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Simultaneous determination of naphazoline, diphenhydramine and phenylephrine in nasal solutions by capillary electrophoresis

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Abstract

A capillary zone electrophoresis (CZE) method has been developed to separate and quantitate naphazoline (NAPH), dyphenhydramine (DIP) and phenylephrine (PHE) in nasal solutions. Samples were diluted 1:25 in ultrapure water and injected at the anodic end. A central composite design has been used to optimise the experimental conditions for a complete and fast separation of the active ingredients studied. Critical parameters such as voltage, pH and buffer concentration have been studied to evaluate how they affect responses such as resolution and migration times. Separation was performed on a silica capillary with 75 μm I.D. and 70 cm total length at an applied voltage of 17.7 kV with a phosphate run buffer of pH 3.72 and 0.063 mol l^{-1}. Calibration curves were prepared for NAPH, DIP and PHE. For each analyte, the correlation coefficients were >0.999 ($n=15$). The RSD% of six replicate injections for each analyte were reasonably good. The method was applied to the quantitation of the three components in a commercial dosage form. The proposed method has the advantage of needing a very simple sample pretreatment and being faster than a typical HPLC chromatographic method.

Keywords: Capillary zone electrophoresis; Experimental design; Naphazoline; Diphehydramine; Phenylephryne

1. Introduction

Phenylephrine hydrochloride (PHE) [(*R*)-2-methylamino-1-(3-hydroxyphenyl)ethanol hydrochloride], is an α-adrenergic (sympathomimetic) agent which stimulates α-adrenergic receptors,

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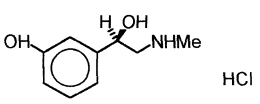
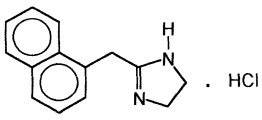
producing pronounced vasoconstriction. When used in ophthalmologic solutions, it yields temporary relief of the eye redness associated with cold, hay fever, wind, dust, sun, smog, smoke and contact lenses [1]. Diphenhydramine hydrochloride (DIP) [2-(Diphenylmethoxy)-*N,N*–dimethylethylamine hydrochloride] is an effective antihistaminic, and has been used for the treatment of motion sickness and extrapyramidal symptoms, as well as an antitussive and nighttime sleep-aid [1]. Recently, its use has been reported, in combination with other drugs, as antiemetic for the prevention of cisplatin-induced emesis in chemotherapy treatment. It has also been used as sedative in dentistry for children and in local anaesthesia [1]. Naphazoline hydrochloride (NAPH) [2-(1-naphthylmethyl)-2-imidazoline monohydrochloride] is a sympathomimetic, which belongs to the imidazole group. It is a vasoconstrictor of relatively long-lasting action that acts on the α receptors of the vascular smooth muscle [1]. Several pharmaceuticals containing the three active ingredients are currently commercialised in our country as nasal drops.

Several methods are available for the determination of the latter compounds by high-performance liquid chromatography (HPLC) in different pharmaceutical preparations, either alone or with other active ingredients [2–4]. PHE has been determined by using first-derivative spectroscopy in binary and ternary mixtures [5,6], by spectrophotometry coupled to a classical least squares method [7], a net analyte based method applied to UV spectra (HLA/GO) [8] and also by spectrofluorimetry [9].

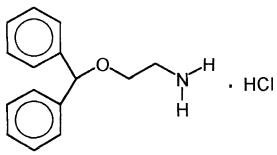
NAPH was analysed by heavy-atom-induced room-temperature phosphorescence [10], by atomic absorption and emission [11], by capillary electrophoresis in presence of its degradation products [12] and by first-derivative UV spectrophotometry [13]. Very recently we reported the simultaneous determination of the three analytes by using spectrophotometry and chemometrics analysis with excellent results [14].



