Journal of Chromatography A, 1192 (2008) 301–305

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Development and validation of a method for quantitative determination of econazole nitrate in cream formulation by capillary zone electrophoresis

Angel Arturo Gaona-Galdos[a], Luiz Antonio Zanolli Filho[b], Marina Franco Maggi Tavares[b], María Segunda Aurora-Prado[a], Maria Inês Rocha Miritello Santoro[a], Érika Rosa Maria Kedor-Hackmann[a,*]

[a] Faculty of Pharmaceutical Sciences, University of São Paulo, Av. Prof. Lineu Prestes 580, 05508-900 São Paulo, SP, Brazil
[b] Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes 748, 05508-900 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 7 January 2008
Received in revised form 18 March 2008
Accepted 25 March 2008
Available online 28 March 2008

Keywords:
Capillary zone electrophoresis
Econazole nitrate
Validation
Antifungals

ABSTRACT

A simple, fast, inexpensive and reliable capillary zone electrophoresis (CZE) method for the determination of econazole nitrate in cream formulations has been developed and validated. Optimum conditions comprised a pH 2.5 phosphate buffer at 20 mmol L^{-1} concentration, +30 kV applied voltage in a 31.5 cm × 50 μm I.D. capillary. Direct UV detection at 200 nm led to an adequate sensitivity without interference from sample excipients. A single extraction step of the cream sample in hydrochloric acid was performed prior to injection. Imidazole (100 μg mL^{-1}) was used as internal standard. Econazole nitrate migrates in approximately 1.2 min. The analytical curve presented a coefficient of correlation of 0.9995. Detection and quantitation limits were 1.85 and 5.62 μg mL^{-1}, respectively. Excellent accuracy and precision were obtained. Recoveries varied from 98.1 to 102.5% and intra- and inter-day precisions, calculated as relative standard deviation (RSD), were better than 2.0%. The proposed CZE method presented advantageous performance characteristics and it can be considered suitable for the quality control of econazole nitrate cream formulations.

1. Introduction

Despite the successes and achievements of modern medicine, competent immunity remains the best hope for effective and long-lasting protection against infection and disease. Inherited and acquired causes, however, render the immune system deficient. The expansion of the immunocompromised patient population, including the rising number of acquired immunodeficiency syndrome (AIDS) patients, organ transplant recipients receiving immunosuppressive therapy, cancer patients with chemotherapy and the increasing amount of invasive devices (catheters, artificial joins and valves), has originated a growing rate of invasive fungal infections [1,2]. The Food and Drug Administration (FDA) has approved several antifungal agents belonging to different chemical classes (polyenes, pyrimidines, azoles, and echinocandins) as therapeutic options for fungal infections. Azole antifungal drugs is perhaps the most rapidly expanding group of antifungals. The inhibition of fungal growth by azoles derivatives was described in the 1940s and the fungicidal properties of N-substituted imidazoles were described in the 1960s. More than 40 β-substituted 1-phenethylimidazole derivatives are known to be potent against fungi, dermatophytes and Gram-positive bacteria.

Imidazoles are five membered ring structures containing two nitrogen atoms with a complex side chain attached to one of the nitrogen atoms. Imidazoles in current clinical use are clotrimazole, miconazole, econazole and ketoconazole. Econazole, miconazole and sulconazole are used in the treatment of superficial dermatophytic and yeast infections [1,3].

Econazole nitrate (1-[2-(4-chlorophenyl) methoxy]-2-(2,4-dichlorophenyl) ethyl)-1*H*-imidazole mononitrate (Fig. 1A), is a potent broad-spectrum antifungal agent applied topically in the treatment of skin infections. In addition, econazole as well as clotrimazole were found to have strong antimycobacterial potential action against latent *Mycobacterium tuberculosis* under "in vitro" conditions. It was found that econazole prevented the formation of drug induced latency and significantly reduced bacterial burden from lungs and spleens of latent tuberculosis infected mice [4]. Recent studies have shown that econazole reduces bacterial burden by 90% in lungs and spleen of mice infected with cells of *M. tuberculosis* [5]. Furthermore, econazole was found to be equipo-

* Corresponding author at: University of Sao Paulo, Department of Pharmacy, Faculty of Pharmaceutical Sciences, Av. Prof. Lineu Prestes 580 B-13, Butanta, 05508-900 Sao Paulo, SP, Brazil. Tel.: +55 11 3091 3655; fax: +55 11 3091 3648.
E-mail address: ermkedor@usp.br (É.R.M. Kedor-Hackmann).

