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Research Article

Determination of ciclopirox olamine in pharmaceutical products by capillary electrophoresis with capacitively coupled contactless conductivity detection

This paper describes the determination of ciclopirox olamine in pharmaceutical formulations using capillary electrophoresis with capacitively coupled contactless conductivity detection. In an alkaline medium, ciclopirox olamine is converted into an anionic species and its detection is possible in capillary electrophoresis with capacitively coupled contactless conductivity detection without an electroosmotic flow modifier, because it is a low-mobility species. A linear working range from 2.64 to 264 μg/mL in sodium hydroxide electrolyte as well as low detection limit (0.39 μg/mL) and a good repeatability (RSD = 3.4% for 264 μg/mL ciclopirox solution ($n = 10$)) were achieved. It was also possible to determine olamine in its cationic form when acetic acid was used as the electrolyte solution. The results obtained include a linear range from 26.4 to 184.8 μg/mL and a detection limit of 2.6 μg/mL olamine. The proposed methods were applied to the analysis of commercial pharmaceutical products and the results were compared with the values indicated by the manufacturer as well as those obtained using a titrimetric method recommended by American Pharmacopoeia.

Keywords:
Antimycotic drugs / Capillary zone electrophoresis / Ciclopirox olamine / Contactless conductivity detection / Pharmaceutical products
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1 Introduction

Ciclopirox olamine belongs to the group of antimycotic drugs used for the treatment of superficial fungal infections and seborrhoeic dermatitis. The active ingredient is the ciclopirox (6-cyclohexyl-1-hydroxy-4-methyl-2(1H)-pyridone) associated with olamine, which is another name for ethanolamine. It differs structurally from other available antifungal compounds and has a unique and complex mode of action, which mainly affects iron-dependent enzymatic systems [1], by actuation on cytoplasmatic membranes or via damage to the cell membranes, producing disorganisation of internal structure [2]. Ciclopirox has an excellent activity against most pathogenic fungi, to non-pathogenic fungi, and on seborrhoeic dermatitis. It is available in many forms, among them, cream, lotion, gel, spray, powder, ophthalmic solution, topical suspension, shampoo, and nail lacquer [3, 4]. The structural representation of ciclopirox olamine is presented in Fig. 1.

Some methods have been described in the literature for the quantification of ciclopirox olamine in topical solutions and in raw materials, among them high-performance liquid chromatography [5–7], gas chromatography [8], MEKC [9], and polarography [10]. Some of these reported methods require time-consuming sample preparation or expensive instrumentation. Studies to evaluate the ciclopirox penetration across human nails [11], bovine [12] and porcine hooves [13, 14] were also reported.

According to several authors [6, 7, 11], the use of silica-based material during chromatographic analysis leads to strong interaction with the analyte (*N*-hydroxy-pyridone group), resulting in the formation of tailing peaks and non-linear calibration curves.

In an alkaline medium (pH = 12), ciclopirox olamine is converted into an anionic species and its detection in the cathodic side is possible due to the strong electroosmotic flow (EOF), because ciclopirox has low mobility. Moreover, conductivity detection also allows the determination of ethanolamine in its cationic form at low pH. The possibility of performing the analysis using two different ways is advantageous, because it minimises significantly the chances of obtaining results affected by interfering species. In a previous study, we have described similar procedure in a study invol-

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Abbreviation: CE-C^{4}D, capillary electrophoresis with capacitively coupled contactless conductivity detection

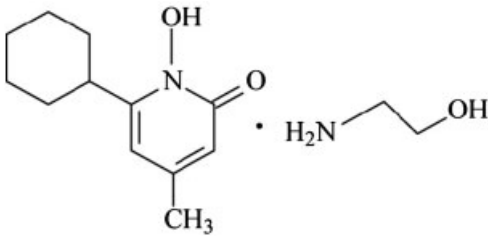

Figure 1. Structural formula of ciclopirox olamine.

ving the determination of salbutamol in syrups [15]. There, the cationic form of salbutamol was quantified and its indirect quantification via its counter ion (sulphate) was demonstrated.

In this paper, a new way to determine ciclopirox olamine in pharmaceutical formulations by using capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) is presented. C^4D is an attractive alternative form of detection, mainly due to its ease of implementation and operation. The good performance of this technique has been demonstrated previously for many applications in different matrices [16–19]. The advantages of this way of analysis are discussed in the next sections.

