mode, as an additional delivering system is needed. For these reasons, in the present study the elimination of the excess of NaClO was not tested. Since a

minor consumption of reagents is desirable in order to avoid baseline distortions, the concentrations of NaClO and OPA–NAC finally selected were 6 mM and 10 mM, respectively.

3.1.4. Effect of the temperature

In previous experiments with primary amphetamines we observed that reactions with OPA are very rapid, and maximum conversion of the analytes can be obtained at ambient temperature in a few minutes. However, the temperature has been indicated to be an important parameter affecting the oxidation process of secondary amines with NaClO [9,10]. Consequently, the effect of the temperature on the oxidation step was investigated within the 20–80°C range. The results obtained are presented in Fig. 4d. An increase in the temperature has a favorable effect up to 40°C. However, at higher temperatures the analytical signal decreased. In accordance with these results, a temperature of 40°C was chosen for subsequent work.

3.1.5. Effect of the pH

Another parameter markedly affecting the destruction of secondary amines with NaClO is the pH of the reaction medium. For this reason, we studied the influence of the pH on analyte responses by varying the pH of the NaClO solution within the 9.5–12.5 range. The results obtained (Fig. 4e) indicated that the optimum pH for the transformation of the secondary amino group into the primary one was 10.5. At higher pH values, the analytical signals decreased, most probably due the hydrolysis of the OPA–NAC reagent [4].

On the basis of the above results, conditions finally selected for the derivatization of ephedrine were those summarized in Table 1. It should be noted that the absolute percentage of ephedrine derivatized was not established, as external standards of the derivatives were not available.

