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Enantiomeric determination of econazole and sulconazole by electrokinetic chromatography using hydroxypropyl-β-cyclodextrin combined with ionic liquids based on L-lysine and L-glutamic acid

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Abstract

Two analytical methodologies based on the combined use of hydroxypropyl-β-cyclodextrin and two different amino acid-based chiral ionic liquids (tetrabutylammonium-L-lysine or tetrabutylammonium-L-glutamic acid) in electrokinetic chromatography were developed in this work to perform the enantioselective determination of econazole and sulconazole in pharmaceutical formulations. The influence of different experimental variables such as buffer concentration, applied voltage, nature and concentration of the ionic liquid, temperature and injection time, on the enantiomeric separation was investigated. The combination of hydroxypropyl-β-cyclodextrin and tetrabutylammonium-L-lysine under the optimized conditions enabled to achieve the enantiomeric determination of both drugs with high enantiomeric resolution (3.5 for econazole and 2.4 for sulconazole). The analytical characteristics of the developed methodologies were evaluated in terms of linearity, precision, LOD, LOQ and recovery showing good performance for the determination of both drugs which were successfully quantitated in pharmaceutical formulations. This work reports the first analytical methodology enabling the enantiomeric determination of sulconazole in pharmaceutical formulations.

Keywords: Electrokinetic chromatography, amino acid based chiral ionic liquids, cyclodextrin, enantioseparation, econazole, sulconazole.

1. Introduction

The big impact of chirality in the pharmaceutical field is well known. In fact, the different biological activity that the enantiomers of a chiral drug may have makes paramount the development of stereoselective analytical methodologies enabling the individual determination of each enantiomer in raw materials and pharmaceutical formulations [1-3]. An important part of the commercialized chiral drugs is nowadays marketed as pure enantiomers which makes necessary an adequate quality control to assess that the enantiomeric impurity is not present at a percentage higher than 0.1 % of the majority enantiomer, as established by the ICH guidelines [4]. However, even in the case of drugs marketed as racemates it is desirable to develop analytical methodologies enabling their enantiomeric determination since those drugs could be marketed in a near future as pure enantiomers in those cases where the enantiomers have different biological activities.

Chromatographic and electrophoretic techniques are the most widely used to carry out the enantiomeric separation of drugs. Among them, Capillary electrophoresis (CE) has proven to possess many advantages such as the possibility of changing easily the nature and concentration of the chiral selector in the separation buffer (in the most frequently employed separation mode in chiral CE which is Electrokinetic Chromatography (EKC)) avoiding the need of using a chiral stationary column, the low volume of reagents and samples necessary, and the high enantioresolution and peak efficiency that can be attained. Among the different chiral selectors that can be used in EKC (cyclodextrins (CDs), macrocyclic antibiotics, proteins, crown ethers, etc.) [5, 6], the most employed have been CDs, whether as the sole chiral selectors in the separation buffer or in dual systems constituted by a mixture of CDs or a mixture of one CD and other selector such as a chiral ionic liquid (CILs) although other combinations have also

been described [6, 7]. Ionic liquids (ILs) are molten organic salts with melting points below 100ºC constituted by a bulky organic cation and an inorganic or organic anion. Among their most important properties, negligible vapor pressure, high thermal stability, low surface tension and high electrical conductivity can be cited [8]. CILs are a group of ILs containing at least one chiral center in their structure and can be used in CE as sole chiral selectors, as chiral ligands or as chiral selectors in dual systems. In the last years, almost half of articles published on CILs dealt with the synergistic effect observed when CILs are combined with other chiral selectors such as CDs [9-10]. In fact, some works revealed that dynamic interactions (hydrogen bonding interaction, charge-charge, dipole-dipole, π-π, host-guest inclusion) taking place inside the capillary between CILs and chiral selectors modified the enantiorecognition process between the chiral selectors and analytes, as well as their electrophoretic mobilities, giving rise to different migration times and enantiomeric resolutions [11].