Phenylephrine hydrochloride



Naphazoline hydrochloride



Diphenhydramine hydrochloride

Due to its high selectivity, high resolution liquid chromatography is the most frequently used technique for determining active ingredients in pharmaceuticals [15]. The use of capillary electrophoresis (CZE) is becoming widely spread and even replacing HPLC in some cases, because it provides a faster way of analyzing complex preparations, lower costs and simpler ways of preparing samples, although it is less precise than the latter method. Thus, a large number of methods for pharmaceutical analysis based on that technique has been reported [16,17]. The aim of this paper is to enhance experimental conditions and study the performance characteristics of a method based on CZE for simultaneously determining three active ingredients (diphenhydramine, naphazoline and phenylephrine) in nasal drops. Despite the fact that the above mentioned pharmaceuticals are within a complex matrix of excipients, their determination has been successfully approached.

2. Experimental

2.1. Equipment

Two capillary electrophoresis equipments were used: (a) Spectra PHORESIS 100 Variable UV/Vis Detector (Thermo Separation Products, San Jose, California, USA), with silica capillary with 75 μm I.D. and 70 cm (total length) and 62 cm (effective length), and (b) BECKMAN P/ACE 5000, with diode array detector and a silica capillary with 75 μm I.D. and 67 cm (total length) and 60 cm (effective length).

UV spectra were carried out on a PERKIN–ELMER UV–Vis Lambda 20 spectrophotometer. Sartorius cellulose acetate filters (0.2 μm porous size) were used. Unscrumbler 6.11 CAMO (Trandheim, Norway) program was used for processing data.

2.2. Reagents and commercial samples

All experiments were performed with ultrapure distilled water. Stock solutions of the three active components were prepared in a synthetic matrix, containing excipients commonly found in pharmaceutical preparations (1.00 g l^{-1}). Reference substances were provided by INAME (Instituto Nacional de Medicamentos, Argentina). Several phosphate buffer solutions were also prepared in order to reach the concentration and pH values established by the experimental design (see Table 1). A commercial sample (Alvonasal, Lafedar Laboratories, Paraná, Argentina) containing the following active ingredients and excipientes was tested: diphenhydramine hydrochloride l.00 g l^{-1}, naphazoline hydrochloride l.00 g l^{-1}, phenylephrine hydrochloride l.00 g l^{-1}, sodium chloride 40.0 g l^{-1}, disodium hydrogen phosphate 30.0 g l^{-1}, sodium dihydrogen phosphate 68.0 g l^{-1} and methyl hydroxybenzoate 0.10 g l^{-1}. It is remarkable that sample preparation is not needed, since only 1:25 dilution with distilled water is required.

3. Results and discussion

3.1. Optimisation of the experimental conditions

The wavelength used for detection was selected by analyzing individual UV spectra of active ingredients, obtained between 200 and 350 nm. As a result of this study, a 210 nm value was chosen since the three compounds show suitable molar extinction coefficients at this value. On the other hand, and due to high reproducibility values, capillary washing and conditioning were carried out for both equipments. This was performed following these steps: (a) at the beginning of the day, 5 min with NaOH 0.1 mol l^{-1} and 10 min with water and 5 min with the buffer solution. (b) between samples, 1 min with Na OH 0.1 mol l^{-1}, 1 min with water, 1 min with the buffer solution. Three buffer solutions (pH 9.0, 7.0 and 3.0) were tested in order to determine the most convenient zone of pH to be used in the subsequent optimization. The synthetic sample (diluted to l:25) was run using these buffer solutions and the components separation degree was observed. The region 2.3–

Table 1
Central composite design used to optimise resolution between peaks and first compound migration time

Experiment	Voltage (kV)	Buffer concentration (mol l^{-1})	pH	Resolution[a]	First compound migration time (min)
1	13.64	0.06	4.00	1.22	12.57
2	20.36	0.06	4.00	1.29	6.48
3	17.00	0.01	4.00	0.00	9.11
4	17.00	0.10	4.00	1.12	7.55
5	17.00	0.06	2.32	1.23	8.07
6	17.00	0.06	5.68	0.89	6.50
7	15.00	0.03	3.00	0.00	12.35
8	19.00	0.03	3.00	0.98	8.24
9	15.00	0.08	3.00	1.14	10.44
10	19.00	0.08	3.00	1.20	9.01
11	15.00	0.03	5.00	0.00	10.50
12	19.00	0.03	5.00	0.00	7.53
13	15.00	0.08	5.00	0.86	10.34
14	19.00	0.08	5.00	0.81	6.61
15	17.00	0.06	4.00	1.43	8.18

[a] The resolution was assigned as zero when the three compound were not separated.