3.1.6. Determination of ephedrine

Conditions listed in Table 1 were applied to the derivatization of aqueous solutions of ephedrine within the 0.2–20.0 μg/ml concentration interval. Under such conditions the mole ratios of OPA–NAC to analyte were in the 98 500–985 range. In these studies the peak area of the derivative eluted at 4.2
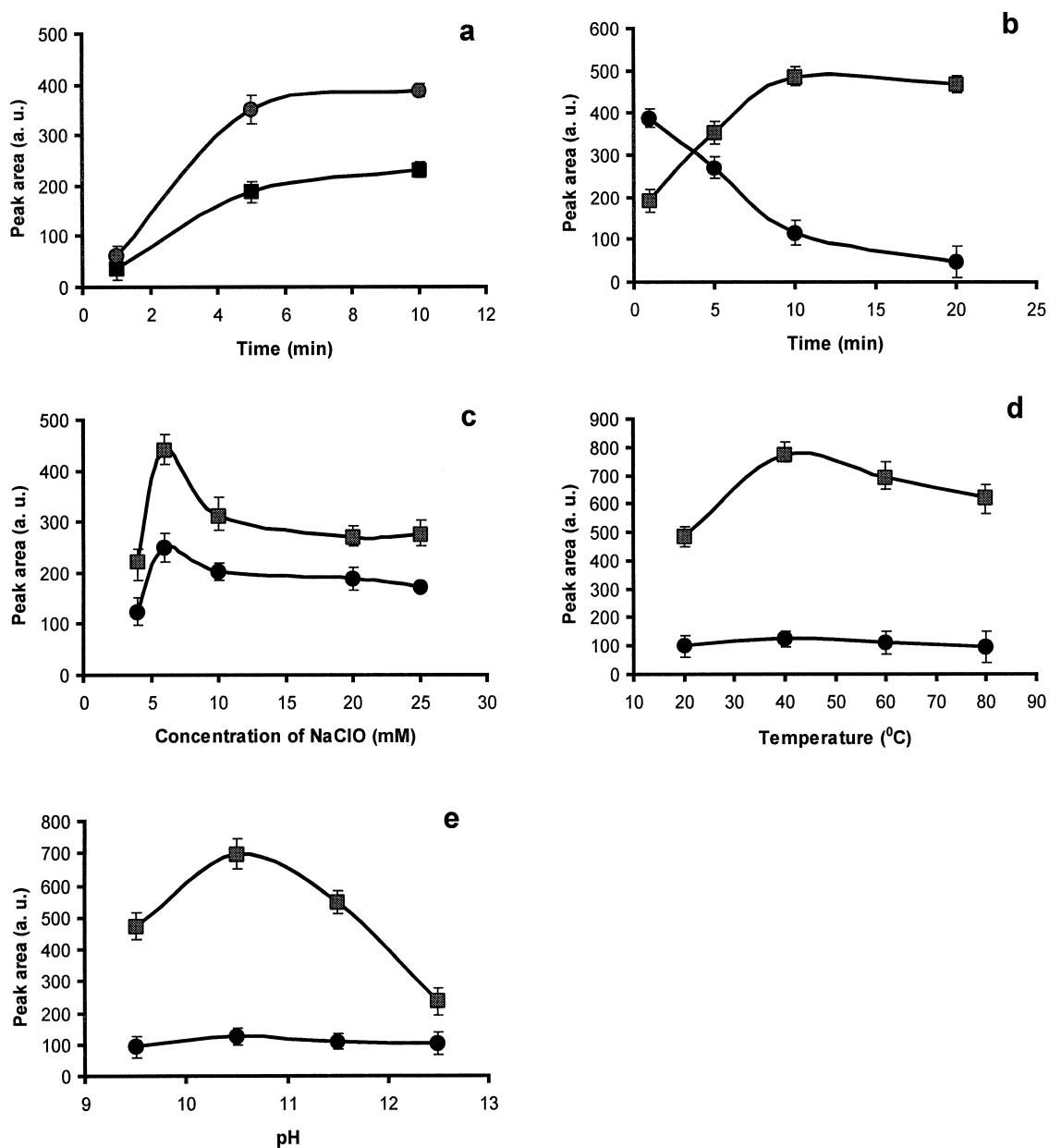
Fig. 4. Effect of experimental conditions in the derivatization of ephedrine (20.0 μg/ml) in the pre-column mode. (a) Effect of the time of reaction with NaClO; concentration of NaClO and OPA–NAC, 6 mM and 10 mM, respectively; time of reaction with OPA–NAC, 2 min; both reactions were performed at ambient temperature. (b) Effect of the time of reaction with OPA–NAC; concentration of NaClO and OPA–NAC, 6 mM and 10 mM, respectively; time of reaction with NaClO, 5 min; both reactions were performed at ambient temperature. (c) Effect of the concentration of NaClO; concentration of OPA–NAC, 10 mM; times of reaction with NaClO and OPA–NAC, 5 and 10 min, respectively; both reactions were performed at ambient temperature. (d) Effect of the temperature of reaction with NaClO; concentration of NaClO and OPA–NAC, 6 mM and 10 mM, respectively; times of reaction with NaClO and OPA–NAC, 5 and 10 min, respectively. (e) Effect of the pH of the NaClO solution; concentration of NaClO and OPA–NAC, 6 mM and 10 mM, respectively; times of reaction with NaClO and OPA–NAC, 5 and 10 min, respectively; temperature of reaction with NaClO, 40°C. First-eluting derivative (●); last-eluting derivative (■). For other experimental details, see text.

Table 1
Conditions selected for derivatization of ephedrine in the off-line pre-column mode

	Reaction with NaClO	Reaction with OPA–NAC
Conditions	125 µl sample+75 µl of 6 mM NaClO (pH 10.5)	+800 µl of OPA–NAC 10 mM (pH 10.5)
Time of reaction (min)	5	10
Temperature	40°C	Ambient