Imidazole derivatives are a class of heterocycles that could possess antifungal and antibacterial activity. Two of them are econazole and sulconazole that are widely used for the treatment of skin fungal infections. They are available as nitrate salt in pharmaceutical formulations such as creams, powders and solutions at low concentrations (1%). Both drugs are commercialized as racemic mixtures but there are some studies showing the different activity that each azole enantiomer could possess [12]. For instance, the antifungal activity of S-econazole against several microorganisms is higher than that of R-econazole and the racemic mixture, above all against *Aspergillus niger* [13].

In a previous work of our research team, the potential of the combination of the two amino acid-based CILs, tetrabutylammonium L-lysine ([TBA][L-Lys]) or tetrabutylammonium L-glutamic acid ([TBA][L-Glu]), with hydroxypropyl-β-cyclodextrin (HP-β-CD) was

demonstrated for the stereoselective separation of a group of drugs [14] including the antifungals econazole and sulconazole. The previous use of these CILs has very scarcely been reported. In fact, [TBA][L-Lys] and [TBA][L-Glu] were employed combined with β-CD for the enantioseparation of phenethylamine [15] while the separation of two diastereoisomers of corynoxine was described with [TBA][L-Glu] combined with HP-β-CD [16]. However, as far as we know, no applications to the analysis of real samples have been reported.

Although the enantiomeric separation of econazole by CE has been achieved in some research works, using whether CDs as sole chiral selectors in the separation buffer [17-26] or CD/CILs dual systems [14, 27, 28], the analysis of this drug in real samples has scarcely been carried out. Thus, an analytical methodology based on the use of a 25 mM phosphate buffer (pH 3.0) with 2% (v/v) sulfated-β-CD at 20 ºC and applying -20 kV was developed by our research team enabling the simultaneous enantiomeric separation of econazole and other six drugs in 16 min and it was applied to the analysis of wastewater samples [24]. Econazole enantiomers were also quantitated in a pharmaceutical formulation (a cream) by MEKC using 20 mM phosphate buffer (pH 8.0) containing 50 mM sodium dodecyl sulfate and 40 mM hydroxypropyl-γ-CD obtaining a resolution of 2.2 in 9 min [23]. In the case of sulconazole, although a few works used this compound as model drug to study by CE the potential of different chiral selectors [14, 18, 19, 29-35] including dual systems constituted by CILs and α-CD [30], HP-β-CD [14, 28] or a dextrin [29], the analysis of real samples has not been reported.

Chromatographic methodologies were also developed enabling the enantiomeric separation of econazole and sulconazole. In these works, LC [36-43], nano-LC [44, 45] and Supercritical Fluid Chromatography [46] were employed with chiral stationary

phases. However, only in one of these articles the developed methodology was applied to the analysis of real samples like wastewater and sludge [38].

The aim of this work was to develop EKC methodologies based on the use of the dual systems HP-β-CD/[TBA][L-Lys] or HP-β-CD/[TBA][L-Glu] as chiral selectors for the enantioselective determination of econazole and sulconazole in pharmaceutical formulations.

2. Materials and methods

2.1. *Reagents and samples*

Chemicals and reagents of analytical grade were employed to perform all experiments. Water used to prepare solutions was purified with a Milli-Q system from Millipore (Bedford, MA, USA). Racemic econazole (1-(2-[(4-chlorophenyl)methoxy]-2-[2,4-dichlorophenyl]ethyl)-1H-imidazole nitrate salt), racemic sulconazole (1-(2-[p-chlorobenzylthio]-2-[2,4-dichlorophenyl]ethyl)-1H-imidazole nitrate salt), sodium hydroxide, and cartridges (DSC-diol sorbent, 500 mg/6 mL tube) for solid phase extraction (SPE) were provided by Sigma-Aldrich (Saint-Louis, MO, USA). Methanol, hexane, hydrochloric acid, and ortho-phosphoric acid were purchased from Scharlau (Barcelona, Spain). Dichloromethane and HP-β-CD (DS~0.6) were from Merck (Darmstadt, Germany) and Fluka (Buchs, Switzerland), respectively.