5.7 was selected, since only in in this range complete separation of the compounds was enabled. In order to identify peak values, each of the components was added to the injected solution. This test determines the order in which substances come out, in terms of the area growth of one of the peaks. Fig. 1 shows the order found: first NAPH, second PHE and finally DIP. Considering that this separation order corresponds to an acid pH, one can analyze if the order matches the mass/charge ratio. The pKa's and molecular weight of the active ingredients are: NAP = 10.9 (210.3 g mol^{-1}), PHE = 10.1 and 8.9 (167.2 g mol^{-1}) and DIP = 9.0 (255.4 g mol^{-1}). As can be seen, the most charged compound is NAP, the second one is PHE and finally DIP. This fact supports the separation order. On the other hand, the high mass of the last compound is probably the cause of the better resolution between DIP and PHE.

Experimental design allows a large number of factors to be tested simultaneously and precludes the use of a large number of independent runs when the traditional step-by-step approach is used. Systematic optimisation procedures are carried out by selecting an objective function, finding the most important factors and investigating the relationship between responses and factors by the so-called response surface methods (RSM) [18]. Two experimental responses were chosen as objective functions to be optimised [19]: (a) resolution between peaks and (b) first active ingredient migration time. The former is defined as:

$$R = \frac{2(t_2 - t_1)}{w_1 + w_2} \quad (1)$$

where t_1 and t_2 represent the positions in the center of the zone. This data is obtained drawing a perpendicular from the center to the baseline. w_1 and w_2 are the width of the zones on the baseline. When the resolution is higher than 1.5, the two species are said to be resolved at the baseline [19]. On the other hand, time is an important factor to be optimised, since speed is one of most important advantages of a CE method.

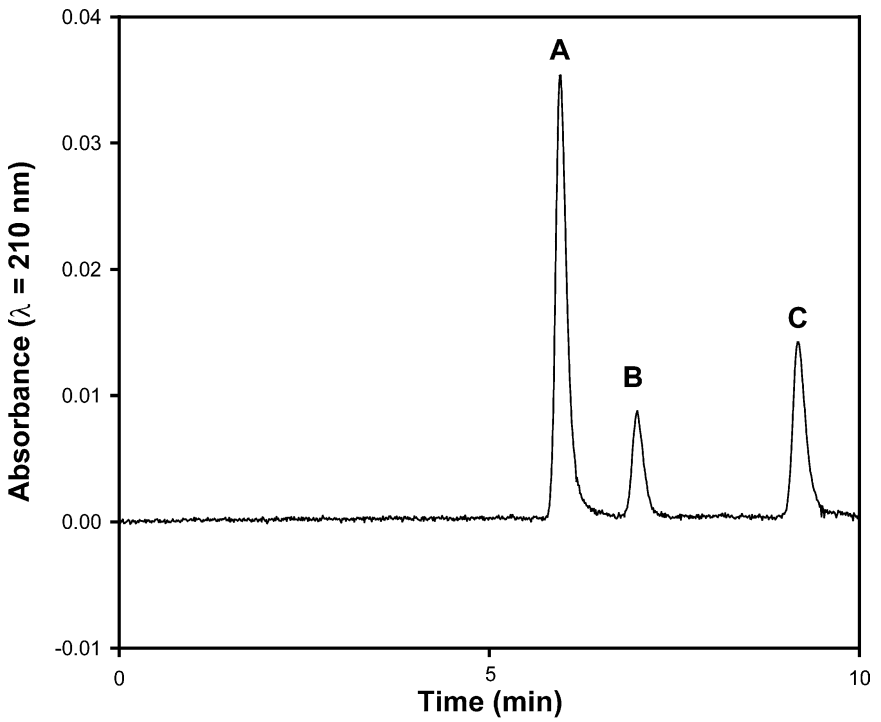


Fig. 1. Electropherogram of a sample containing the three components: A, NAPH; B, PHE and C, DIP, using a buffer phosphate solution of 0.063 mol l^{-1}, pH 3.72 and a voltage of 17.7 kV.