min (last-eluting compound) was used as the analytical signal, as this peak provided the best sensitivity. Table 2 lists the relevant analytical data of the procedure. These data demonstrate that linear and reproducible responses are obtained for ephedrine in the tested concentration range. The estimated limit of detection procedure (for a signal-to-noise ratio of 3) was 50 ng/ml. This value can be considered satisfactory taking into account the limits of detection reported by most HPLC methods based on pre-column chemical derivatization, which are typically in the 5–100 ng/ml [1,3]. On the other hand, the limit of detection found by the present procedure corresponds to an amount of ephedrine injected in the chromatograph of 1 ng. We have recently reported an LC method for the direct quantification of underivatized amphetamines based on UV detection at 210 nm [13]. In such method, the minimum detectable amount of ephedrine was of about one-order of magnitude higher than that obtained under the present conditions. Therefore, the NaClO/OPA–NAC method can be used to improved sensitivity, and also to improve selectivity as many compounds are adsorptive at 210 nm whereas only compounds containing amino groups would react under the described conditions.

3.1.7. Derivatization of other amphetamines

Conditions summarized in Table 1 were also applied to the derivatization of other primary, secondary and tertiary amphetamines. In all cases, derivatization led to two peaks, which eluted at the same retention times that were observed for ephedrine derivatives. This behavior suggests that oxidation with NaClO of all the amphetamines lead to the same products of reaction. However, the extent of the reaction depends on the chemical structure of the amphetamine derivatized. This is illustrated in Fig. 5, which compares the peak areas of the last-eluting derivative obtained for the different compounds assayed. As can be deduced from this figure, the primary amines amphetamine and norephedrine originated the highest responses (indeed, for these compounds the reaction with NaClO is not necessary). Similar behavior has been reported for other compounds containing amino-groups [9]. Ephedrine-originated responses significantly greater than those observed for the other secondary amphetamines pseudoephedrine and methamphetamine. The latter compounds and the tertiary amphetamines N-methylephedrine and N-methylpseudoephedrine lead to relatively low responses. For these compounds, the employment of more drastic conditions in the

Table 2
Comparison of the analytical properties obtained for ephedrine with the pre-column and the post-column methods

Method	Concentration interval (µg/ml)	Linearity			Reproducibility[a] (%)		Limit of detection (ng/ml)
		$y=ax+b$	$t_{calculated}$[b]	n	Intra-day ($n=3$)	Inter-day ($n=6$)	
Pre-column derivatization	0.2–20.0	$a=18.1\pm0.3$ $b=12\pm3$	60.3	22	4	7	50
Post-column derivatization	2.0–50.0	$a=3.3\pm0.1$ $b=9\pm6$	33.0	12	7	5	500

[a] Determined at concentration of 10.0 µg/ml and 25.0 µg/ml in the pre-column method and post-column methods, respectively.

[b] $t_{calculated}=a/S_a$; $t_{tabulated}=2.845$ for a confidence level of 99% and 20 degrees of freedom; $t_{tabulated}=3.17$ for a confidence level of 99% and 10 degrees of freedom.
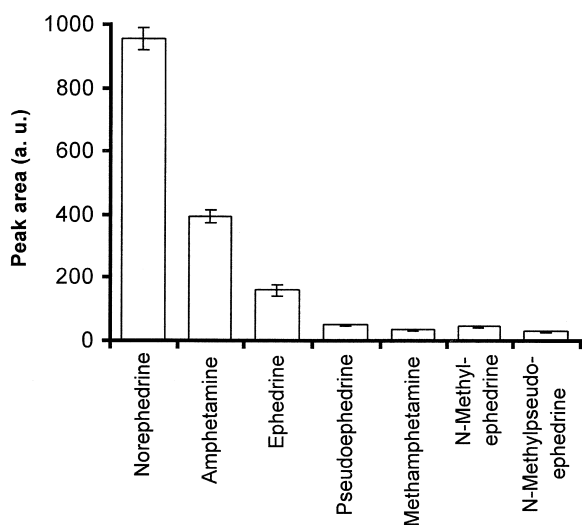
Fig. 5. Peak areas obtained for the amphetamines (20 μg/ml) derivatized in the pre-column mode under conditions summarized in Table 1.

oxidizing step would most probably increase the extent of the reaction with NaClO. This was demonstrated for methamphetamine by increasing the temperature of the oxidation and by increasing the concentration of NaClO, as can be seen in Fig. 6. Whereas the oxidation of ephedrine is maximum at 40°C, the analytical signal for methamphetamine increased with the temperature within the studied interval (Fig. 6a). Similarly, the concentration of NaClO necessary to achieve maximum analyte responses was higher than that required for ephedrine under similar conditions (Fig. 6b). In other words, the working conditions should be adjusted according with the compound to be analyzed.