2 Materials and methods

2.1 Reagents and solutions

Standard ciclopirox olamine salt was kindly donated by Arventis Manipulation Pharmacy (São Paulo, Brazil) and utilised without further purification. Other reagents were of analytical grade. Sodium hydroxide, hydrochloric acid, ethanol, acetic acid, 2-amino-2-hydroxymethyl-propane-1,3-diol (Tris), and *N*-cetyl-*N*,*N*-trimethylammonium bromide (CTAB) were obtained from Merck (Darmstadt, Germany). Ciclopirox olamine should be stored away from light and at low temperatures [3]. All solutions were made with ultra pure water from a Millipore Milli-Q system (resistivity $\geq$18 MΩ.cm). The pharmaceutical products analysed in this study were purchased in a local drugstore.

2.2 Preparation of standard solutions

A standard stock solution (2.64 mg/mL) of ciclopirox olamine was prepared in ultra pure water with 2.0% ethanol. Just before the measurements, the ciclopirox olamine solutions (2.64–264 and 26.4–184.8 μg/mL) were conveniently diluted in the background electrolyte used for the analysis of ciclopirox (10 mmol/L sodium hydroxide) and ethanolamine (2.0 mol/L acetic acid).

2.3 Sample preparation

Ciclopirox olamine solution: an aliquot of 250 μL of ciclopirox olamine sample was transferred to a 5 mL volumetric flask and 100 μL of ethanol was added. The content of the flask was conveniently diluted with background electrolyte to obtain the final concentration of 100 μg/mL.

Ciclopirox cream: a portion of the cream, equivalent to 0.5 mg of ciclopirox olamine, was transferred to a 5 mL volumetric flask and 100 μL of ethanol was added. The content of the flask was sonicated for 15 min and centrifuged (7000 rpm for 10 min). The supernatant solution was diluted with background electrolyte to final concentrations of 13.4 and 55 μg/mL for the determination of ciclopirox and ethanolamine, respectively.

2.4 CE

The CE equipment interfaced to a microcomputer was built in our laboratory and details of the construction and the detector developed have been reported elsewhere [20, 21]. The detector was operated at 600 kHz and 2 Vpp [21] and the fused-silica capillary (50 μm inner diameter, 375 μm outer diameter, 56 cm long and effective length of 46 cm) was purchased from Agilent Technologies (São Paulo, Brazil). The samples were injected into the capillary from the anodic reservoir by hydrodynamic injection (9.8 mBar for 30 s). The separation potentials of 15 and 25 kV were applied during the analysis of ciclopirox (anionic form) and ethanolamine (cationic form), respectively.

2.5 Potentiometric titration

The potentiometric titration of the pharmaceutical formulation samples was done following the procedure described in the American Pharmacopoeia [3] with an alteration; ethanol was used instead of methanol due its toxicity. A known amount of ciclopirox olamine was dissolved in 2 mL of ethanol and the final volume was completed with ultra pure water. The mixture was then titrated with a standardised (0.1 mol/L) sodium hydroxide solution. The titrant volume referring to the equivalence point was used to calculate the concentration of ciclopirox olamine.

3 Results and discussion

3.1 Optimisation of the CE conditions for ciclopirox analysis

Different electrophoretic conditions were tried out for the detection of ciclopirox as an anion. A common practice for the analysis of anions is the addition of a cationic surfactant to invert the direction of the EOF. However, the use of 0.2 mmol/L CTAB in background electrolyte did not allow the determination of ciclopirox (Fig. 2). Most likely, this was due to the interaction of the anionic analyte with the cationic CTA^+ to form an ion-pair [22]. Thus, the EOF modifier was removed and a counter-flow approach was adopted. The first

experiments were performed in 20 mmol/L Tris/10 mmol/L HCl solution pH 8.1, which is a favourable condition for the deprotonation of the *N*-hydroxy-pyridone group. It was possible to verify a peak after the EOF around 4.0 min (Fig. 2). However, for ten repetitive measurements of a solution containing 264 μg/mL of ciclopirox olamine, the SD of the peak area was 11.4% and different migration times were also observed. These results were probably due to interactions (mainly adsorptive processes) of the cationic form of ethanolamine on the negative inner capillary wall. Thus, the pH was raised in order to deprotonate ethanolammonium and, consequently, to reduce its adsorption on the wall.