In order to calculate quadratic regression model coefficients, each design variable has to be studied at least at three distinct levels, and consequently the central composite design is often used to provide estimation of a second-order equation. Among the standard designs used in response surface methodology (RSM), this design represents a good choice because of its high efficiency with respect to the number of required runs and also because they are built considering five levels of the factors being studied. Variables used during the optimization process were voltage, pH and buffer solution concentration. Table 1 shows the levels of each variable studied for finding out the optimum values and responses. As can be seen in this table, the ranges used were: pH (2.32–5.68), voltage (13.64–20.36 kV) and buffer concentration (0.01–0.10 mol l^{-1}). These ranges were selected taking in consideration previous studies, as the one mentioned for pH, as well as instrumental limitations. All experiments were performed in randomised order to minimise the effects of uncontrolled factors that may introduce a bias on the measurements. This design allowed us to obtain the surface response fitting the data to the following polynomial mathematical model:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \varepsilon_i \quad (2)$$

with X_1, X_2 and X_3 being the analyzed factors (voltage, buffer concentration and pH respectively), where β_1 represent the model coefficients and ε_i the experimental error. After fitting (Eq. (2)) by a least-squares regression, the parameters obtained for both responses (Y_1 = resolution and Y_2 = first compound migration time) were the following:

$$Y_1 = 20.2 + 1.6X_1 + 103.1X_2 + 2.5X_3 - 2.6X_1X_2 - 0.1X_1X_3 + 1.9X_2X_3 - 0.1X_1^2 - 480.1X_2^2 - 0.2X_3^2 \quad (3)$$

$$Y_2 = 73.4 - 6.6X_1 - 122.7X_2 + 1.5X_3 + 4.3X_1X_2 - 0.1X_1X_3 + 0.8X_2X_3 + 0.1X_1^2 + 288.9X_2^2 - 0.1X_3^2 \quad (4)$$

The significance of the parameters estimated by least-squares can be assessed by using classical statistical tools as ANOVA. This analysis showed that the most significant factor is the buffer concentration ($P < 0.01$) and that its quadratic term has a high influence when both responses are chosen as objective function. The second factor, with a significantly lower effect, is the pH. On the other hand, the ratios between the variance originated for the factors and the total variance, the coefficients of multiple determination (R^2), that totally explain the variance in the data, were 0.882 and 0.908 respectively, showing a reasonably good fitting of the experimental data (88.2 and 90.8%). According to the adjustment performed, and deriving Eq. (3), the variable values corresponding to maximum Y_1 response (resolution = 1.60), were: buffer concentration = 0.063 mol l^{-1}, pH 3.72, and voltage = 17.7 kV. These values correspond to a first compound migration of 7.45 min, indicating that the total time that would be necessary to perform the separation is approximately 10 min, and can be considered as acceptable to be employed in pharmaceutical analysis.

Based on the mathematical model, the response surface can be explored graphically. In this case, one can plot the response surface against two of the factors, while the third is held constant at a specified level, usually the center value. Fig. 2-A shows the response surface of resolution, obtained plotting pH versus buffer concentration (these factors were found to be the most important ones in the previous study). One alternative way to find the maximum can be seen in Fig. 2-B. It shows the corresponding contour plot in which it can be easily seen the optimum value of resolution when both pH and buffer concentration are evaluated maintaining a constant voltage. The results obtained by means of the graphical analysis are comparable to those obtained when Eq. (3) and Eq. (4) were derived. Although the former is a faster way to reach the optimization values, the latter is more intuitive and simple.

