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Quantification of terbinafine in pharmaceutical tablets using capillary electrophoresis with contactless conductivity detection and batch injection analysis with amperometric detection

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ABSTRACT

Terbinafine hydrochloride (TerbHCl) is an allylamine derivative with fungicidal action, especially against dermatophytes. Different analytical methods have been reported for quantifying TerbHCl in different samples. These procedures require time-consuming sample preparation or expensive instrumentation. In this paper, electrochemical methods involving capillary electrophoresis with contactless conductivity detection, and amperometry associated with batch injection analysis, are described for the determination of TerbHCl in pharmaceutical products. In the capillary electrophoresis experiments, terbinafine was protonated and analyzed in the cationic form in less than 1 min. A linear range from 1.46 to 36.4 μg mL^{-1} in acetate buffer solution and a detection limit of 0.11 μg mL^{-1} were achieved. In the amperometric studies, terbinafine was oxidized at +0.85 V with high throughput (225 injection h^{-1}) and good linear range (10–100 μmol L^{-1}). It was also possible to determine the antifungal agent using simultaneous conductometric and potentiometric titrations in the presence of 5% ethanol. The electrochemical methods were applied to the quantification of TerbHCl in different tablet samples; the results were comparable with values indicated by the manufacturer and those found using titrimetry according to the Pharmacopoeia. The electrochemical methods are simple, rapid and an appropriate alternative for quantifying this drug in real samples.

1. Introduction

Terbinafine hydrochloride ((E)-N-(6,6-dimethyl-2-hepten-4-ynyl)-N-methyl-1-naphthalene methanamine hydrochloride), or simply TerbHCl, is an allylamine derivative used to treat fungal infections [1]. The drug inhibits ergosterol biosynthesis by inhibiting squalene epoxidase, an enzyme present in fungal and mammalian cell systems. During this process, the fungicidal action is the result of a combination of sterol deficiency and the toxic accumulation of squalene inside the fungal cell wall [2,3]. Terbinafine is especially effective against dermatophytes, a group of filamentous fungi that invade keratinized tissues (nails, hair, skin) of humans and animals [4,5]. TerbHCl is available in the form of nail varnish, creams, tablets and solutions.

Several analytical methods have been described in the literature for the determination of TerbHCl, alone or combined with other drugs belonging to different therapeutic groups, in various samples [6–17]. Most of the reported methods are laborious and generally require expensive instrumentation, which are not appropriate for fast and simple analysis in quality control laboratories. On the other hand, the British and European Pharmacopoeias recommend the use of titrimetry in an alcoholic medium to determine TerbHCl in raw materials [18,19].

Both electrochemical methods explored in this study—capillary electrophoresis with conductivity detection and voltammetry+BIA–have proved to be effective for the analysis of pharmaceutical formulations, because these procedures offer many advantages such as simple manipulation, rapid response time, repeatability and good selectivity [20,21]. Capillary electrophoresis with capacitively coupled contactless conductivity detection (CE–C^4D) has emerged as an alternative and fast analytical technique for the separation and quantification of a large variety of ionic compounds [22–25]. CE–C^4D usually provides a short analysis time, high separation efficiency and low consumption of reagents and samples [26]. Amperometry associated with batch injection analysis (BIA) has demonstrated differentiated performance in routine analysis due to its main electrochemical characteristics, which can be summarized as high sample frequency, small sample volume, high speed and sensitivity and good reproducibility [27,28].

In this paper, efficient electrochemical procedures for the quantification of TerbHCl in pharmaceutical products are presented.

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The first one is based on separation by free solution capillary electrophoresis (FSCE), associated with a C^4D detector for quantification of terbinafine in its cationic form. The second method involves electrochemical oxidation of terbinafine just after injection directly onto the surface of a glassy carbon electrode, using an applied potential of +0.85 V (Amperometry–BIA). To evaluate the results obtained by both methods, the potentiometric methodology described in the Pharmacopoeia [18] was applied, and the results obtained presented very good concordance. The results obtained by all these procedures, including both potentiometry and conductometry in medium with only 5% ethanol, are presented in the following sections.