[TBA][L-Lys] and [TBA][L-Glu] were synthesized by the Center for Applied Chemistry and Biotechnology (CQAB) from the University of Alcalá following the method previously described by Salido-Fortuna et al [14].

Pharmaceutical creams of sulconazole (10 mg sulconazole nitrate/g cream) and econazole (containing 1% econazole nitrate) were acquired in online drug stores from Netherlands or Italy, respectively.

2.2. *CE conditions*

All analyses were carried out with a 7100 CE system from Agilent Technologies (Palo Alto, CA, USA) equipped with a diode array detector (DAD) and controlled by the Agilent ChemStation software. Uncoated fused-silica capillaries of 50 µm ID (362 µm OD) with a total length of 48.5 cm (effective length of 40 cm) from Polymicro Technologies (Phoenix, AZ, USA) were used to carry out the electrophoretic experiments. Detection wavelength was set at 200 nm with a bandwidth of 4 nm and injections were made by applying a pressure of 50 mbar for 10 s. Analyses were performed using a voltage of 30 kV at a working temperature of 25ºC or 15ºC for econazole and sulconazole, respectively.

New capillaries were conditioned (applying 1 bar) with 1 M sodium hydroxide for 30 min, Milli-Q water for 15 min and phosphate buffer for 60 min. At the beginning of each working day, the capillary was rinsed for 5 min with 0.1 M sodium hydroxide, followed by 5 min of Milli-Q water and 30 min of phosphate buffer. Between injections, the capillary was preconditioned during 5 min with 1 M sodium hydroxide, 2 min with 0.1 M hydrochloric acid, 1 min with Milli-Q water and 5 min with background electrolyte (BGE).

2.3. *Preparation of solutions and samples*

Buffer solutions were prepared by diluting the appropriate amount of ortho-phosphoric acid with Milli-Q water to obtain a concentration of 50 mM and adjusting the pH to 2.5 with 1 M sodium hydroxide. Background electrolytes (BGEs) were prepared dissolving the adequate amount of HP-β-CD and CILs in phosphate buffer. The pH of BGEs containing mixtures of CD and CILs were adjusted to 2.5 with ortho-phosphoric acid.

Stock standard solutions of econazole and sulconazole were prepared dissolving the adequate amount of each racemic drug standard in methanol up to a final concentration of 1000 mg/L. These solutions were stored at -20ºC and different aliquots were diluted in Milli-Q water to get solutions with concentrations from 5 to 75 mg/L of each enantiomer. Before CE analysis, each working solution was filtered through 0.45 µm Nylon syringe filters (Scharlau, Barcelona, Spain) and sonicated.

To prepare sample solutions, econazole and sulconazole were extracted from the pharmaceutical creams using a methodology previously reported by Hermawan et al. with some modifications [23]. Briefly, 2.5 mL of dichloromethane were added to 100 mg cream sample. After sonicating the mixture during 2 min, the volume was adjusted to 5 mL with dichloromethane. Then, 2.0 mL were filtered through 0.45 µm Nylon syringe filters and extracted using SPE. The diol cartridges were preconditioned with 6.0 mL of dichloromethane and washed two times with 3.0 mL n-hexane/dichloromethane (4:1, v/v) after the application of the sample. Then, econazole was eluted with three portions of 1.0 mL of methanol, combined and adjusted to 5.0 mL with methanol. In the case of sulconazole, it was eluted with five portions of 1.0 mL of methanol that were combined. Aliquots of 500 µL were diluted to 1.0 mL with methanol, filtered through 0.45 µm Nylon syringe filters and sonicated before analysis by CE.

187 2.4. *Data treatment*

188 Migration times, peak areas and resolution values between adjacent peaks were obtained
189 using the ChemStation software from Agilent Technologies. The figures of different
190 electropherograms were composed employing the Origin 8.0 software. Data from the
191 analytical characteristics of the developed methodologies and statistical tests were treated
192 using the STATGRAPHICS Centurion XVII-X64 program and Microsoft Excel.

