Artigo

DETERMINATION OF CETIRIZINE IN TABLETS AND COMPOUNDED CAPSULES: COMPARATIVE STUDY BETWEEN CE AND HPLC

Lisiane Bajerski*, Maximiliano da Silva Sangoi, Thiago Barth, Isabel Fração Diefenbach, Sérgio Luís Dalmora e Simone Gonçalves Cardoso

Faculdade de Ciências Farmacêuticas, Universidade Federal de Santa Maria, Av. Roraima, 1000, 97105-900 Santa Maria - RS, Brasil

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A capillary electrophoresis (CE) method was developed and validated for determination of cetirizine dihydrochloride in tablets and compounded capsules. The electrophoretic separation was performed in an uncoated fused-silica capillary (40 cm x 50 μm i.d.) using 20 mmol L^{-1} sodium tetraborate buffer (pH 9.3) as background electrolyte, a hydrodinamic sample injection at 50 mBar for 5 s, 20 KV applied voltage at 25 °C, and detection at 232 nm. The proposed method was compared with the high performance liquid chromatographic (HPLC) method previously validated for this drug, and statistical analysis showed no significant difference between the techniques.

Keywords: cetirizine; capillary eletrophoresis; high performance liquid chromatography.

INTRODUCTION

The cetirizine dihydrochloride (CTZ, Figure 1), second generation of antihistaminic, actuate in relieve of the physical symptoms of allergical rhinites, rinoconjuntivites, chronic urticaria, and another allergical disorders.[1] This drug, freely soluble in water, practically insoluble in acetone and in methylene chloride,[2] is considered a weak acid with three pKa values (2.19, 2.93 and 8.00) and chemically known as (*RS*)-[2-[4-[(4-chlorophenyl)phenylmethyl]-1-piperazinyl] ethoxy] acetic acid dihydrochloride.[3] It is available such as raw material, tablets, solutions (drops and syrup), and compounded capsules.

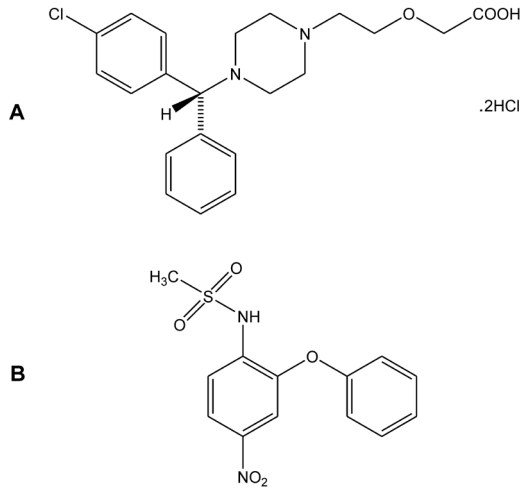

Figure 1. *Chemical structure of cetirizine dihydrocloride (A) and nimesulide (B)*

An official technique for CTZ quantification in oral formulations has not yet been described in the literature but methods such as potenciometry,[2,4] and HPLC[5] are available for its determination in raw material. Different analytical methods for determination of this antihistaminic in biological fluids (urine, plasma, serum) have been reported and include HPLC,[6-9] HPLC-MS,[10-12] CG,[13] HPTLC,[14] and TLC.[15]

In pharmaceutical dosage forms (tablets, compounded capsules, solutions) this drug was analyzed isolated by HPLC,[16-19] HPLC-MS,[20] and spectrophotometry,[4,16,21,22] or combined with multicomponent dosage forms by spectrophotometry,[22] HPTLC,[23] and HPLC.[24]

Traditionally, pharmaceutical analysis relies heavily on HPLC. CE has many advantages over HPLC that are including greater separation, efficiency, small sample and reagent volume, fast separation and better robustness.[25] This technique has emerged specially due to the diversities of separation modes, which can be performed in a single capillary format.[26] These compensations empower CE with great utility to be successfully applied for routine pharmaceutical analysis.[25] CE methods, using different detection wavelengths and buffer systems, are available in the literature for determination of CTZ in syrup and tablets[27,28] or combinated in multicomponent dosage form,[29] as well as in its chiral separation in pharmaceuticals.[30-32] In this paper, we propose develop and validate a CE method for determination of CTZ in tablets and compounded capsules according to official guidance requirements for industry.[33,34]