0021-9673/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2008.03.070

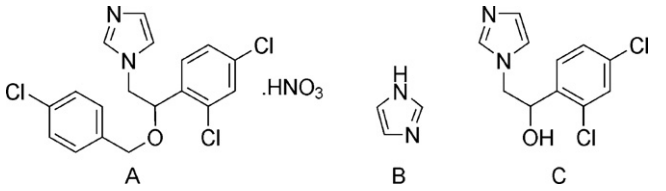

Fig. 1. Chemical structures of econazole nitrate (A), imidazole (B) and alpha-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol (C).

tent to rifampicin and can replace both rifampicin and isoniazid in chemotherapy of murine tuberculosis. Econazole alone or in combination with antitubercular drugs did not produce any hepatotoxicity in normal or *M. tuberculosis*-infected mice [5].

Reported methods for the determination of econazole nitrate in pharmaceutical formulations include titrimetry [6], spectrophotometry [7,8], derivative spectrophotometry [9,10], high performance liquid chromatography (HPLC) [11–16], gas chromatography [17], high performance thin-layer chromatography [18] and capillary electrophoresis (CE) [19]. A variety of enantiomeric separations using HPLC and CE methods were also published [20–29]. Quantification of econazole nitrate in biological samples have been developed using near infrared spectrometry [30,31] and liquid chromatography [32]. Capillary electrophoresis is increasingly regarded as an attractive alternative method for drug analysis because of its high separation efficiency, short analysis time, the relatively low operating and consumable costs, ease of automation and the requirement of small volumes of analytes and low solvent consumption compared with those necessary for HPLC [33]. Nevertheless, in the literature, only a single CE method was described [19], in which the determination of econazole, clotrimazole and ketoconazole in pharmaceutical formulations (pessaries) using pH 5.18 acetic acid–Tris buffer at 75 mmol L^{-1} concentration in (20:80, v/v) methanol–water was accomplished. However, for the determination of econazole nitrate in cream dosage forms, no CE methods were found. Hence, the present work describes a novel, simple, fast, and low cost effective capillary zone electrophoresis (CZE) method for determination of econazole nitrate in creams. Under the optimized conditions, specificity, linearity, detection (DL) and quantitation (QL) limits, precision, accuracy, robustness and assay were validated.

2. Experimental

2.1. Instrumentation

CE experiments were conducted in a capillary electrophoresis system (Agilent Technologies, model HP 3D CE, Palo Alto, CA, USA), equipped with a diode array and a temperature control device. Data acquisition and treatment software was supplied by the manufacturer (HP ChemStation, rev A.06.01). The capillary was thermostated at 25 °C. An uncoated fused-silica capillary of 50 μm I.D. (Polymicro Technologies, Phoenix, AZ, USA) with a total length of 31.5 cm (23 cm effective length) was used for the separation. Samples were kept in the auto sampler and injected hydrodynamically at the anodic side by pressure of 50 mbar for 5 s. A constant voltage of +30 kV was applied throughout analysis. Detections were performed at 200 nm. For the robustness experiments an additional CE system (model P/ACE MDQ, Beckman Coulter Instruments, Fullerton, CA, USA), equipped with a variable UV–vis, software for data acquisition and treatment (Beckmann P/ACE System Gold Software) was used.

New capillaries were conditioned by rinsing with 0.1 mol L^{-1} sodium hydroxide (NaOH) for 20 min, water for 10 min and finally buffer solution for 20 min. At the beginning of each working day, the capillary was rinsed with 0.1 mol L^{-1} NaOH for 10 min, then with deionized water for 10 min and finally with running buffer for 10 min. In between injections, the capillary was rinsed with buffer solution for 2 min. The electrolyte was a 20 mmol L^{-1} phosphate buffer adjusted to pH 2.5 with *o*-phosphoric acid.