193

194 **3. Results and discussion**

195 The potential of chiral dual systems based on the combination of HP-β-CD with the amino
196 acid based chiral ionic liquids [TBA][L-Lys] or [TBA][L-Glu] for the enantioseparation
197 of econazole and sulconazole by EKC was recently demonstrated by our research group
198 [14]. Using 100 mM phosphate buffer (pH 2.5) containing 30 mM [TBA][L-Lys] or
199 [TBA][L-Glu] with HP-β-CD (5 mM for the analysis of econazole and 2 mM in the case
200 of sulconazole), enantiomeric resolutions of 4.1 or 4.3 for econazole and 2.7 or 2.8 for
201 sulconazole were reached, respectively, in analysis times close to 30 min.
202 Bearing in mind that short analysis times are always a priority in the development of
203 analytical methodologies to be used in routine analysis, a systematic study of the
204 influence of different experimental variables on the enantiomeric resolution and analysis
205 time was performed with the aim of developing a fast methodology to carry out the quality
206 control of econazole and sulconazole in pharmaceutical formulations.

207

208 3.1. *Optimization of EKC methodologies for the enantiomeric determination of econazole*
209 *and sulconazole*

The first step to decrease the migration time of the enantiomers was to shorten the effective capillary length from 50 to 40 cm under the experimental conditions developed in our previous work [14]. However, the current generated into the capillary was too high (~ 200 μA) which gave rise to capillary breakage. For this reason, the influence of different variables, such as buffer concentration, applied voltage, CILs concentration, temperature, and injection time, was investigated. The selection of the most appropriate conditions was made establishing a compromise between migration time, enantiomeric resolution, and current generated into the capillary.

First, the effect of the buffer concentration (50, 75, 100 mM) and the applied voltage (20, 25, 30 kV) was evaluated using the two different chiral dual systems (HP-β-CD/[TBA][L-Lys] and HP-β-CD/[TBA][L-Glu]) in the appropriate concentrations for the enantioseparation of both compounds. As **Table 1** shows, the application of 20 kV in all the buffer concentrations tested originated the longest analysis times (> 50 min). Increasing the applied voltage, it was possible to shorten the migration times what also increased the current generated. In fact, currents between 130-190 μA were obtained when voltages higher than 20 kV were applied in the 100 mM phosphate buffers and around 115 μA when a value of 30 kV was employed in buffers of 75 mM. The effect that the presence of the CILs ions can have on the conductivity of the separation buffer [30] was shown to contribute to the high currents obtained, then influencing the selection of the buffer concentration and the applied voltage (lower currents were observed in absence of the CILs). From these results, a buffer concentration of 50 mM was considered the most adequate to carry out the chiral separation of both compounds. Using this buffer and a voltage of 30 kV, a decrease not only in the analysis time but also in the resolution values was observed (see **Table 1**). However, the enantiomeric resolutions achieved using

234 the highest voltage were still high enough (Rs > 2.8). For this reason, a 50 mM phosphate
235 buffer and 30 kV were chosen for further experiments.
236 The effect of CILs concentration was also studied. It was observed that an increase in the
237 CILs concentration generally gave rise to higher migration times. These results can be
238 justified considering the effect of CILs on the characteristics of the separation buffer such
239 as viscosity that influence migration times [30, 47, 48]. Moreover, enantiomeric
240 resolutions values also increased with the CILs concentration due to the expected
241 synergistic effect between the CILs and HP-β-CD [14]. In fact, a change in the
242 interactions between the CILs ions and the CD [30], which in turn can modify the
243 interactions between the analyte and the CD, can explain the observed synergistic effect
244 as reported by other authors [49-51].