It is important to note that since all amphetamines originate the same reaction products, the pre-column derivatization approach can only be applied to the analysis of the single compounds. If mixtures of amphetamines are going to be analyzed, a post-column method is mandatory.

3.2. Post-column derivatization

3.2.1. Optimization of the experimental conditions

In a previous work we investigated the chromatographic separation of underivatized amphetamines on C_{18} stationary phases [13]. In accordance with such a
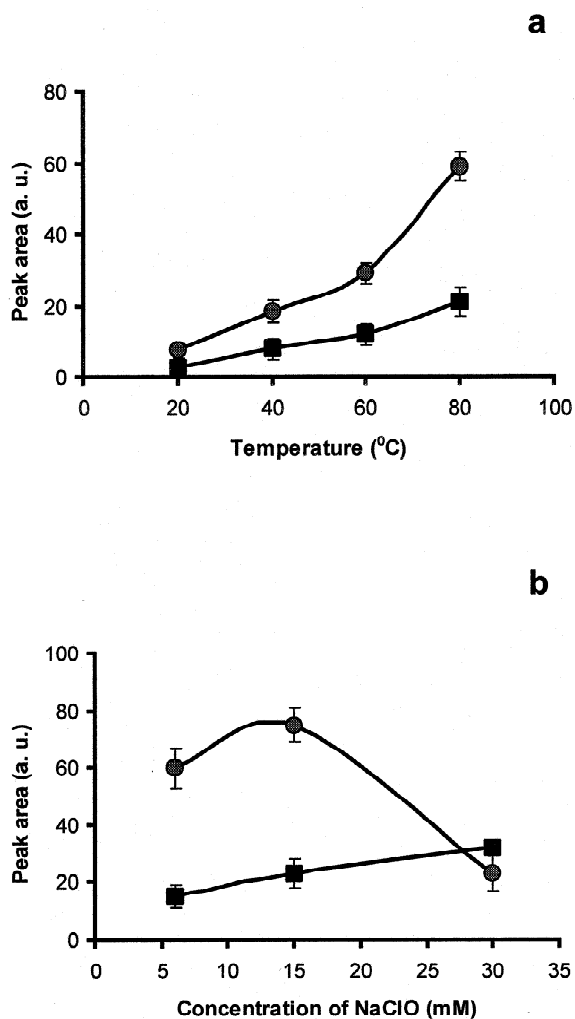

Fig. 6. Effect of experimental conditions in the derivatization of methamphetamine (20.0 μg/ml) in the pre-column mode. (a) Effect of the temperature of the reaction with NaClO; concentration of NaClO and OPA–NAC, 6 mM and 10 mM, respectively; times of reaction with NaClO and OPA–NAC, 5 min and 10 min. (b) Effect of the concentration of NaClO; concentration OPA–NAC, 10 mM; times of reaction with NaClO and OPA–NAC, 5 min and 10 min, respectively; temperature of reaction with NaClO, 80°C. First-eluting derivative (●); last-eluting derivative (■). For other experimental details, see text.

study, the best resolution and peak profiles are obtained with an acidic mobile phase and in the presence of TEA. Based on those results, the mobile phase used in the present study was a mixture of acetonitrile–0.01 M phosphate buffer (pH 3) containing 2% (v/v) TEA at a flow-rate of 0.5 ml/min.