Our attention was focused on the development of an alternative technique for CE method to be applied in the quality control of this drug, not only in tablets, but also in compounded capsules, that is considered a cheap alternative if compared to the official solid oral preparation. The results obtained by the developed method were compared with the HPLC method previously developed and validated by our group.[18] Until now, there is only one comparative method between CE and HPLC for CTZ determination in biological fluids available in the literature, while no one in pharmaceutical forms.[35]

EXPERIMENTAL

Chemical

CTZ, reference substance (assigned purity, 99.9%) and nimesulide, (NI, Figure 1) internal standard (assigned purity, 99.4%) were obtained from Sintética and Galena, respectively (São Paulo, Brazil). Zyrtec 10 mg tablets (Glaxo Welcome, Brazil) and compounded capsules 10 mg were purchased from local pharmacies. The excipients of tablets (lactose, magnesium stearate, talc, titanium dioxide, povidone, hydroxypropylmethylcellulose, polyethyleneglycol, corn starch) and compounded capsules (lactose, magnesium stearate, corn starch, polyvinylpirrolidone, hydroxypropylmethylcellulose) were acquired from local distributors. Acetonitrile HPLC grade from Tedia Company

*e-mail: lisianebajerski@yahoo.com.br

(Fairfield, USA), *ortho*-phosforic acid, 1 mol L^{-1} sodium hydroxide, sodium tetraborate decahydrate and 0.1 mol L^{-1} sodium hydroxide solutions were obtained from Merck (Darmstadt, Germany).

Instrumentation

The CE instrument used was HP[3D] CE instrument Agilent Technologies (Santa Clara, USA) equipped with a diode-array detector (DAD). Sample injections were made in a hydrodynamic mode, performed at 50 mBar for 5 s. A constant voltage of 20 KV, with an initial ramping of 1 KV s^{-1}, was applied during analysis. The DAD was set at 232 nm. The capillary temperature was maintained constant at 25 °C. All experiments were carried out applying positive mode. CE ChemStation software (version A 09.01) was used for instrumentation control, data acquisition, and analysis. The separation was carried out using a conventional fused silica capillary (40 cm x 50 μm i.d.) Agilent Technologies (Santa Clara, USA). pH was recorded with a digital pHmeter Schott (Stafford, UK). Purified water was prepared using a Water Purification Unit Labconco (Kansas City, USA). The solvents were filtered in a 0.45 μm membrane filter Millipore (Belford, USA) and degassed daily.

All HPLC experiments were carried out on Shimadzu LC-10 A system equipped with a model LC-10 AD_{VP} pump, a SPD-10 A_{VP} UV-VIS detector, a SCL-10 A_{VP} system controller, SIL-10 A_{VP} auto injector and a degasser module, data were acquired and processed by Shimadzu class-$_{VP}$5.0 software. The chromatographic separation was performed on an endcapped Luna Phenomenex® RP-18 column (250 mm x 4.6 mm i.d., 5 μm) with a guard cartridge system LiChroCart RP-18 (4 x 4 mm i.d.). The mobile phase consisted of *ortho*-phosforic acid 1% pH 3.0-acetonitrile (60:40 v/v). The flow rate was 1.0 mL min^{-1}. Detection was performed at 232 nm at room temperature. The injection volume was 20 μL.

Analytical procedure

Optimized background electrolyte (BGE) solution was 20 mmol L^{-1} sodium tetraborate decahydrate buffer adjusted to pH 9.3 with 1 mol L^{-1} sodium hydroxide solution. Before the first use, the fused-silica capillary was sequentially rinsed with 1 mol L^{-1} sodium hydroxide for 30 min, followed by deionized water and BGE solution, both by 15 min. The preconditioning was consisted the washing the capillary between analyses with 0.1 mol L^{-1} sodium hydroxide for 2 min, followed by deionized water for 2 min, then equilibrated with BGE for 3 min.

Preparation of stock solutions

Stock solution of CTZ reference substance (1000 μg mL^{-1}) was prepared by weighing accurately 10 mg of this drug and dissolving in BGE solution. Working solutions (2-150 μg mL^{-1}) were prepared by diluting appropriately the stock solutions with BGE solution. To each flask was added NI solution, previously prepared in acetonitrile, in a constant aliquot of 50 μg mL^{-1}.