245 Based on the effect of the CILs concentration on the migration times, decreasing the CIL
246 concentration can be useful to decrease migration times although a loss in enantiomeric
247 resolution can also take place. In the case of econazole, migration times for its
248 enantiomers were significantly shortened when the concentration of both CILs was
249 decreased while a slightly decrease in resolution was obtained. Using the dual system 5
250 mM HP-β-CD plus 20 mM [TBA][L-Lys], a resolution value of 4.0 in 15.3 min was
251 achieved, while a resolution of 4.3 in 16.8 min was obtained when adding 30 mM of
252 [TBA][L-Lys]. On the contrary, when the dual system was based on the combined use of
253 5 mM HP-β-CD plus 20 mM [TBA][L-Glu] or 30 mM [TBA][L-Glu], resolution values
254 of 3.7 and 4.3 in 15.0 min and 18.4 min, respectively, were reached. In order to obtain
255 shorter analysis times with an adequate resolution, a concentration of 20 mM of each CIL
256 was selected for the enantiomeric determination of econazole. Regarding sulconazole, the
257 enantiomeric resolution decreased from a value of 2.8 (in 14.8 min) to 2.6 (in 16.0 min)

when 20 mM [TBA][L-Lys] instead of 30 mM was used in combination with 2 mM HP-β-CD. In the same way, employing 20 mM instead of 30 mM of [TBA][L-Glu], resolution values decreased significantly from 3.1 (in 19.7 min) to 2.4 (in 17.1 min). As the current generated into the capillary using a concentration of 30 mM for both CILs was high (data shown in **Table 1**), the use of this CILs concentration was discarded. Nevertheless, enantiomeric resolutions were improved to 2.9 and 3.1 with a slightly increase in analysis time (16.9 min and 20.1 min) when BGEs containing 25 mM of each CIL, [TBA][L-Lys] and [TBA][L-Glu], were used. Establishing a compromise between migration times, resolution and generated current inside the capillary, 25 mM of [TBA][L-Lys] or [TBA][L-Glu] was selected as the optimum concentration of CILs in the dual systems for the enantiomeric separation of sulconazole.

Once selected the best BGEs to perform the enantiomeric separation of econazole and sulconazole, the effect of the temperature was studied. As it can be seen in **Figure 1**, as expected, modifying the temperature from 15 to 25 ºC, both the analysis time and the enantiomeric resolution decreased. In the case of econazole (**Figures 1A** and **1C**), its enantiomers were separated with high resolution in only 10 min using the two different dual systems (i.e. HP-β-CD/[TBA][L-Lys] or HP-β-CD/[TBA][L-Glu]) at 25ºC. The system based on the combination of HP-β-CD with [TBA][L-Lys] was chosen because the resolution was slightly higher and BGEs with this CIL showed to originate a greater current stability. On the contrary, the chiral separation of sulconazole employing HP-β-CD/[TBA][L-Lys] (**Figure 1B**) or HP-β-CD/[TBA][L-Glu] (**Figure 1D**) at 25ºC gave rise to a more significant loss of resolution. With the purpose of reaching enantiomeric resolution values around 3.0, a temperature of 15 ºC was selected. In addition, as it can be observed in **Figures 1B** and **1D**, lower migration times were obtained using a BGE

containing HP-β-CD with [TBA][L-Lys] instead of [TBA][L-Glu]. Thus, this dual system was chosen as the most appropriate to obtain the chiral separation of sulconazole with high resolution in a relatively short analysis time.

Finally, with the aim of improving the sensitivity of the developed methodologies for the chiral analysis of econazole and sulconazole, the effect of the injection time was investigated. First, the racemic concentration of each compound was decreased from 150 mg/L (racemic concentration employed to carry out the optimization of all the experimental conditions described above) to 40 mg/L. Then, the injection was made by applying 4 or 10 s at a pressure of 50 mbar. When the longest injection time was employed, an improvement in the sensitivity was also observed. Since an injection time of 10 s enabled to reach high resolutions for both compounds (values of 3.5 and 2.4 for econazole and sulconazole respectively) this value was selected.

3.2. Analytical characteristics of the developed EKC methodologies

The potential of the methodologies developed in this work to be applied for routine quality control of pharmaceutical formulations was evaluated in terms of different analytical characteristics such as linearity, selectivity, accuracy, precision, limits of detection (LOD), and limits of quantification (LOQ).