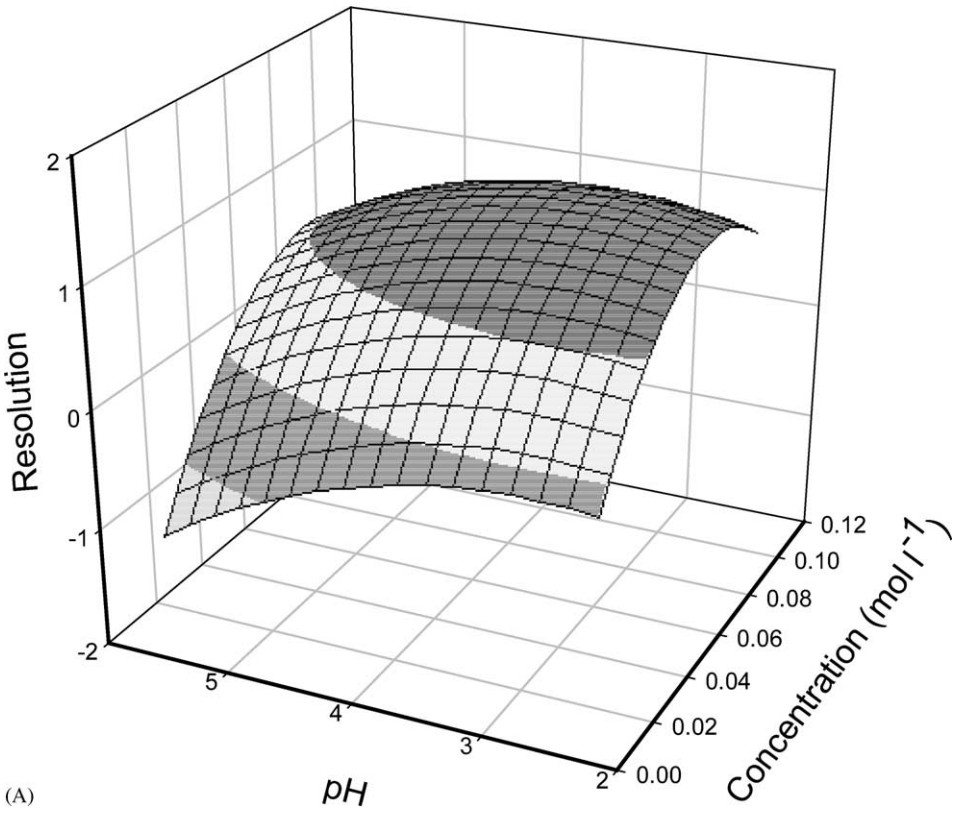


Fig. 2. A, Response surface of resolution, estimated from the central composite design plotted for pH vs. buffer concentration while keeping voltage constant at its central value; B, Contour plot.

3.2. Performance

3.2.1. Linearity

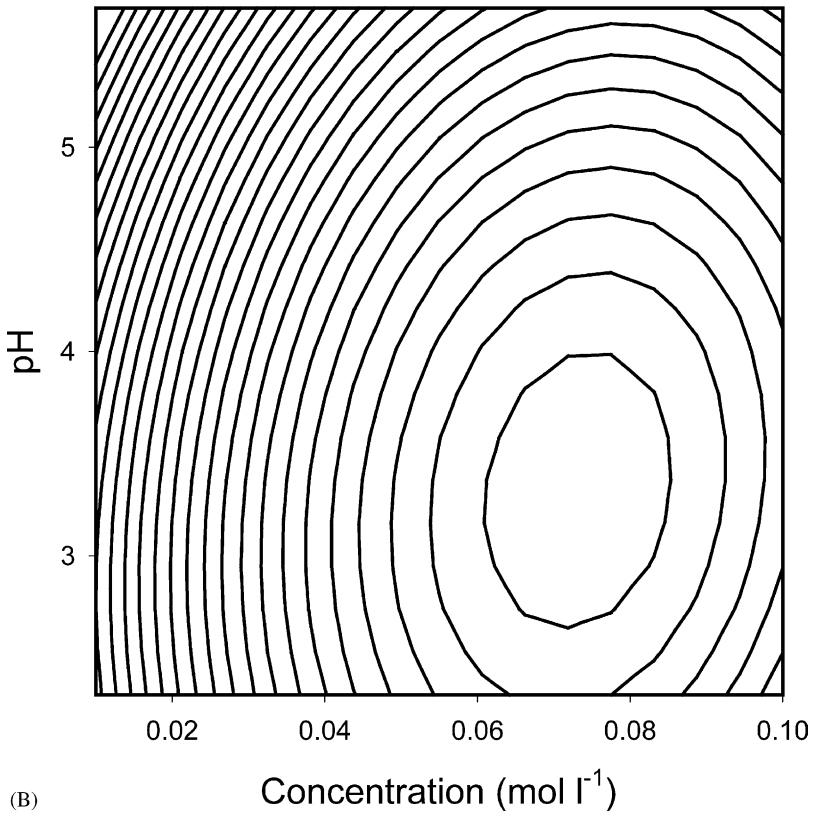
In order to verify the method linearity within a concentration range of 80–l20% of the target analyte concentration [20], three replicates were prepared at five concentration levels: 40.0, 45.0, 50.0 and 60.0 μg ml^{-1}. A least square fitting was performed with the data obtained and the results are shown in Table 2. Analysis of the residual variance proof was performed in all cases showing that linearity exists for the three components within the range studied [21].