2. Experimental

2.1. Reagents and solutions

Standard TerbHCl was kindly donated by Arventis Manipulation Pharmacy (São Paulo, Brazil) and used without further purification. All other reagents were of analytical grade. Acetic acid, sodium acetate, sodium hydroxide, lithium hydroxide, ethanol, tris (hydroxymethyl) aminomethane (Tris), and hydrochloric acid were purchased from Merck (Darmstadt, Germany). L-histidine (His) and potassium hydrogen phthalate were obtained from Sigma (St. Louis, MO). Pharmaceutical products were purchased from a local drugstore. All solutions were prepared using ultra pure water from a Millipore Milli-Q system (resistivity $\geq$ 18.2 MΩ cm). For potentiometric and conductometric experiments, a sodium hydroxide solution was used as the titrant. This basic solution was potentiometrically standardised against carefully weighted amounts of potassium hydrogen phthalate (primary standard).

2.2. Instrumentation

The electrophoretic analyses were carried out using home-made CE–C^4D equipment; details of its construction and its detector were described previously [29,30]. A fused silica capillary with dimensions of 75 μm inner diameter, 375 μm outer diameter, and 65 cm long with an effective length of 10 cm (Agilent Technologies, São Paulo, Brazil) was used. Before starting work, the capillary was flushed with 0.1 mol L^{-1} NaOH solution (15 min), ultra pure water (10 min) and background electrolyte (20 min). The samples were injected hydrodynamically for 4 s at 25 kPa and the separation potential adopted was −30 kV.

All voltammetric measurements were performed using a PGSTAT 20 potentiostat (EcoChemie, The Netherlands). A glassy carbon (geometric area=0.071 cm^2) was used as the working electrode, a platinum wire as the auxiliary electrode and Ag/AgCl $_{(KCl\ sat)}$ as the reference electrode. Cyclic voltammetry experiments were done in a conventional 10 mL cell. Batch injection analysis was carried out using an electrochemical cell with a volume of 40 mL which was constructed in our laboratory and described previously [31]. Injections of the standard solutions and commercial samples of TerbHCl were performed using a motorized electronic micropipette EDP Plus EP-100, from Rainin Instruments [32].

Potentiometric measurements were performed using pH metre model Q 400 M1S (Quimis, Diadema, Brazil) connected to a combined glass electrode DME–CV1 (Digimed, São Paulo, Brazil). Titrations were conducted in a 60 mL glass cell under magnetic stirring (300 rpm). A 5 mL manual burette was used during experiments. Following each addition of the titrant, experimental pH values and conductivity values were collected at 15 s intervals. In the first potentiometric measurements, the conditions suggested in the Pharmacopoeia were followed [18]. During these studies, it was observed that 5% ethanol was sufficient for the dissolution of terbinafine. Under this new condition, greater stability of the potential and of conductivity was observed. Simultaneous potentiometric and conductometric measurements in 5% ethanol were performed using the same procedure and arrangement described for potentiometric experiments in medium containing 100% ethanol.

Conductometric experiments were carried out using a conductivity metre (Digimed, model DM-3P) equipped with a conductivity cell with k=1.0 cm^{-1} (Digimed, model DMC-010 M). The conductivity results were corrected according to Sartori et al. [33].

2.3. Preparation of standard solutions

CE–C^4D: a standard stock solution (500 μg mL^{-1}) of TerbHCl was prepared in ultra pure water with 5% ethanol and protected from light. During CE analysis, working standard solutions of TerbHCl from 1.46 to 36.4 μg mL^{-1} were prepared by proper dilutions of the stock solution in the background electrolyte (10 mmol L^{-1} acetic acid/sodium acetate buffer, pH 4.7).

Amperometry–BIA: a standard stock solution (1.0×10^{-2} mol L^{-1}) of TerbHCl was prepared in ethanol, protected from light, and used for all voltammetric studies. Just before the measurements, the TerbHCl solutions (from 10 to 100 μmol L^{-1}) were conveniently diluted in the electrolyte (0.1 mol L^{-1} LiOH in EtOH/H_2O, 1:1 v/v).