The best condition for ciclopirox detection was achieved by using 10 mmol/L NaOH solution without CTAB (Fig. 2). In this electrolyte, the signal of ciclopirox was higher and the migration time did not increase significantly (about 4.5 min). Since the NaOH solution provided a short analysis time and a good S/N, this solution was selected as the background electrolyte for all experiments involving the ciclopirox determination.

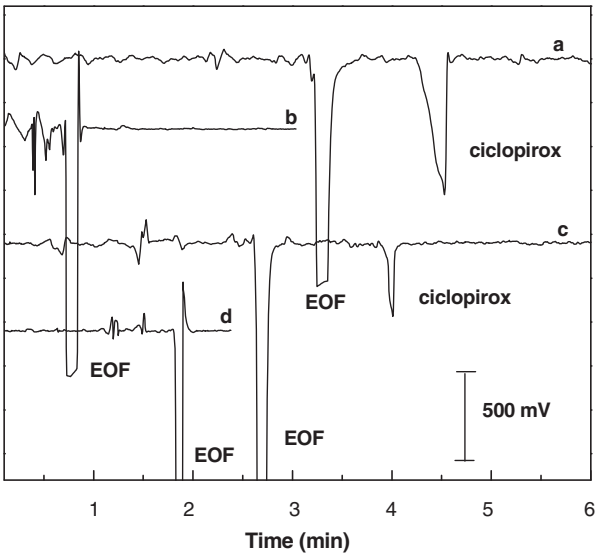

Figure 2. Electropherograms obtained for 264 μg/mL ciclopirox by using different solutions: (a) 10 mmol/L NaOH; (b) 10 mmol/L NaOH +0.2 mmol/L CTAB; (c) 20 mmol/L Tris/10 mmol/L HCl pH 8.1; (d) 20 mmol/L Tris/10 mmol/L HCl pH 8.1+0.2 mmol/L CTAB. Separation voltage: 15 kV (10 mmol/L NaOH) and 28 kV (20 mmol/L Tris/10 mmol/L HCl).

In order to obtain the best conditions in the CE-C^4D experiments, it was important to study the effect of NaOH concentration, the applied potential and the injection time. The effect of the concentration was verified by using NaOH solutions ranging from 10 to 30 mmol/L. The results showed that the higher the NaOH concentration, the smaller the S/N and the broader the peak. This behaviour is clearly related to Joule heating inside the capillary. Therefore, the NaOH concentration was kept at 10 mmol/L.

The impact of changing the applied potential up to 25 kV with the 10 mmol/L NaOH solution was studied. At a first glance, the higher the potential, the higher the efficiency. However, there is an increase in the Joule heating, which impairs the efficiency. An applied potential of 15 kV yielded the best compromise in terms of the SNR and analysis time.

The width and height of the ciclopirox peak was affected by variation of the injection time (5–30 s). Figure 3A shows that the peak area increased with the increasing injection time and a good linear relationship was observed in this range. Therefore, 30 s was selected as the optimum injection time. In short, the final conditions chosen for the quantification of ciclopirox were 10 mmol/L NaOH solution as background electrolyte, a separation voltage of 15 kV and sample injection time fixed at 30 s. Figure 3B presents a repeatability study for ten consecutive injections of 264 μg/mL ciclopirox (RSD = 3.4%) using the selected conditions.

3.2 Calibration plots and determination of ciclopirox olamine in pharmaceutical formulations

Under the optimised conditions, a series of experiments was carried out in triplicate using standard solutions of ciclopirox in different concentrations (2.64–264 μg/mL) to construct the analytical curve, presented in Fig. 4. The inset