Table 1
Composition of simulated sample containing econazole nitrate

Ingredient	Amount (g)
Econazole nitrate	1.0
Cetilic alcohol	1.5
Glycerin monostearate	8.0
Sorbitan monostearate	2.0
Glycerine	10.0
Isopropyl palmitate	3.0
Emulsifying Wax NF	8.0
Methylparaben	0.15
Propylparaben	0.05
Imidazolidinyl urea	0.20
Water q.s.p.	100.0

2.2. Chemicals

All reagents were of analytical grade and no further purification was required. Methanol (HPLC grade), hydrochloric acid and *o*-phosphoric acid were obtained from Merck (Darmstadt, Germany). Sodium dihydrogenphosphate dihydrate was purchased from J. T. Baker (Phillisburg, NJ, USA). Water was deionized and purified on a Milli-Q® water purification system (Millipore, Bedford, MA, USA) and used to prepare all solutions.

2.3. Standards

Econazole nitrate (100.55% purity) was kindly donated by Formil Química Ltda. (São Paulo, Brazil). Imidazole (99% purity), used as internal standard (IS), was obtained from Merck (Darmstadt, Germany) (Fig. 1B) and alpha-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol (99% purity) (econazole nitrate impurity) was obtained from Acrõs Organics (Geel, Belgium) (Fig. 1C).

2.4. Samples

Samples 1 and 2: Two available batches cream samples were supplied by Laboratory Stiefel Ltda. (São Paulo, Brazil) containing 1% of econazole nitrate and excipients in sufficient quantity for a tube of 45 g capacity. *Sample 3*: Simulated cream formulation containing 1% of econazole nitrate and excipients in sufficient quantity for 100 g of cream (Table 1).

2.5. Preparation of standard solution

Standard stock solutions of econazole nitrate (200 μg mL^{-1}) and imidazole (1000 μg mL^{-1}) were prepared in 37 mmol L^{-1} HCl. The solutions were sonicated for 10 min to aid dissolution and kept under refrigeration. Working standard solutions were prepared fresh daily by appropriately diluting the stock solutions with the same solvent.

2.6. Preparation of sample solution

A portion equivalent to about 0.25 g of cream was accurately weighed into a 25 mL beaker and extracted with 10 mL of a 37 mmol L^{-1} HCl solution in a water-bath at 60 °C for 5 min. After this period, the solution was quantitatively transferred to a

25 mL volumetric flask. A 2.5 mL aliquot of the imidazole solution (1000 μg mL^{-1}, stock solution) was transferred to the volumetric flask. Then the sample solution was homogenized, cooled to ambient temperature and the volume adjusted to 25 mL with the diluted HCl. The sample solution was then filtered using blue strip filter paper (Schleicher & Schull, Germany) and analyzed by CZE. Final concentrations of econazole nitrate and imidazole solutions were 100 μg mL^{-1}.

2.7. Method validation

The method was validated according to the United States Pharmacopeia requirements [34]. The following validation characteristics were addressed: linearity, detection limit, quantitation limit, precision, accuracy, robustness and specificity.

2.7.1. Linearity

Appropriate aliquots of econazole nitrate and imidazole (IS) standard stock solutions were transferred separately into 10 mL volumetric flasks and diluted to volume with 37 mmol L^{-1} HCl. Concentration ranges from 80 to 120 μg mL^{-1} of econazole nitrate and 100 μg mL^{-1} of imidazole were obtained. The solutions were sonicated for 10 min and filtered using a 0.22 μm filter (Millipore) and injected on the CE instrument. Each solution was injected in triplicate. Peak area ratios, PAR (econazole nitrate/imidazole) were plotted versus the respective concentrations of econazole nitrate.