Preparation of samples

Twenty units of tablets and compounded capsules were weighed and the average weight was calculated for each. The tablets were crushed to a fine powder and the contents of the compounded capsules were completely removed from shells and homogenized. An amount of powder, equivalent to 10 mg CTZ from each pharmaceutical formulations, were transferred to 50 mL volumetric flask. After, the volume was adjusted with BGE solution and sonicated by 5 min, and filtered through quantitative filter paper (Schleicher & Schuell, Dassel, Germany). Aliquots of this solution were diluted to give a final concentration of 50 μg mL^{-1}. The solutions were filtered through a 0.45 μm membrane filter before injection. For all quantitative determination, a constant amount of NI (50 μg mL^{-1}), previously solubilized in acetonitrile, was added to the drug solution.

Validation procedure

Specificity

The method specificity was investigated by observing all interferences encountered from excipients, cited at *Experimental Section*, present in tablets and compounded capsules of CTZ. Their concentration in these formulations was based on the literature[36] and calculated in relation of medium weight of each pharmaceutical form. The electropherograms of excipients placebo solution (without drugs) and the CTZ reference substance and NI (50 μg mL^{-1}), prepared in BGE solution, were compared to verify the probable interference of the excipients in the quantitative determination of these drugs.

Linearity and limits of detection (LOD) and quantitation (LOQ)

The linearity was studied by injecting solutions in the concentration range of 2-150 μg mL^{-1} of CTZ and fix concentration of NI (50 μg mL^{-1}). Reference substance solutions were prepared at seven concentrations (2, 5, 10, 25, 50, 100, 150 μg mL^{-1}) and injected in triplicate every day, during three consecutive days. The ratio of peak area values of CTZ and NI was computed and analyzed using linear least-squares regression parameters (correlation coefficient, slope, intercept).

The LOD and LOQ values were mathematically determined through calibration curve. The aforementioned factors (3.3 and 10) were multiplied by the ratio from the residual standard deviation and the slope (corresponding to the standard error of slope), according to the guideline.[33]

Precision

The precision was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) studies. Repeatability was analyzed through the preparation of six samples containing 50 μg mL^{-1} of CTZ and NI, injected in duplicate, in the same day. Intermediate precision was tested of repeating the same procedure in two different days (n = 9) and comparing the results between them. The data of tablets and compounded capsules precision were expressed as the percent relative standard deviation (RSD %).

Accuracy

The accuracy of the method was evaluated by preparing a synthetic excipients representative of CTZ tablets and compounded capsules, and spiking three samples with CTZ reference substance in the concentration levels corresponding to 40 μg mL^{-1} (low), 50 μg mL^{-1} (medium) and 60 μg mL^{-1} (high). The CTZ was extracted from the excipients and determined. Each solution was prepared in triplicate and injected three times. The concentrations and recoveries were calculated against the added concentration.

Stability of solutions

Solutions of CTZ and NI, tablets and compounded capsules were prepared in BGE solution at 50 μg mL^{-1} and stored at 8 ± 2 °C (in the refrigerator) and 25 ± 1 °C (room temperature). The stability of these solutions was checked after 48 h and compared against freshly prepared solutions. The initial peak area was considered 100% and the recoveries in followed days were evaluated.

Robustness

Robustness of the proposed method was examined by evaluating the influence of small variations of some of the most important procedure variable such buffer concentration (19, 20 and 21 mmol L^{-1}), BGE pH solution (9.2, 9.3 and 9.4) and temperature system (23, 25 and 27 °C). Analyses were carried out with reference substance solution at 50 μg mL^{-1} of CTZ and NI, in triplicate. Only one parameter was changed in the experiments at a time and the effects were studied based on RSD (%) values obtained among the parameters analyzed.

RESULTS AND DISCUSSION

Optimization of the electrophoretic conditions

Changes in the analytical procedure were tested to develop a fast system capable of analyze the CTZ in pharmaceutical preparations. One important parameter for CE separation is the buffer system, particularly pH applied. The optimization of electrophoretic separation and migration time of analyte was conducted using different buffers, such as borate and phosphate in distinct pH (8.8-9.8) and concentration (20-50 mmol L^{-1}) ranges. Various parameters such as migration time, peak area, peak shape, height, width, and symmetry of CTZ and NI were evaluated. The best results were obtained with 20 mmol L^{-1} sodium tetraborate decahydrate buffer pH 9.3.