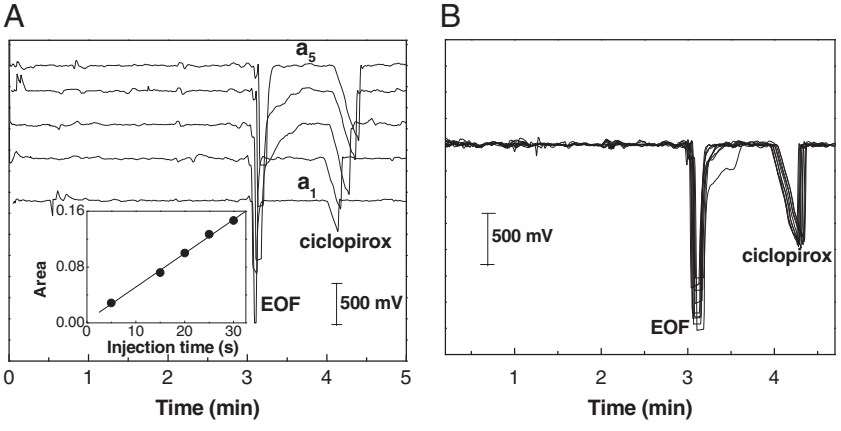

Figure 3. Electropherograms obtained for ciclopirox by using 10 mmol/L NaOH of electrolyte solution: (A) in different injection times: a_1 – 5, a_2 – 15, a_3 – 20, a_4 – 25, and a_5 – 30 s. (B) repeatability study for ten consecutive injections of analyte, injections of 30 s. Concentration of ciclopirox: 264 μg/mL. Separation voltage: 15 kV.

of this figure shows a linear relationship between the total area under the peaks and the analyte concentration as well as the linear regression of these points, which generates a straight line ($A = 8.02 \times 10^{-4} + 5.23 \times 10^{-4}\ C$; $r = 0.999$, where A is the area in V × min and C is the concentration of ciclopirox in μg/mL). The detection limit (S/N = 3) was estimated as 0.39 μg/mL and quantification limit was calculated as 1.30 μg/mL. This LOD value is lower than that one obtained by Li et al. [9] using MEKC with UV detection (LOD = 31.3 μg/mL).

To estimate the effect of the concomitant species present in the commercial samples, nominally lactic acid, propylene glycol, ethylene glycol, propylparaben, methylparaben, and glycerin were added to a solution containing 264 μg/mL ciclopirox, in 10 mmol/L NaOH. Among these concomitant species, only propylparaben caused significant interference on the signal.

According to Soni et al. [23], the maximum content of parabens in commercial samples is 0.8% w/w. Taking into account that ciclopirox is about 10 mg/mL in such products, we spiked the standard 264 μg/mL ciclopirox solution with 106 μg/mL of each paraben to evaluate the interference on the ciclopirox determination. A decrease of 4% in the peak area was observed for propylparaben. In similar conditions, methylparaben caused an interference of less than 1%. Other compounds (propylene glycol, ethylene glycol, and glycerin) did not affect the peak shape of ciclopirox.

Figure 5 presents electropherograms corresponding to standard solutions of ciclopirox, ranging from 7.9 to 184.8 μg/mL (a–g), and pharmaceutical products (s_1–s_3). A linear relationship between peak areas and concentrations of analyte was verified in this interval (Fig. 5 inset) and the total analysis time (for each run) was less than 5 min with good peak shape for ciclopirox samples.

Ethanolamine was also identified and quantified in the pharmaceutical products using CE-C^4D. A preliminary study similar to the one described for ciclopirox was performed to obtain the best experimental conditions. The results showed that a separation voltage of 25 kV, injection time of 30 s and 2.0 mol/L acetic acid as running electrolyte were the most favourable conditions. These parameters were adopted for all subsequent experiments.

Figure 6 presents typical electropherograms of ethanolamine standard solutions in acetic acid. The electropherograms (Fig. 6a–f) corresponds to standard solutions of ethanolamine, ranging from 26.4 to 184.8 μg/mL. The following electropherograms (Fig. 6 s_1–s_3) correspond to commercial pharmaceutical samples used during the quantification of ciclopirox (anionic form).

The inset on Fig. 6 shows the linearity obtained between peak areas and analyte concentrations. The straight line could be represented by the equation $A = 0.96 \times 10^{-4} - 2.11 \times 10^{-4}\ C$ ($r = 0.998$, for $n = 6$).

The detection limit (S/N = 3) was estimated as 2.6 μg/mL and the quantification limit was calculated as 8.6 μg/mL. Although this methodology presents a higher limit of detection and a narrower linear range than those for ciclopirox, it is important to stress the versatility of CE to perform analyses of cation and anion in the same samples without increasing considerably the analysis time (for both analyses, the total time required is shorter than 12 min).