2.7.2. Detection and quantitation limits

Detection (DL) and quantitation limits (QL) were calculated from the residual standard deviation of the regression line (σ) of the analytical curve and its slope (S) in accordance with the equations DL = 3.3 (σ/S) and QL = 10 (σ/S) [35].

2.7.3. Precision

2.7.3.1. Repeatability (intra-day). Sample solutions were prepared at 100 μg mL^{-1} of econazole nitrate and 100 μg mL^{-1} of imidazole (IS). Ten determinations were performed to establish the intra-day precision.

2.7.3.2. Intermediate precision (inter-day). The method inter-day precision was evaluated by injecting sample solutions prepared at lower, middle and higher concentrations of the analytical curve (80–120 μg mL^{-1} econazole nitrate) containing 100 μg mL^{-1} of imidazole (IS), in three consecutive days. Three determinations for each concentration were performed.

Precision was expressed as the percentage relative standard deviation (%RSD) for PAR of econazole nitrate/IS.

2.7.4. Accuracy

To determine the method accuracy, recovery experiments were performed according standard addition procedures [36]. Accuracy was calculated as the percentage recovery of a known amount of standard added to the sample. The accuracy of method was evaluated in triplicate using three concentration levels 80, 100 and 120 μg mL^{-1}. Econazole nitrate standard solution was added to commercial sample solution and analyzed by the proposed method.

2.7.5. Robustness

The robustness of the developed method was established by comparison of the quantitative results of two commercial samples (1 and 2), by two different CE instruments. Significance tests, *t*-test and *F*-test [37,38], were performed to compare the accuracy and precision of the results, respectively.

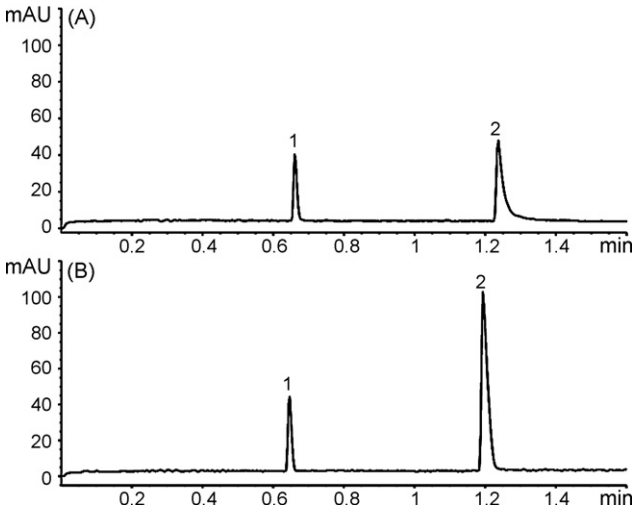

Fig. 2. Electropherograms of standard solutions of econazole nitrate (EN) in pH 2.5 phosphate buffer at 20 mmol L^{-1} concentration. (A) EN dissolved in methanol, (B) EN dissolved in 37 mmol L^{-1} HCl. Conditions: fused-silica capillary, 31.5 cm (23 cm to detector) × 50 μm I.D.; hydrodynamic injection: 50 mbar for 5 s; applied voltage: +30 kV; detection at 200 nm; room temperature. Peaks: (1) imidazole (IS) and (2) econazole nitrate.

2.7.6. Specificity

The specificity of the method was tested by analyzing placebo and for spiking the commercially sample with appropriate levels of impurities, according USP 31 for assay [34]. A mixture of the inactive ingredients (placebo), the commercial samples of econazole nitrate before and after being spiked with a known impurities of econazole nitrate alpha-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol were analyzed by the proposed methodology.

3. Results and discussion

3.1. Method development

Econazole nitrate exhibits a pK_a value of 6.6 [39]. Consequently, at pH <5.6 econazole is increasingly protonated, making it suitable for capillary zone electrophoresis analysis. Therefore, a proper electrolyte system for econazole nitrate determination would be a low pH phosphate buffer (p$K_{a1} \cong 2.5$). Moreover, econazole nitrate is soluble in methanol but slightly soluble in water [34], however, the protonation of the imidazole nitrogen in acidic medium enhances its solubility.