2.4. Sample preparation

Two pharmaceutical products containing TerbHCl and other compounds (microcrystalline cellulose, polyvinylpyrrolidone, colloidal silica, magnesium stearate, talcum powder, starch, sodium lauryl sulphate and lactose) were analyzed by the electrochemical methods. Three tablets of each sample (S_1 and S_2) were weighed and pulverized. Portions corresponding to 28.39% (from S_1) and 23.53% (from S_2) were transferred to a 100 mL volumetric flask and filled with ultra pure water in the presence of 5% ethanol. Subsequently, the resulting solutions were filtered through quantitative filter paper and kept in polyethylene bottles. For the CE–C^4D studies, 40 μL of the resulting solutions of TerbHCl (500 μg mL^{-1}) were diluted in background electrolyte (10 mmol L^{-1} acetic acid/sodium acetate buffer, pH 4.7) to a final concentration of 20 μg mL^{-1}.

To perform the amperometric analysis, 300 μL of resulting solutions of analyte were transferred to a 10 mL volumetric flask and filled with electrolyte (0.1 mol L^{-1} LiOH in EtOH/H_2O, 1:1 v/v). For the simultaneous potentiometric and conductometric titration experiments, 30 mL aliquots of the resulting solutions of drug were transferred into a titration cell, which were titrated with a NaOH solution (C_{NaOH}=0.01 mol L^{-1}).

2.5. Titrimetry according to pharmacopoeia

The potentiometric titration of the commercial samples was performed following the procedure described in the British Pharmacopoeia [18]. A known amount of TerbHCl was dissolved in 50 mL of ethanol. The mixture was titrated with a 0.1 mol L^{-1} standardised NaOH solution. The titrant volume required to reach the equivalence point was used to calculate the TerbHCl concentration. This procedure was compared with the methodologies proposed in this paper, which include amperometry, capillary electrophoresis and simultaneous potentiometric and conductometric titration using only 5% ethanol.

3. Results and discussion

3.1. CE–C^4D

Different electrophoretic conditions were tried for analysis of terbinafine in its cationic form. According to data in the literature [34], the pKa value of the amino group of this compound is 7.1. It means that in aqueous solution, below pH 7.1, terbinafine exists mainly in its protonated form and can be separated as a cation. To identify a convenient background electrolyte for the determination of terbinafine, three solutions —10 mmol L^{-1} acetic acid/His, pH 4.6; 10 mmol L^{-1} acetic acid/sodium acetate, pH 4.7; and 10 mmol L^{-1} acetic acid/Tris, pH 4.9—were evaluated. Under these conditions, the drug migrated out at similar times (~48 s). However, it was possible to obtain a higher signal when the 10 mmol L^{-1} acetic acid/sodium acetate buffer was used (signal increase >61%, compared with 10 mmol L^{-1} acetic acid/Tris solution). Since the 10 mmol L^{-1} acetic acid/sodium acetate buffer also provided a good signal-to-noise ratio and a short analysis time, this background electrolyte was selected for the following experiments.

The influence of the acetic acid/sodium acetate concentration was also evaluated in the interval from 10 to 30 mmol L^{-1}. With increasing background electrolyte concentration, an improvement in the signal-to-noise ratio was observed. However, the peak became broader due to Joule heating inside the capillary. Therefore, the acetic acid/sodium acetate concentration was kept at 10 mmol L^{-1}.

In order to obtain the best instrumental conditions in the EC experiments, it was important to study the effect of the applied potential and the injection time in 10 mmol L^{-1} acetic acid/sodium acetate (pH 4.7). The dependence of the migration time of terbinafine on changes to the applied potential was examined up to −30 kV. As expected, there was an increase in the migration time and peak broadening for low potentials. Based on these results, a potential of −30 kV was adopted. The analytical signal of terbinafine was also affected by variations in the hydrodynamic injection time (from 1 to 10 s). The results demonstrated that well-defined peaks were obtained at 4 s (under a pressure of 25 kPa).

Under the optimized conditions (potential: −30 kV and injection time: 4 s), a series of experiments were performed in triplicate, using standard solutions of TerbHCl at different concentrations to build the analytical curve presented in Fig. 1A. A linear relationship was observed between peak areas and concentrations of the drug in the range from 1.46 to 36.4 μg mL^{-1}, with a calibration plot intercept at -1.13 ± 2.28 min and a slope of 7.56 ± 0.12 min/(μg mL^{-1}) for $r = 0.999$.

The detection limit estimated for this interval was 0.11 μg mL^{-1} (S/N = 3) and the quantification limit was calculated as 0.35 μg mL^{-1}. Fig. 1B presents electropherograms corresponding to standard solutions of analyte, ranging from 3.48 to 27.8 μg mL^{-1} (a–e), and tablet samples (S_1 and S_2). The inset depicts the linearity of the response obtained for TerbHCl with good peak shape for pharmaceutical products. The total analysis time (for each run) was around 60 s.