3.2.2. Precision

The precision (repeatability) was determined by total analysis of ten replicates samples on three different levels of concentrations under the same conditions, by the same analyst, and on the same day. The RSD% values obtained are shown in Table 3. As can be seen, these values are lower than 2%, indicating an excellent precision and the capability of the present method to be used in pharmaceutical analysis.

3.2.3. Accuracy

Three concentration levels (80, 100 and 120% of the commercial sample) were evaluated after performing the corresponding dilution. Samples were prepared adding the same excipients which are present in commercial samples in order to evaluate the effect of these on the method performance. Determinations were made in triplicate and the average recovery values are shown in Table 3. These results show the high accuracy obtained in the three concentration levels studied.



(B)

Fig. 2 (*Continued*)

Table 2
Statistical parameters for the best fitted line when analysing phenylephrine, diphenhydramine and naphazoline in nasal solutions by CZE

Statistical parameters[a]	DIP[b]	PHE[b]	NAPH[b]
Intercept (a)	−0.01	−0.009	−0.02
S.D. (s_a)	0.01	0.008	0.03
Slope (b)	0.067	0.036	0.171
S.D. (s_b)	0.002	0.001	0.006
Fit S.D. (s_{fit})	0.012	0.0087	0.031
Correlation coefficient (r^2)	0.9932	0.9991	0.9929

[a] Each analyte with five standards and three replicates.
[b] Concentration levels: 0.00, 40.0, 45.0, 50.0 and 60.0 mg l^{-1}.

Table 3
Figures of merit when analysing phenylephrine, diphenhydramine and naphazoline in nasal solutions by CZE

Figures of merit	DIP	PHE	NAPH
LOD (mg l^{-1})	4.5	6.6	4.7
LQD (mg l^{-1})	14.9	22.0	15.8
RSD% (level: 80%)	1.4	1.7	1.0
RSD% (level: 100%)	1.5	1.1	1.1
RSD% (level: 120%)	0.8	1.9	0.8
Recovery% (level: 80%)	101.9	100.4	100.1
Recovery% (level: 100%)	101.2	101.8	102.0
Recovery% (level: 120%)	99.9	100.5	101.1

3.2.4. Detection and quantification limits

The detection limit (LOD) of a method is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically three times the noise level. The quantification limit is the lowest level of analyte that can be accurately and precisely measured (LOQ). According to IUPAC [22], detection and quantification limits can be established according to the following equations:

$$\mathrm{LOD} = 3 \times s_a / b \quad (5)$$

$$LOQ = 10 \times s_a/b \quad (6)$$

where s_a is the standard deviation (S.D.) for the intercept, and b is the calibration straight line slope. Table 3 shows these figures of merit calculated according to Eq. (5) and Eq. (6).

3.2.5. Commercial samples

A commercial sample containing the three components was analysed performing five replicates with similar qualitative and quantitative results to those obtained for artificial samples. The results obtained when analysing this particular sample were: NAP: 1.02 (0.01) g l^{-1}, PHE: 1.01 (0.01) g l^{-1} and DIP: 1.01 (0.01) g l^{-1}, with the values within parenthesis being the S.D. of the five replicates. It is important to considerate that excipients do not interfere in the determination of the three active ingredients since the samples used to evaluate recovery were prepared with excipients.

4. Conclusions

The content in the three active components present in nasal solutions currently commercialised in our country (phenylephrine, diphenhydramine, naphazoline) was determined using capillary electrophoresis. A synthetic sample was used to study the performance characteristics, with excellent quality indexes. A commercial sample was also analyzed, giving results comparable to those reported by the manufacturer. As shown in this study the method allows fast and simple simultaneous identification and quantification of the three compounds, being very suitable for quality control analyses of pharmaceuticals containing such compounds.

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