Fig. 2 depicts electropherograms of econazole nitrate standard dissolved in methanol (Fig. 2A) and 37 mmol L^{-1} HCl (Fig. 2B) in 20 mmol L^{-1} phosphate buffer adjusted to pH 2.5 with *o*-phosphoric acid. As observed, the analysis is fast (less than 1.4 min), but when the sample is dissolved in methanol, the econazole peak is tailing (conductance inside of solute zone is smaller than that in the carrier electrolyte when sample is dissolved in methanol causing electrodispersion and thus tailing) and peak area is smaller, whereas better sensitivity and peak shape are obtained for acidic sample dissolution.

The influence of the applied voltage was also evaluated. With a voltage of 30 kV, the analysis time was shorter and the current was not too excessive, so this voltage was selected as the optimum running voltage.

The detection wavelength was selected at 200 nm, because at this wavelength econazole nitrate and imidazole (IS) present their maximum absorption, so that the sensitivity of the method would be enhanced.

Table 2
Linearity, detection and quantitation limits for the proposed CZE method for econazole nitrate

Parameter	Statistical data
Concentration range[a] ($\mu g\,mL^{-1}$)	80.0–120.0
Intercept	0.0688
Slope (S)	0.0470
Coefficient of correlation (r)	0.9995
Residual SD of the regression line (σ)	0.0264
Detection limit ($\mu g\,mL^{-1}$)	1.85
Quantitation limit ($\mu g\,mL^{-1}$)	5.62

[a] Five data points, triplicate injection at each concentration level.

Thus, Fig. 2B shows the electropherogram of standard solutions of econazole nitrate and imidazole (IS) under optimized chemical and instrumental conditions.

Arranz et al. [19] have used the acetic acid–Tris buffer at pH 5.18, as the electrolyte, and the econazole nitrate was separated from the mixture of standards (ketoconazole, clotrimazole) in approximately 4.5 min. The migration time of econazole nitrate is three times higher when compared with the presented capillary zone electrophoresis method. In the method described by Di Pietra et al. [14], the HPLC separation of triamcinolone acetonide and econazole was obtained in 11 min.

3.2. Method validation

Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications [34].

3.2.1. Linearity, DL and QL

The linearity of an analytical procedure is its ability within a definite range to obtain results directly proportional to the concentrations (amount) of the analyte in the sample [40].

An analytical curve was obtained using five data points and triplicate injection of standards at each concentration level. The results, summarized in Table 2 showed an excellent linearity ($r > 0.9995$) between peak area ratios (econazole nitrate/IS) and econazole nitrate concentration over the concentration range of 80–120 $\mu g\,mL^{-1}$. DL and QL were 1.85 and 5.62 $\mu g\,mL^{-1}$, respectively.

3.2.2. Precision

The precision of an analytical method gives information on the random error. It expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under prescribed conditions [41–43]. The intra-day precision calculated as %RSD for 10 sample (solutions of econazole nitrate, 100 $\mu g\,mL^{-1}$ and imidazol (IS) 100 $\mu g\,mL^{-1}$) was 1.7%. The inter-day precision of the proposed analytical method calculated for lower, middle and higher concentrations (80, 100 and 120 $\mu g\,mL^{-1}$ econazole nitrate and 100 $\mu g\,mL^{-1}$ imidazol (IS)) were 1.9, 1.4 and 0.6, respectively. Data indicated a good agreement among the individual test results. The criterion for intra-day and inter-day precisions demands a RSD better than 2.0% [44].

The instrumental precision of the mixture (econazole nitrate 100 $\mu g\,mL^{-1}$ and imidazol (IS) 100 $\mu g\,mL^{-1}$) were performed to demonstrate the repeatability for migration time of the system and PAR. The repeatability calculated as %RSD for migration time of 25 runs was 0.61 for imidazole and 0.91 for econazole nitrate and a repeatability for peak area ratio was 0.89. The precision of injection was therefore considered satisfactory [44].

The repeatability of the complete validation, calculated as %RSD, for migration time (approximately 200 runs) was 1.46 for imidazole and 2.42 for econazol nitrate.