A repeatability study with 15 consecutive injections of a 20.9 μg mL^{-1} TerbHCl standard was performed. The standard deviations were calculated as 1.1% for peak area and 0.43% for migration time.

3.2. Cyclic voltammetry

The electrochemical behaviour of TerbHCl at the glassy carbon electrode was initially studied in 0.1 mol L^{-1} acetic acid/sodium acetate (pH 4.7) and in the presence of 5% ethanol, similar to the conditions utilized in the electrophoresis studies. The exploratory voltammetric experiments done under these conditions demonstrated that after 10 cycles, the decrease in the signal was greater than 10%. The reason for this signal decrease is not clear, but it is likely at this pH in the predominantly aqueous medium, the products generated during the oxidation process remain partially deposited on the electrode, blocking its surface.

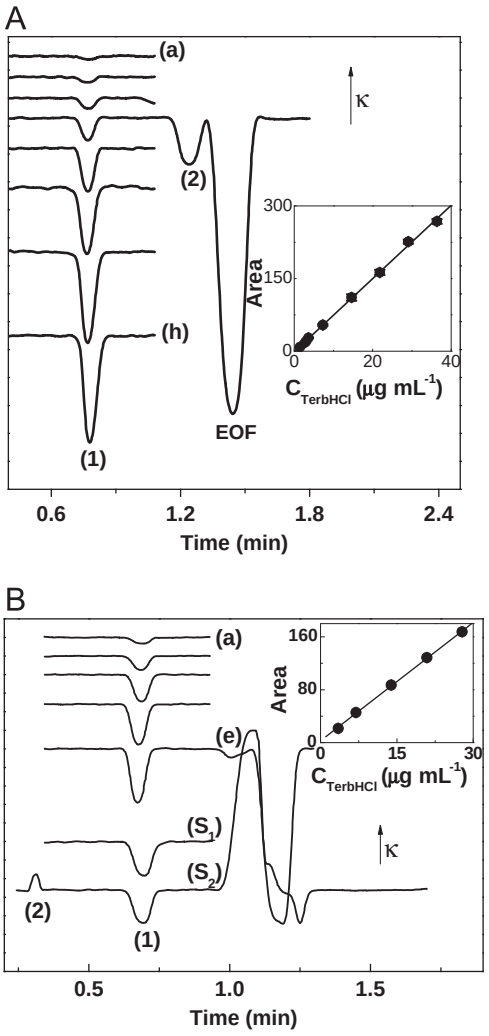

Fig. 1. Electropherograms of (A) standard solutions containing: (a) 1.46, (b) 2.91, (c) 3.64, (d) 7.28, (e) 14.6, (f) 21.8, (g) 29.1, and (h) 36.4 μg mL^{-1} terbinafine in 10 mmol L^{-1} acetic acid/sodium acetate (pH 4.7). Peak 1: terbinafine peak, Peak 2: system peak; (B) standard solutions containing: (a) 3.48, (b) 6.96, (c) 13.9, (d) 20,9, and (e) 27,8 μg mL^{-1} terbinafine and diluted commercial samples (S_1 and S_2) in 10 mmol L^{-1} acetic acid/sodium acetate (pH 4.7). Peak 1: terbinafine signal, Peak 2: unknown peak. Separation voltage: 30 kV. Hydrodynamic injection: 25 kPa and 4 s.

Other electrolytes were also evaluated. Experiments under alkaline conditions indicated a considerable lowering in the oxidation potential and a significant decrease in the electrode fouling process. The best results obtaining in a series of analytes were obtained utilizing LiOH 0.1 mol L^{-1}. Even so, some decrease in signal was observed after many experiments using 5% ethanol in the solution. Seeking more favourable conditions, in the next step, the ratio between alcohol and water was varied from 10 to 80% v/v of ethanol. No apparent displacement of the peak potential was observed, and a stable current value was still verified even when the alcohol concentration was increased up to 50% ethanol. Above this proportion, the peak was dislodged to a more positive potential, attaining the region where the electrolyte