The results of three different commercial pharmaceutical samples obtained through both proposed methods

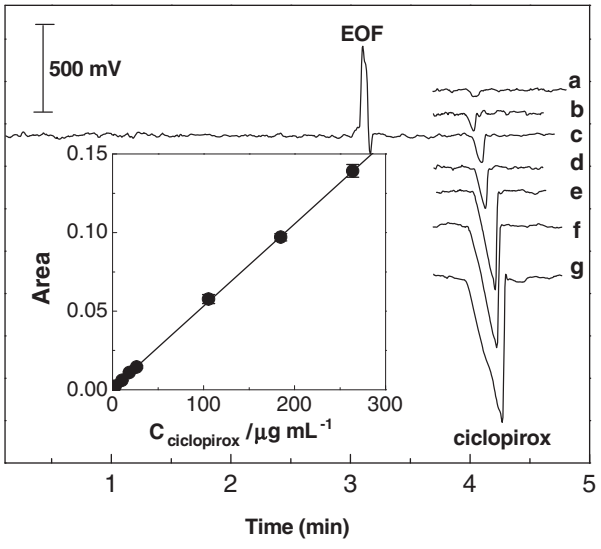

Figure 4. Electropherograms of standard solutions containing: (a) 2.64, (b) 10.6, (c) 18.5, (d) 26.4, (e) 105.6, (f) 184.8, and (g) 264 μg/mL ciclopirox in 10 mmol/L NaOH. Separation voltage: 15 kV. Hydrodynamic injection: 9.8 mBar and 30 s. For the sake of clarity, all the curves but c present only the peak correspondent to the ciclopirox.

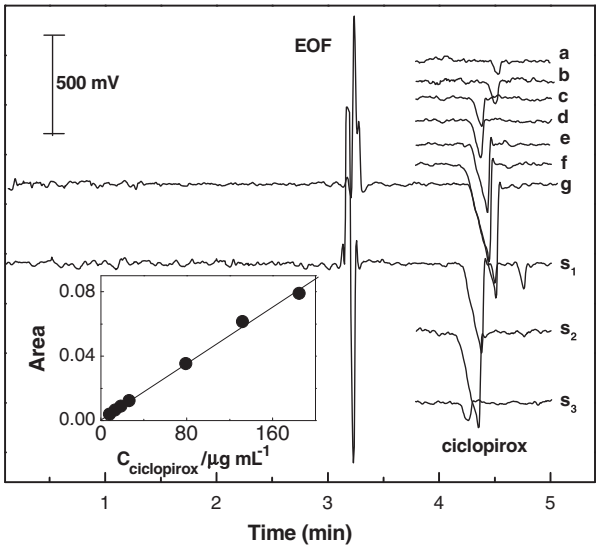

Figure 5. Electropherograms of standard solutions containing: (a) 7.9, (b) 13.2, (c) 18.5, (d) 26.4, (e) 79.2, (f) 132, and (g) 184.8 μg/mL ciclopirox and diluted commercial samples (s_1–s_3) in 10 mmol/L NaOH. Separation voltage: 15 kV. Hydrodynamic injection: 9.8 mBar and 30 s. For the sake of clarity, the curves (a–f and s_2–s_3) present only the peak correspondent to the ciclopirox.

(ciclopirox and ethanolamine determination) are compared with those based on potentiometric titration [3] and are summarised in Table 1. The composition of each pharmaceutical formulation is also included in order to show the components present in each unit and the amount of ciclopirox olamine expected in each product. The fourth, fifth, and sixth columns are the results of the electrophoretic and titrimetric methods as well as the respective SDs determined using three independent measurements for each sample. Finally, the three last columns of Table 1 contain the relative differences between the results obtained by the three procedures and the labelled values.