As **Table 2** shows, linearity was evaluated for both EKC methodologies by injecting in triplicate six standard solutions at different concentration levels in two days. Data obtained showed adequate correlation coefficients (> 0.990). Moreover, the data fit properly to a linear model since the p-values obtained for all linear regressions in an ANOVA test were higher than 0.05. Also, in both cases, confidence intervals (at a 95% confidence level) for the intercept and slope included and did not include the zero value,

306 respectively. For determining possible matrix effects, the standard addition calibration
307 method was performed adding four known amounts of econazole or sulconazole standard
308 (10, 50, 100 and 125 % of the drug concentration in the sample) to the pharmaceutical
309 cream containing a racemic concentration (40 mg/L) of each drug. Comparing the
310 confidence intervals for the slopes obtained using the external standard calibration
311 method and the standard addition calibration method, no statistically significant
312 differences were observed for a 95% confidence level. Thus, under the optimal
313 conditions, matrix interferences were not found. For this reason, the external standard
314 calibration method was used for the determination of econazole and sulconazole in
315 pharmaceutical creams.
316 Accuracy of the methods was evaluated as the recoveries obtained for sulconazole and
317 econazole enantiomers when the cream samples were spiked with a 40 mg/L (racemic
318 concentration) of the corresponding chiral drug. Recovery values ranged from 96 ± 7%
319 to 101 ± 5% (see **Table 2**).
320 To evaluate the method precision in terms of instrumental repeatability, method
321 repeatability and intermediate precision, a standard solution of 40 mg/L of each racemic
322 drug was employed. Instrumental repeatability was determined from six consecutive
323 injections of the standard solution, method repeatability from three replicate standard
324 solutions injected in triplicate on the same day, and intermediate precision from three
325 standard solutions injected in triplicate in three different days. As it can be seen in **Table**
326 **2**, the RSD values were acceptable for both methods, with RSD values for migration times
327 lower than 3.9% for econazole and 2.7% for sulconazole, and RSD for peaks area lower
328 than 9.1% and 7.2% for econazole and sulconazole, respectively.

LODs and LOQs were experimentally determined as the minimum concentration yielding a S/N ratio of 3 and 10, respectively. LODs of 1.3 mg/L for both econazole enantiomers, 1.6 mg/L for the first-migrating sulconazole enantiomer and 1.5 mg/L for the second-migrating sulconazole enantiomer, were achieved. Regarding LOQs, values of 4.3 mg/L for both econazole enantiomers, 5.3 mg/L the first-migrating sulconazole enantiomer and 5.0 mg/L for the second-migrating sulconazole enantiomer, were obtained. Relative LODs for one enantiomer with respect to the other could not be determined as pure enantiomers of econazole and sulconazole were not commercially available.

3.3. Quantitative analysis of econazole and sulconazole in pharmaceutical formulations

Demonstrated the suitability of the two EKC methodologies developed, they were applied to the quantitative analysis of econazole and sulconazole enantiomers in pharmaceutical cream formulations.

Figure 2A shows the electropherograms corresponding to the enantiomeric analysis of a racemic standard solution and a econazole pharmaceutical cream (both at 40 mg/L) using as chiral selector the dual system composed of 5 mM HP-β-CD and 20 mM [TBA][L-Lys]. In both electropherograms interfering peaks were not found, demonstrating the apropriate selectivity of the developed methodology. The application of this method to the quantitative analysis of econazole in the cream sample enabled to achieve an average amount of econazole of 0.49±0.10 mg/100 mg cream and 0.48±0.01 mg/100 mg cream for the first and the second enantiomer, respectively. Thus, the total econazole concentration determined (1.01±0.02 mg/100 mg cream) corresponded to a percentage of 101±2% of the labeled amount showing a good agreement between the amount of

econazole determined by the EKC method developed and that declared in the label of the pharmaceutical cream.