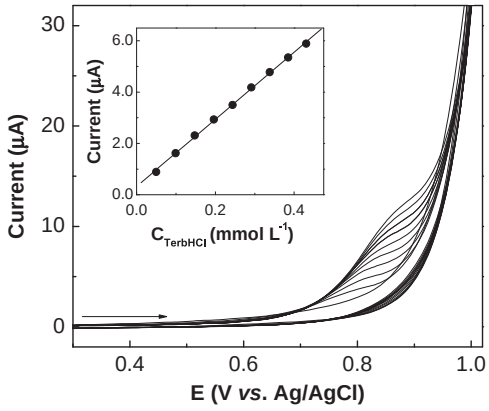

Fig. 2. Cyclic voltammograms obtained with a glassy carbon electrode (A=0.071 cm^2) in EtOH/H_2O (1:1) medium containing 0.1 mol L^{-1} LiOH in the presence of different concentrations of terbinafine standard solutions (from 4.9×10^{-5} to 4.3×10^{-4} mol L^{-1}). Scan rate=100 mV s^{-1}.

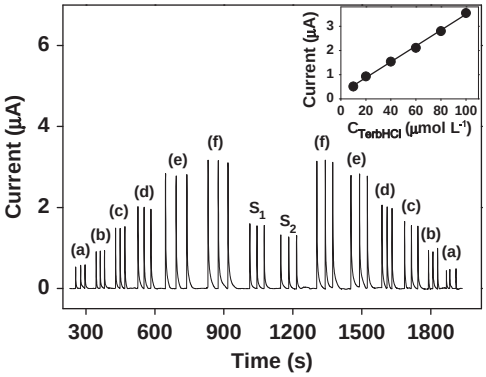

Fig. 3. BIA results for a glassy carbon electrode (A=0.071 cm^2) in EtOH/H_2O (1:1) medium containing 0.1 mol L^{-1} LiOH after injections of (a) 10, (b) 20, (c) 40, (d) 60, (e) 80, and (f) 100×10^{-6} mol L^{-1} of standard solutions, S_1 and S_2 corresponding to tablet samples. Applied potential=+0.85 V, sampling volume=80 μL, injection speed=75.2 μL s^{-1}.

is oxidized. Working with solutions containing 0.1 mol L^{-1} LiOH and 50% ethanol, it was possible after numerous cycles to observe a decrease of 1% in the signal. Therefore, a 0.1 mol L^{-1} LiOH with EtOH/H_2O (1:1) solution was chosen for the following amperometric studies.

Fig. 2 present cyclic voltammograms recorded after increasing additions of TerbHCl in 0.1 mol L^{-1} LiOH with EtOH/H_2O (1:1) solution, at scan 100 mV s^{-1}. The signal obtained for each concentration of analyte was very stable and reproducible. A very good linear relationship between anodic current and terbinafine concentration (from 4.9×10^{-5} to 4.3×10^{-4} mol L^{-1}) is shown in the inset of this figure. The compound was oxidized presenting only one oxidation peak close to +0.9 V. Moreover, no peak was observed in the reverse scan, suggesting that the oxidation process is not reversible. These results confirm the usefulness of a solution of LiOH in EtOH/H_2O (1:1) as the supporting electrolyte for the analysis of TerbHCl.

3.3. Batch injection analysis with amperometric detection

Amperometry–BIA involves the injection of a relatively small amount of analyte onto the surface of an electrode positioned inside a cell filled with electrolyte. The elevated transport of analyte to the electrode followed by its rapid dispersion generates sharp transient peaks similar to the ones obtained by FIA. Parameters such as distance of the pipette to the electrode surface, volume injected, speed of injection and potential of the electrode were evaluated. The optimal distance between the glassy carbon electrode and the pipette tip was found to be 2 mm. The most favourable injection sample volume was 80 μL; the best injection speed of the programmable pipette was the fastest one tried (75.2 μL s^{-1}). The most favourable electrode potential was +0.85 V (vs. Ag/AgCl) and these conditions were fixed for this study.

Fig. 3 presents the signals obtained with commercial samples, preceded and followed by a series of injections of standard solutions of TerbHCl in 0.1 mol L^{-1} LiOH with EtOH/H_2O (1:1) under the optimized conditions. It is possible to verify in the inset of this figure a linearity between BIA current and analyte concentration over a concentration range from 10 to 100 μmol L^{-1}, with a calibration plot intercept at 0.022 ± 0.006 μA and a slope of 0.033 ± 0.001 μA/μmol L^{-1} (r=0.998). The estimated detection limit was 2.4 μmol L^{-1} (S/N=3) and the quantification limit was calculated as 7.99 μmol L^{-1}. A frequency of 225 injections per hour was calculated, considering the rise-up and decreasing the peak signal.