Table 3
Accuracy (recovery test) of the proposed CZE method for econazole nitrate quantitative determination

Commercial sample ($\mu g\,mL^{-1}$)	Standard added to commercial sample ($\mu g\,mL^{-1}$)	Standard found ($\mu g\,mL^{-1}$)	Recovery (%)[a]
40.0	40.4	39.64	98.14
50.0	50.5	49.60	98.22
60.0	60.6	62.11	102.48

[a] Average of three determinations.

3.2.3. Accuracy—recovery test

Accuracy is calculated as the percentage of recovery [34]. Table 3 illustrates the method accuracy for econazole nitrate with recoveries from 98.14 to 102.50% (commercial samples). Mean recoveries should be $100 \pm 2.5\%$ at each concentration over the range of 80–120% of the target concentration [44].

3.2.4. Robustness

The comparison of the accuracy and precision of the results generated by the two CE equipments are shown in Table 4. Means and variances were not found to be significantly different at a confidence level of 95%.

3.2.5. Specificity

The specificity of the method was demonstrated by the absence of interference among econazole nitrate, imidazole, alpha-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol (impurity) and excipients in the samples, criterion defined in the USP 31 for assays [34]. A mixture of the inactive ingredients (placebo), (Fig. 3C), the commercial sample 1 of econazole nitrate, (Fig. 3B), and the commercial sample 1 spiked with standard of a known impurity of econazole nitrate (alpha-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol) [45,46], (Fig. 3A), were analyzed by the proposed methodology. As it can be observed, neither the cream excipients nor the impurity

Table 4
Results obtained in the comparison of the accuracy and precision of econazole nitrate determination, using the two CE equipments[a]

Samples	Results obtained				Comparison between HP[3D]CE and P/ACE MDQ	
	HP[3D]CE		P/ACE MDQ		Accuracy, calculated t-value[b]	Precision, calculated F-value[c]
	Mean value (%)[d]	SD	Mean value (%)[d]	SD		
1	101.0	2.0	101.2	2.2	0.19	1.22
2	98.6	1.7	98.5	1.4	0.15	1.42

HP[3D]CE: capillary electrophoresis system from Agilent Technologies P/ACE MDQ: capillary electrophoresis system from Beckman Coulter Inc.

[a] Method robustness.

[b] Tabulated Student's t-value with $P = 95\%$ and 16 d.f.; $t = 2.1199$ [45].

[c] Tabulated Snedecor F-value with $P = 95\%$; $F9/9 = 4.433$ [45].

[d] Average of nine determinations.

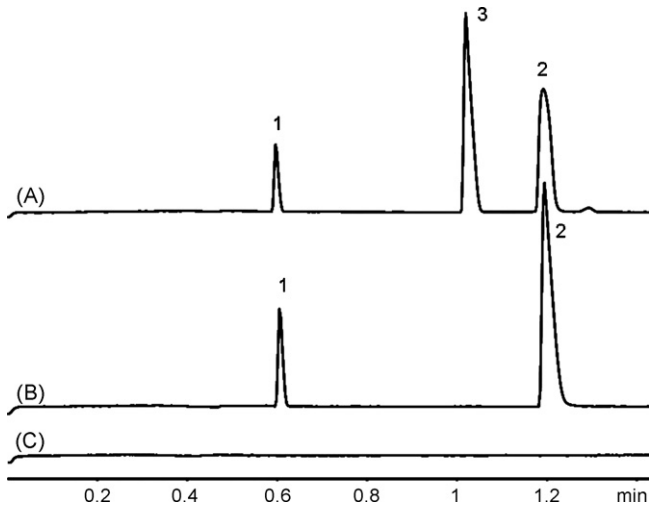

Fig. 3. Electropherograms of placebo (C), commercial sample of econazole nitrate (B) and commercial sample spiked with a known impurity (A). Conditions as in Fig. 2B. Peaks: (1) imidazole (IS), (2) econazole nitrate (EN) and (3): alpha-(2,4-dichlorophenyl)-1*H*-imidazole-1-ethanol.