Nevertheless, the pH resulting by merging the mobile phase with the stream of NaClO (0.1 M, pH 10.5) was appropriate for the oxidizing step, and no significant differences in the responses were observed when using a solution of NaClO buffered at pH 11.5 (data not shown). In order to simplify the experimental work, the optimization studies were performed by injecting the samples after the column (position B in Fig. 2).The parameters optimized were the concentration of NaClO and OPA–NAC, the temperature of the reaction with NaClO (the OPA–NAC reactions were performed at ambient temperature), and the length of the reactor coils in the post-column reactor (L_1 and L_2 in Fig. 2). The results of these studies are depicted in Fig. 7.

As can be observed, the concentrations of NaClO and OPA–NAC required to provide maximum analyte responses are higher than those required in the pre-column mode under equivalent conditions (see Fig. 4). This can be explained by the dilution introduced by merging the NaClO and OPA–NAC streams with the mobile phase. However, no differences in the optimum temperature for the oxidation reaction were observed between the pre-column and the post-column approaches. On the other hand, the optimum coils lengths were $L_1=750$ cm and $L_2=100$ cm. Longer coil lengths did not significantly improve the analytical signals, while resulting in unacceptable peak profiles due to the dispersion of the analyte. According with these results, the conditions finally selected for derivatization of ephedrine are those indicated in Table 3.

3.2.2. Determination of ephedrine

The described conditions were applied to the quantification of ephedrine. In these studies, samples were injected into the chromatographic column (position A in Fig. 2), and then derivatized. Fig. 8 shows the chromatograms typically obtained for a blank (water) and solution of ephedrine.

Table 2 compares the analytical data obtained under the present conditions with those obtained with the previously described pre-column derivatization method. It can be deduced that the described post-column method also provided suitable linearity and reproducibility for the quantification of ephedrine in the tested concentration interval. However, although the two derivatives originated by the analyte are simultaneously detected, the post-column approach is less sensitive. This can be explained by the dispersion of the analyte in the post-column reactor and also by the fact that the estimated time of residence of the analyte in the post-column reactor (3.9 min) was clearly lower than the time required to achieve maximum conversion of the analyte with both NaClO and OPA–NAC reagents. For these reasons, the present approach could only be applied for the quantification of ephedrine at concentrations higher than 2.0 μg/ml.

3.3. Comparison with other LC methods

The NaClO/OPA–NAC derivatization method is a valid alternative for the chromatographic determination of ephedrine. This method can also be applied to derivatize other primary, secondary and tertiary amphetamines. Although the two-stage reaction gives multiple products, the pre-column derivatization approach has been proved to be suitable for the quantification of ephedrine at low concentration levels. The sensitivity is comparable to most LC methods using other UV or fluorescence derivatization agents. The present procedure involves conditions (time of reaction, temperature) similar to those required with other common reagents proposed for secondary amphetamines (e.g. NQS or dansyl chloride); however, it is more time consuming than methods based on the employment of other reagents such as FMOC or DNB-Cl [11,14]. The time required for derivatization is also higher than that required for the formation of the OPA derivatives of primary amphetamines [4,5,11].