A repeatability study of alternate 80 μL injections of 10 and 100 μmol L^{-1} TerbHCl in 0.1 mol L^{-1} LiOH with EtOH/H_2O (1:1) solution was carried out. In this study, relative standard deviations (R.S.D.) of 5.2 and 1.1% for 10 and 100 μmol L^{-1} TerbHCl solutions, respectively, were obtained. These results demonstrate that there is no memory effect between successive injections. In addition, no decrease in the signal from the glassy carbon electrode was observed after 20 injections, attesting to the absence of a fouling effect in medium containing 50% ethanol.

3.4. Conductometric and potentiometric titration

The British Pharmacopoeia [18] states that TerbHCl can be potentiometrically titrated with sodium hydroxide, which neutralizes its amino group in ethanol medium. Using its suggested conditions, a slower response time of the pH electrode and a higher instability of the signal were observed after several titrations. The literature also describes how repeated dehydration and re-use can dramatically reduce the lifetime of a glass electrode. During the capillary electrophoresis and voltammetric studies, it was observed that TerbHCl could be completely dissolved with much less ethanol. Therefore, 5% ethanol was adopted in the following studies. This condition is advantageous not only for preventing deleterious dehydration of the glass electrode, but because it is also significantly minimizes the quantity of organics in the waste generated.

The effect of the concentration of NaOH titrant (from 0.005 to 0.04 mol L^{-1}) was also verified during titrations. A solution of 0.01 mol L^{-1} NaOH was chosen as the optimum concentration, because it represented the best compromise in terms of analysis time and precision.

Taking advantage of the experimental arrangement used for measurements of pH, simultaneous conductometric titrations of TerbHCl were also performed under the same conditions. In these experiments, characteristic conductometric curves were obtained. Fig. 4 presents typical simultaneous potentiometric and conductometric curves obtained for 500 μg mL^{-1} TerbHCl in: (A) standard solution, (B) sample A_1 and (C) sample A_2, titrated with a 0.01 mol L^{-1} NaOH solution. Similar curve shapes were obtained for the samples and standard solutions. However, sample A_2 presented a higher initial conductivity than the previous sample. This increase in signal was attributed to the presence of other compounds in the pharmaceutical sample.

In the conductometric titrations, the conductivity measured before the addition of the titrant is related to the concentration of the pure drug solution (Fig. 4A), or of TerbHCl plus other ionic

components (Fig. 4B and 4C) present in the case of a commercial drug. Until the equivalence point, all OH^- ions added to the titration cell are consumed in the process of neutralizing the protonated terbinafine, resulting in the formation of a white precipitate. During this stage, the gradual increase in Na^+ ions in the solution is mainly responsible for the small increase in the conductivity. Beyond the equivalence point, all the terbinafine is neutralized and the dramatic increase in conductivity is due to the contribution of excess Na^+ ions, mainly as a result of the presence of free OH^- ions in the solution.