Table 5
Results obtained in the analysis of commercial sample containing econazole nitrate using the proposed CZE method

Results	Sample (econazole nitrate)
Amount declared (mg)	2.50
Amount found[a] (mg)	2.56
Percentage found (%)	102.64
Response factor RSD (%)	1.92

[a] Average of three determinations.

interfere in the analysis of econazole nitrate, establishing therefore the method specificity.

3.2.6. Assay

Sample of econazole nitrate was assayed against a reference standard solution of econazole nitrate. Both solutions were prepared at 100 μg mL^{-1} of econazole nitrate. The results in Table 5 demonstrate that the CZE method was suitable for determination of econazole nitrate. The assay was found to be satisfactory, the sample analyzed was within tolerance limits (90–110% label claim) and the RSD of the response factor was 1.92%.

4. Conclusions

A CZE method for econazole nitrate has been developed and validated with respect to specificity, injection precision, linearity, detection and quantitation limits, precision, accuracy and robustness, presenting adequate performance characteristics. The proposed method can be considered as an alternative to HPLC methods and can be used for routine analysis of econazole nitrate in pharmaceutical preparations.

The advantages of the method in comparison with other methodologies described in the literature, is a short time analysis and the simple sample extraction of the drug with hydrochloric acid for a complex formulation as creams, besides the known benefits of capillary electrophoresis, such as use of small amount of solvents, leading to a non-polluting method.

Acknowledgements

The authors thank the “Fundação de Amparo à Pesquisa do Estado de São Paulo” of Brazil (FAPESP 04/08503-2) and the “Conselho Nacional de Desenvolvimento Cientifico e Tecnológico of Brazil” (CNPq, 130652/2007-5) for financial support and fellowship.