A potencial of 20 KV, with ramping of 1 KVs^{-1}, was the best compromise in terms of run time and current generated. As expected, on increasing the applied voltage there is an increase in the electroosmotic flow (EOF), leading to shorter analysis time and higher efficiencies. However, higher applied voltages exhibit higher currents and increased Joule heating.[37]

Control of capillary temperature is important in capillary electrophoresis. Changes in capillary temperature can cause variations in EOF, efficiency, viscosity, electrophoretic mobilities, migration time, injections volume and detector response. The effect of temperature on analysis was investigated at 20, 25 and 30 °C. The temperature giving the best compromise between resolution and run time was 25 °C, and it was selected as optimum temperature.

Under these optimized conditions, the migration times of CTZ and NI were 3.5 and 4.2 min, respectively. The total time of analysis was less than 5 min.

Validation procedure

The analytical method was validated in relation to specificity, linearity, LOD, LOQ, precision, accuracy, stability, and robustness.[33,34]

Specificity test showed that was no interference in CE results from the tablets and compounded capsules excipients indicating that the reported method is selective. Typical eletropherograms of the blank and samples are represented in Figure 2. As it can be observed none impurities interfered in the analysis of CTZ.

The statistical parameters of the analytical curve and estimates of LOD and LOQ for both methods are represented by Table 1. The calibration curves proved to be linear over the 2-150 μg mL^{-1} range. Linear regression of concentration versus peak area ratio plots resulted in an average of coefficient correlation (r) greater than 0.9999. The slope and intercept of calibration curve (± standard deviation, n = 3) were 0.0119±1.7 and 0.0064±70.5, respectively. The validity of the assay was verified by means of analysis of variance (ANOVA), which demonstrated no significant linear regression ($F_{calculated} = 3.76 < F_{critical} = 4.69$; $p < 0.01$).

The LOD and LOQ were estimated to be 1.2 and 3.8 μg mL^{-1}, respectively, indicating a suitable sensitivity of the method.

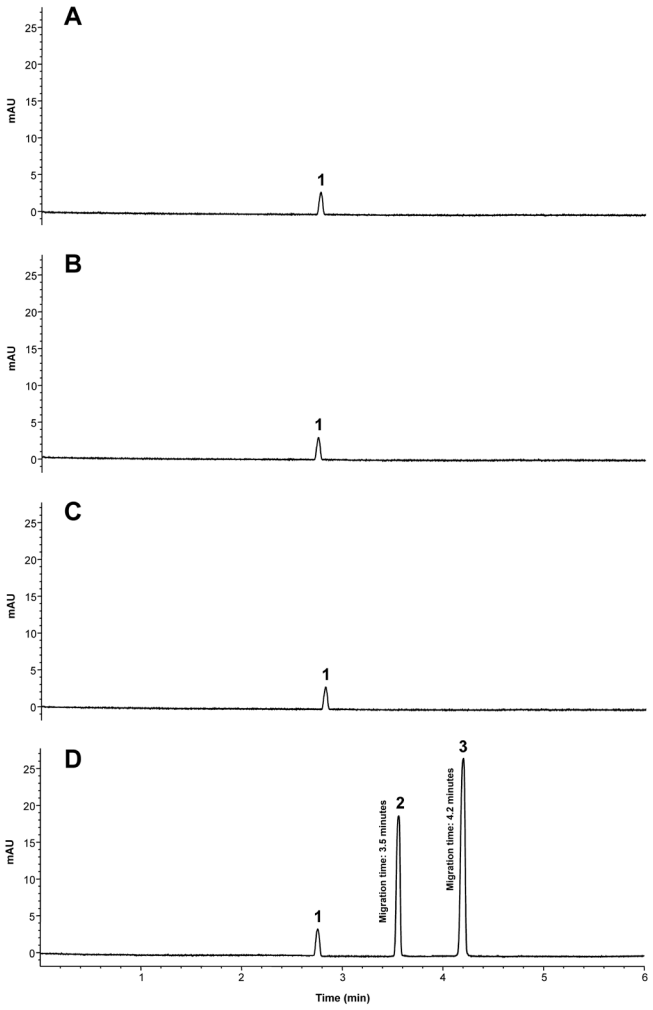

Figure 2. *Electropherograms of specificity test for solutions: blank (A); tablet excipient simulated (B); compounded capsule excipient simulated (C); CTZ reference standard and NI internal standard, both at 50 μg mL^{-1} (D). Peak 1: 20 mmol/L sodium tetraborate decahydrate pH 9.3 buffer; peak 2: CTZ reference standard; peak 3: NI internal standard*