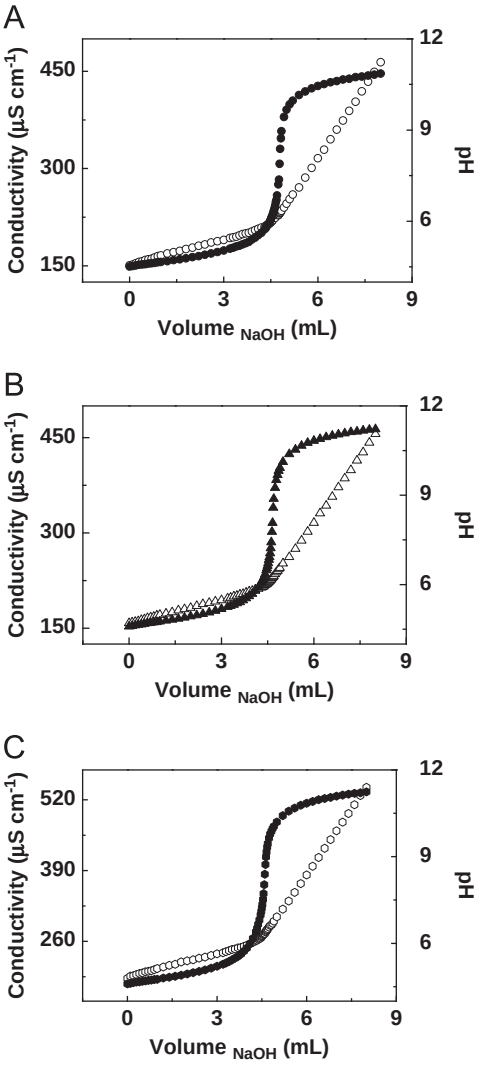

Fig. 4. (●) Conductimetric and (○) potentiometric curves from (A) 500 μg mL^{-1} standard solution of terbinafine; (B and C) pharmaceutical samples containing 500 μg mL^{-1} terbinafine. All solutions were prepared in 5% EtOH and titrated with 0.01024 mol L^{-1} NaOH solution.

In a repeatability study of the titrimetric procedures, a 500 μg mL^{-1} TerbHCl standard solution was titrated with a 0.01 mol L^{-1} NaOH solution; the RSD obtained were about 2.6% and 2.5% for the potentiometric and conductometric titrations, respectively.

3.5. Comparing the different techniques

Table 1 shows the results of the analyses of two commercial samples (in the form of tablets) performed by electrochemical techniques (amperometry–BIA, CE–C^4D and simultaneous conductometric and potentiometric titrations in the presence of 5% ethanol) and the results from titrimetry in alcoholic medium, as described in the British Pharmacopoeia [18].

The results obtained using electrochemical procedures were very close to the labelled values (250 mg of TerbHCl) with a maximum difference of +5.6% (sample 1) for the amperometry–BIA analysis. A significance test (null hypothesis) was applied to the results presented in Table 1, resulting in experimental *t-values* between 0 and 4.0. These results suggest there is no evidence of systematic errors, for either two (CE and amperometric analysis) or one (titrimetric analysis) degrees of freedom (95% of confidence internal), for which the critical values of *t* [35] were 4.30 (CE and amperometry–BIA) and 12.71 (potentiometric and conductometric titrations). These results attest to the good performance of all the measurements performed during this study.

4. Conclusion

In this study we demonstrated that CE–C^4D and amperometry–BIA are suitable electrochemical techniques for the quantification of terbinafine hydrochloride in pharmaceutical products. Major characteristics shared by both techniques, including good accuracy, low consumption of reagents and samples, short analysis times (less than 1 min for CE), and high sample rates (225 injections per hour for amperometric analysis) make them very attractive, especially when many samples need to be analyzed. Moreover, both electrochemical procedures presented precise results and do not require time-consuming sample preparation or expensive instrumentation when compared with several analytical procedures (primarily chromatography, actually the most utilized technique). In addition, conductometric and potentiometric titrations performed simultaneously showed that both alternatives are reliable for terbinafine determination. The simplicity and low cost of the instrumentation required for these techniques are fundamental aspects for quality control in undeveloped countries. The modification in the potentiometric methodology proposed in this study is also of great importance, because pH measurements in alcoholic solutions are intensely affected by dehydration of the electrode membrane. The utilization of only 5% ethanol in terbinafine solutions significantly minimized electrodes problems, and also reduced the content of organic species generated as waste during analysis of this compound.

Table 1
Results obtained (in *g/tablet*) after analysis of terbinafine hydrochloride in pharmaceutical products using capillary electrophoresis, amperometry + BIA, potentiometric and conductometric analysis (in a medium with only 5% ethanol) compared to the titrimetric procedure described by British Pharmacopoeia [18].

Sample	CE–$C^4D \pm SD^a$	AmperometryBIA $\pm$ SD[a]	Potentiometric titration $\pm$ SD[b]	Conductometric titration $\pm$ SD[b]	Recommended procedure $\pm$ SD[b]
A_1	0.252 ± 0.004	0.264 ± 0.006	0.252 ± 0.004	0.251 ± 0.009	0.246 ± 0.006
A_2	0.260 ± 0.007	0.248 ± 0.006	0.257 ± 0.008	0.250 ± 0.006	0.253 ± 0.005

[a] Average ± standard deviation for three determinations,
[b] Average ± standard deviation for two determinations.

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