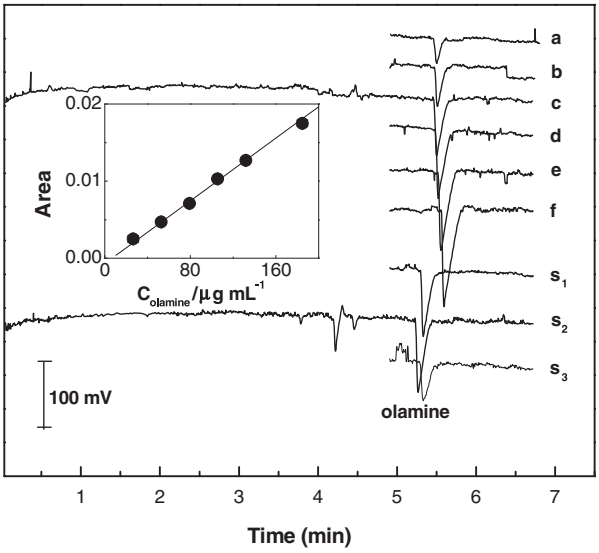

Figure 6. Electropherograms of standard solutions containing: (a) 26.4, (b) 52.8, (c) 79.2, (d) 105.6, (e) 132, and (f) 184.8 μg/mL olamine and diluted commercial samples (s_1–s_3) in 2.0 mol/L acetic acid. Separation voltage: 25 kV. Hydrodynamic injection: 9.8 mBar and 30 s. For the sake of clarity, the curves (a, b, d–f and s_1–s_3) present only the peak correspondent to the olamine.

The results obtained by using CE-C^4D were close to the labelled values and compared favourably with the results obtained by potentiometric titration. Comparing the two electrophoretic means of determining the anionic form (ciclopirox) and the cationic form (ethanolammonium), the results obtained with the latter form were similar for samples 2 and 3. The largest difference was obtained for the first sample, when the anionic form was +14% above the labelled value, which was attributed to matrix interferences (e.g., propylparaben peak close to ciclopirox). The titrimetric results showed good concordance in the case of sample 2 (fewer components), but for more complex samples it was considerably affected.

Another study, indicated in the American Pharmacopoeia [3], involves spectrophotometric analysis with ferrous sulphate solution. We tried to apply this methodology for the determination of ciclopirox olamine in sample 3. During experiments, there was no differentiation between the absorbance signals of the blank and sample solutions. Most probably, the excipients in the cream sample affect the formation of the Fe(II) complexes with *N*-hydroxy-pyridone group, and the method indicated by Pharmacopoeia, therefore, cannot be applied to this complex pharmaceutical formulation.

4 Concluding remarks

The CE with C^4D proved to be a powerful technique to solve many complex samples and demonstrated to be a suitable tool for the quantification of ciclopirox olamine in pharmaceutical products. The proposed methods to quantify both, ciclopirox and olamine, require a relatively short time for analysis (less than 6 min for both analytes),

Table 1. Results obtained after analyses of three different commercial samples of ciclopirox olamine by CE-C^4D and potentiometric titration [3]

Sample	Composition	Labelled value	CE-C^4D[a)] ± SD[b)]	CE-C^4D[c)] ± SD[b)]	Titration ± SD[b)]	Δ (%)[d)]	Δ (%)[e)]	Δ (%)[f)]
s_1	Ciclopirox olamine, glycerol, methylparaben, propylparaben, isopropilic alcohol, polysorbate 80	10 mg/mL	11.4 ± 0.6 mg/mL	10.3 ± 0.8 mg/mL	9.3 ± 0.2 mg/mL	+14	+3.0	−7.0
s_2	Ciclopirox olamine, isopropilic alcohol, macrogol stearate	10 mg/mL	10.2 ± 0.2 mg/mL	10.4 ± 0.7 mg/mL	10.1 ± 0.3 mg/mL	+2.0	+4.0	+1.0
s_3	Ciclopirox olamine, lactic acid, benzyl alcohol, cetostearyl alcohol, isopropyl myristate, diethylene glycol monostearate, polysorbate 60, propylene glycol, capric and caprylic acid triglyceride	10 mg/g	9.6 ± 0.3 mg/g	10.4 ± 0.4 mg/g	4.2 ± 0.2 mg/g	−4.0	+4.0	−58

a) Anion determination (ciclopirox).
b) Average ± SD for three determinations.
c) Cation determination (olamine).
d) Relative difference between labelled value and proposed method (ciclopirox).
e) Relative difference between labelled value and proposed method (olamine).
f) Relative difference between labelled value and potentiometric titration method.

present good accuracy, relatively low cost, and generate small amounts of residues. A wide linear range of concentration was achieved, with good repeatability. Moreover, there are strong evidences that CE with conductivity detection presents better selectivity for ciclopirox in the mixtures of substances found in pharmaceutical samples then the methods recommended by American Pharmacopoeia (potentiometric titration and spectrophotometry). The possibility to perform a double check, by detecting the cationic form of ethanolamine and the anionic form of ciclopirox, is an excellent way to avoid errors caused by interfering species.

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The authors have declared no conflict of interest.

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