References

[1] K.H. Abu-Elteen, M. Hamad, in: K. Kavanagh (Ed.), Fungi Biology and Applications, John Wiley & Sons Ltd., England, 2005, p. 191.
[2] P.K. Mukherjee, D.J. Sheehan, C.A. Hitchcock, M.A. Ghannoum, Clin. Microbiol. Rev. 18 (2005) 163.
[3] J. Loeffler, D.A. Stevens, Clin. Infect. Dis. 36 (2003) S31.
[4] A. Zahoor, S. Sharma, G.K. Khuller, FEMS Microbiol. Lett. 258 (2006) 200.
[5] A. Zahoor, S. Sharma, G.K. Khuller, FEMS Microbiol. Lett. 261 (2006) 181.
[6] M. Massaccesi, Analyst 111 (1986) 987.
[7] S.R. El-Shabouri, K.M. Emara, P.Y. Khashaba, A.M. Mohamed, Anal. Lett. 31 (1998) 1367.
[8] P.Y. Khashaba, S.R. El-Shabouri, K.M. Emara, A.M. Mohamed, J. Pharm. Biomed. Anal. 22 (2000) 363.
[9] D. Bonazzi, V. Cavrini, R. Gatti, E. Boselli, M. Caboni, J. Pharm. Biomed. Anal. 18 (1998) 235.
[10] V. Cavrini, A.M. Di Pietra, R. Gatti, J. Pharm. Biomed. Anal. 7 (1989) 1535.
[11] X.-L. Yang, Z.-F. Xi, J.-F. Sheng, Chin. J. Antib. 29 (2004) 403.
[12] L. Gagliardi, D. De Orsi, P. Chimenti, R. Porra', D. Tonelli, Anal. Sci. 19 (2003) 1195.
[13] E. Kublin, T. Kaniewska, J. Pharm. Belg. 53 (1998) 208.
[14] A.M. Di Pietra, V. Andrisano, R. Gotti, V. Cavrini, J. Pharm. Biomed. Anal. 14 (1996) 1191.
[15] R. Christinat, H.W. Zulliger, Arzneim. Forsch. 34 (1984) 551.
[16] A.M. Di Pietra, V. Cavrini, V. Andrisano, R. Gatti, J. Pharm. Biomed. Anal. 10 (1992) 873.
[17] E. Kublin, T. Kaniewska, Chem. Anal. (Warsaw, Pol.) 41 (1996) 19.
[18] G. Popovic, M. Cakar, K. Vucicevic, S. Vladimirov, D. Agbaba, J. Planar Chromatogr.-Mod. TLC 17 (2004) 109.
[19] A. Arranz, C. Echevarria, J.M. Moreda, A. Cid, J.F. Arranz, J. Chromatogr. A 871 (2000) 399.
[20] L. Toribio, M.J. Del Nozal, J.L. Bernal, C. Alonso, J.J. Jimenez, J. Chromatogr. A 1144 (2007) 255.
[21] X. Lin, C. Zhu, C. Hao, Electrophoresis 26 (2005) 3890.
[22] I. Ali, H.Y. Aboul-Enein, Biomed. Chromatogr. 17 (2003) 113.
[23] H.Y. Aboul-Enein, I. Ali, J. Pharm. Biomed. Anal. 27 (2002) 441.
[24] H.Y. Aboul-Enein, I. Ali, Anal. Bioanal. Chem. 370 (2001) 951.
[25] H.Y. Aboul-Enein, I. Ali, Chromatographia 54 (2001) 200.
[26] A. Van Eeckhaut, S. Boonkerd, M.R. Detaevernier, Y. Michotte, J. Chromatogr. A 903 (2000) 245.
[27] J.Y. Liu, Y.Y. Dong, T.S. Wang, H.W. Liu, A.J. Huang, Y.L. Sun, Z.P. Sun, Chin. Chem. Lett. 10 (1999) 39.
[28] E. Peyrin, Y.C. Guillaume, Chromatographia 49 (1999) 691.
[29] N. Morin, Y.C. Guillaume, J.C. Rouland, Chromatographia 48 (1998) 388.
[30] J.P. Medendorp, K.S. Paudel, R.A. Lodder, A.L. Stinchcomb, Pharm. Res. 24 (2007) 186.
[31] J. Medendorp, J. Yedluri, D.C. Hammell, T. Ji, R.A. Lodder, A.L. Stinchcomb, Pharm. Res. 23 (2006) 835.
[32] P. Chiap, Ph. Hubert, J. Crommen, J. Chromatogr. A 948 (2002) 151.
[33] K.D. Altria, Capillary Electrophoresis Guidebook: Principles, Operation, and Applications, Humana Press, Totowa, 1996, p. 1 [Methods in Molecular Biology Series, 52].
[34] United States Pharmacopeia, 31st ed., The United States Pharmacopeial Convention 12601, Rockville, 2008.
[35] E. McEvoy, S. Donegan, J. Power, K. Altria, J. Pharm. Biomed. Anal. 44 (2007) 137.
[36] F.M. Garfield, Quality Assurance Principles for Analytical Laboratories, 2nd ed., AOAC International, Arlington, 1991, p.75.
[37] M.S. Aurora-Prado, M. Steppe, E.R.M. Kedor-Hackmann, M.I.R.M. Santoro, Rev. Bras. Farm. 82 (2001) 06.
[38] J.E. De Muth, Basic Statistics and Pharmaceutical Statistical Applications, 2nd ed., Chapman & Hall/CRC, 2006, p. 682.
[39] J.P. Remington, The Science and Practice of Pharmacy, 20th ed., Lippincott William &Wilkins, Philadelphia, 2000, p.1554.
[40] International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva,2005.
[41] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, J. Pharm. Biomed. Anal. 36 (2004)579.
[42] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, E. Rozet, J. Pharm. Biomed. Anal. 45 (2007)70.
[43] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Laurentie, N. Mercier, G. Muzard, L. Valat, E. Rozet, J. Pharm. Biomed. Anal. 45 (2007)82.
[44] K.D. Altria, D.R. Rudd, Chromatographia 41 (1995) 325.
[45] British Pharmacopoeia London: The Stationery Office, 1999, pp. 560, 1806.
[46] European Pharmacopoeia, 5th ed., Council of Europe, Strasbourg, 2004, p. 1493.