In the post-column approach the analysis time is drastically reduced and the analysis is simplified, as no manipulations of the sample are involved. However, although the reaction products are simultaneously detected, the limit of detection is relatively high in comparison with the values reported by LC methods based on chemical derivatization with reagents capable of reacting with secondary amphetamines. Thus, the main advantage of the NaClO/OPA–NAC derivatization over such methods is that it can integrated in a post-column configuration. In other words, post-column derivatization would be the method of choice when rapidity or automation rather
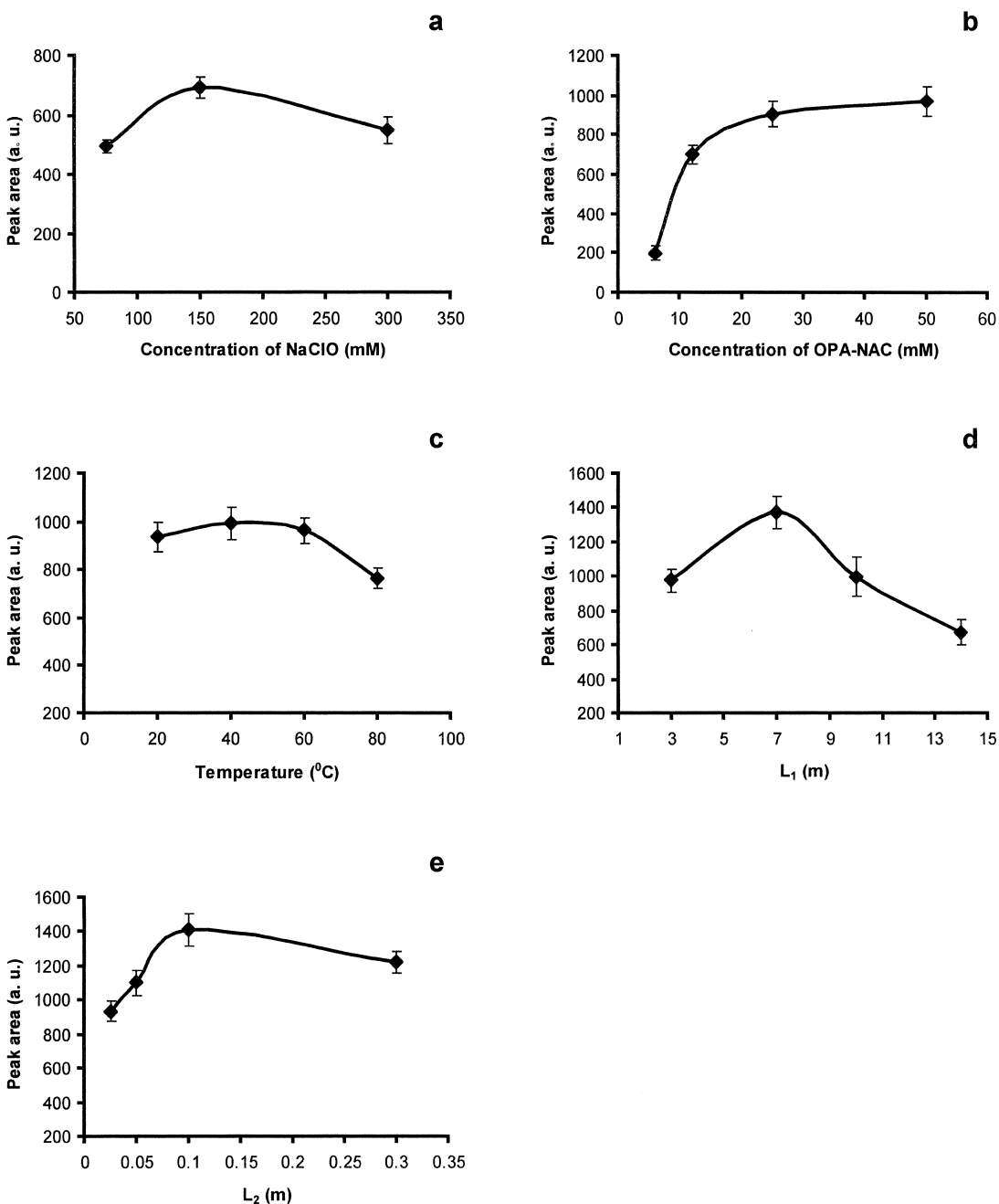
Fig. 7. Effect of experimental conditions in the derivatization of ephedrine (20.0 µg/ml) in the post-column mode. (a) Effect of the concentration of NaClO; concentration of OPA–NAC, 10 mM; L_1=3 m; L_2=0.1 m; temperature of the reaction with NaClO, 40°C. (b) Effect of concentration of OPA–NAC; concentration of OPA–NAC, 0.15 M; L_1=3 m; L_2=0.1 m; temperature of the reaction with NaClO, 40°C. (c) Effect of the temperature of the reaction with NaClO; concentrations of NaClO and OPA–NAC, 15 M and 25 mM, respectively; L_1=3 m; L_2=0.1 m. (d) Effect of L_1; concentration of NaClO and OPA–NAC, 15 M and 25 mM, respectively; temperature of the reaction with NaClO, 40°C; L_2=0.1 m. (e) Effect of L_2; concentration of NaClO and OPA–NAC, 15 M and 25 mM, respectively; temperature of the reaction with NaClO, 40°C; L_1=7.5 m. For other experimental details, see text.