Table 1. Statistical parameters of the analytical curve and estimates of LOD and LOQ for the UV-absorbance detection methods

	HPLC	CE
Linearity range (μg mL^{-1})	10-30	2-150
Intercept	1363.6	0.0064
Slope	34223	0.0119
Correlation coefficient (r)	1.0000	0.9999
LOD (μg mL^{-1})	0.3	1.2
LOQ (μg mL^{-1})	0.8	3.8

The results of precision are summarized in Table 2. The low RSD (%) obtained for the repeatability (< 2.0%) and intermediate precision, 0.1 and 1.2, for tablets and compounded capsules, respectively, showed the good precision of the method.

The accuracy results for both formulations showed good recovery and are listed in Table 3. Results for accuracy of CTZ at three levels by the standard addition technique range from 102.0 to 103.0%, for tablets, and 98.0 to 101.0%, for compounded capsules. These values showed the good accuracy of the proposed method.

Table 2. Analitycal performance of the method regarding precision

	Tablets		Compounded capsules	
	HPLC	CE	HPLC	CE
Repeatability (n = 6)	101.0	100.0	97.0	99.0
RSD (%)	0.9	1.0	1.4	1.3
Intermediate precision (n = 9)	101.0	100.0	98.0	98.0
RSD (%)	0.1	0.1	0.8	1.2

Table 3. Method validation regarding accuracy: recovery test

	Tablets		Compounded capsules	
	HPLC	CE	HPLC	CE
Mean recovery	100.0	102.0	100.0	99.0
RSD (%)	1.9	0.5	1.8	1.7

The RSD (%) of peak area ratio between CTZ and NI was calculated for each parameter proposed (temperature, BGE concentration and pH). The results showed that the most critical parameter was the temperature (RSD > 4.0%). Thus, is possible considerer that the method developed is rugged in relation of BGE concentration and different values of pH, but not with changes in temperature.

The stability of standard and sample solutions was determined by monitoring the peak area ratio of these solutions over a period of two days. The results showed that the peak area RSD (%) was lower than 2.0%, and no significant degradation was observed within this period, indicating that the solutions were stable.

Comparison between methods

The performance parameter comparison for CE and HPLC methods is in Table 4. The results obtained for all items analyzed in suitability test by proposed method were better than HPLC method. Both methods had good linearity, in the range of concentrations studied, with acceptable correlation coefficients for analytical purpose. As can be observed, the LOD and LOQ were lower for HPLC than CE method. However, these results were satisfactory for the aim of this work. The RSD (%) values obtained in the precision by HPLC were higher than CE, for tablets and lower for compounded capsules, but for both methods the precision results were acceptable. The means recoveries for both techniques showed good accuracy.

Table 4. Suitability test parameters

Parameter	HPLC[a]	CE[a]
Peak asymmetry (T)	1.5	1.0
Efficiency (N)	4618	62034
Migration time (min)	6.3	3.5

[a]Mean of five replicates.

The results obtained from CE method were compared statistically with the HPLC method by ANOVA, using F-test, and does not reveal significant difference between the experimental values obtained for tablets and compounded capsules by both methods. The calculated F-value for tablets ($F_{calculated}$ = 5.96) and compounded capsules ($F_{calculated}$ = 3.30) were found to be less than the critical F-value ($F_{critical}$ = 6.36) at 1.0% of significance level.

Thus, both techniques provided suitable results for CTZ quantitation and could be used in the quality control of this drug in tablets and compounded capsules.

CONCLUSION

The proposed CE method, developed and validated for determination of CTZ in tablets and compounded capsules, showed a good performance in relation of specificity, linearity, precision, accuracy, and it offers a precise way to be used for assess the quality of this drug in solid dosage forms. The main advantage that this method offer in relation of the others available in the literature is a short analysis time, approximated 3.5 min. Due to these features, this method can be considered very useful for routine laboratory work.

Comparing to HPLC, the developed CE method was less expensive, low solvent and sample consumption. The results of this study demonstrated that CE is an attractive alternative method to HPLC CTZ determination in pharmaceutical solid dosage forms.

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