Table 3
Conditions selected for derivatization of ephedrine in the post-column mode

Condition	Reaction with NaClO	Reaction with OPA–NAC
Concentration of reagent (mM)	0.15	0.025
Coil length (cm)	750	100
Temperature	40°C	Ambient

than sensitivity are required (for example, in pharmaceutical analysis).

The NaClO/OPA–NAC post-column derivatization method can be considered an alternative to other methods proposed for the on-line derivatization of primary and secondary amphetamines with either conventional solid supports (solid-support assisted derivatization) or solid-phase reagents [1,11]. Indeed, each methodology involves additional equipment. For example, in the present NaClO/OPA–NAC post-column derivatization method, only a post-column reactor is required over a simple chromatographic system. In contrast, solid-support assisted derivatization methodology requires an additional pumping system, a programmable sample injector, a pre-col-

umn and a switching arrangement; however, the latter methodology can also perform on-line sample clean-up.

Compared with our previous on-line method based on preconcentration of the analyte into a trapping column and detection of underivatized ephedrine at 210 nm, the present approach provides better selectivity [13]. Moreover, problems encountered when working at such a low wavelengths, especially those derived from baseline distortions, are avoided. In our experience, sample clean-up can be integrated in the chromatographic system by using pre-column technology without affecting significantly the derivatization process, especially when a large excess of reagent (as in the proposed post-column derivatization mode) is used [4,11,15]. Therefore, the described post-column derivatization method could be adapted to the determination of ephedrine in biological fluids, thus combining advantages of on-line sample clean-up and derivatization (excellent sensitivity and selectivity without sample manipulation).
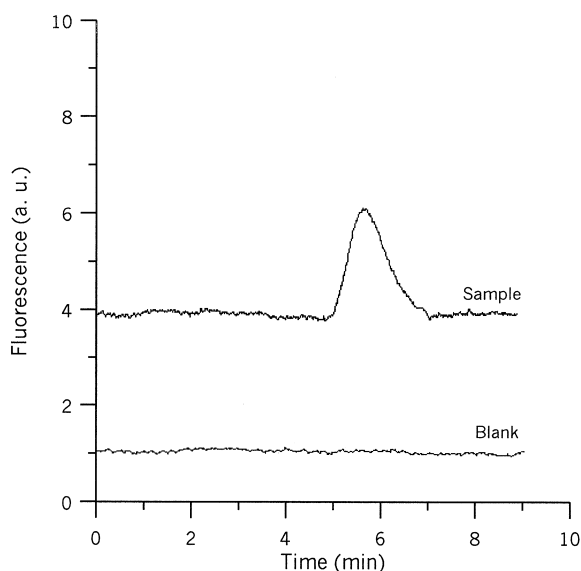
4. Conclusions

The present study illustrates the possibility of extending the derivatizations with OPA–NAC to secondary or tertiary amphetamines after their oxidation with NaClO. The NaClO/OPA–NAC approach can be used in pre-column derivatization mode to analyze the single amphetamines. The analysis of complex samples (for example mixtures of amphetamines or biological fluids) would require a post-column approach. The experimental conditions should be selected according to the amphetamine to be analyzed.

As regards the determination of ephedrine, the proposed methods provide suitable linearity and reproducibility within the tested concentration inter-

Fig. 8. Chromatograms obtained for a blank (water) and a solution containing ephedrine (50 μg/ml) under conditions summarized in Table 3.

vals. However, the post-column derivatization meth-
od is less sensitive; therefore, preconcentration of the
analyte may be required for the quantification of
ephedrine at trace levels. Post-column derivatization
would be the method of choice when rapidity or
automation rather than sensitivity are required.

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