Regarding sulconazole, **Figure 2B** shows the electropherograms corresponding to the enantiomeric analysis of a racemic standard solution and a sulconazole pharmaceutical cream (both at 40 mg/L) using as chiral selector the dual system based on the combination of 2 mM HP-β-CD and 25 mM [TBA][L-Lys]. As in the case of econazole, the absence of interfering peaks demonstrates the adequate selectivity of the developed methodology. The quantitative determination of sulconazole in the pharmaceutical cream enabled to obtain amounts of 3.8±0.1 mg/g cream and 3.85±0.07 mg/g cream for the first- and second-migrating enantiomers, respectively, what makes a total amount of 7.7±0.2 mg/g cream. This value corresponded to a 77 % of the labeled amount of sulconazole in the analyzed sample. This could be due to the fact that the protocol employed to carry out the extraction of sulconazole from the cream sample was optimized for econazole [23] and not for sulconazole. In order to improve this result, the methodology described by Hermawan *et al* [23] was slightly modified. Thus, the amount of methanol used to elute sulconazole from the SPE cartridge was increased to five portions of 1.0 mL instead of three portions of 1.0 mL. In this way, the amount of sulconazole determined in the cream sample was 10.0±0.1 mg/g cream (5.07±0.05 mg/g cream and 4.93±0.05 mg/g cream for the first- and the second-migrating enantiomer, respectively) which corresponded to a percentage of 100±1% of the labeled amount.

4. Conclusions

The two chiral EKC methodologies developed in this work using a dual system of HP-β-CD and [TBA][L-Lys] allow the enantiomeric determination of econazole and

sulconazole in pharmaceutical formulations (antifungal creams). The use of 50 mM phosphate buffer at pH 2.5 containing HP-β-CD and [TBA][L-Lys] applying 30 kV enabled the enantioseparation with resolution values of 3.4 and 2.4 in an analysis time of 12 and 18 min for econazole and sulconazole, respectively. Moreover, the evaluation of the analytical characteristics of both methods demonstrated adequate linearity, precision and accuracy, as well as LODs under 1.6 mg/L and LOQs under 5.3 mg/L for each enantiomer. Thus, these methods are suitable for the enantiomeric quantitation of econazole and sulconazole in pharmaceutical formulations. Taking into account that no chiral methodologies have previously been reported for the analysis of sulconazole in pharmaceutical formulations and just one work described the enantiomeric analysis of econazole in pharmaceutical formulations (using MEKC), the results obtained in the present work constitute an interesting tool for the quality control of antifungal creams based on these two drugs. The individual determination achieved for each enantiomer supports the possibility to apply the developed methodologies to the analysis of pharmaceutical formulations that could be marketed in a near future as pure enantiomers. Moreover, compared to the MEKC method in which an enantiomeric resolution of 2.2 in 9 min was obtained for econazole, in our case, a more sensitive methodology was developed (LOQ 4.3 mg/L instead of 14.3 mg/L) with an enantiomeric resolution of 3.4 in 12 min using a more cost-effective CD (HP-β-CD instead of HP-γ-CD) and a lower CD concentration (5 mM instead of 40 mM) in the EKC separation medium.

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Declaration of interest

Declarations of interest: none.

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Figure captions.

Figure 1. Electropherograms corresponding to the enantioseparation of racemic standard solution of econazole and sulconazole (150 mg/mL) using as dual systems A) 5 mM HP-β-CD and 20 mM [TBA][L-Lys]; B) 2 mM HP-β-CD and 25 mM [TBA][L-Lys]; C) 5 mM HP-β-CD and 20 mM [TBA][L-Glu]; D) 2 mM HP-β-CD and 25 mM [TBA][L-Glu] at different temperatures. Other CE conditions: 50 mM phosphate buffer (pH 2.5); uncoated fused-silica capillary, 50 µM ID x 48.5 cm (40 cm of effective length); UV detection at 200 nm; applied voltage, 30 kV; injection by pressure, 50 mbar for 4 s.

Figure 2. Electropherograms corresponding to the enantiomeric separation of a standard solution (40 mg/mL) and cream samples of econazole and sulconazole. CE conditions: 50 mM phosphate buffer (pH 2.5) containing mixtures of A) 5 mM HP-β-CD with 20 mM [TBA][L-Lys] at 25 ºC; B) 2 mM HP-β-CD with 25 mM [TBA][L-Lys] at 15 ºC. Other conditions: uncoated fused-silica capillary, 50 µM ID x 48.5 cm (40 cm of effective length); UV detection at 200 nm; applied voltage, 30 kV; injection by pressure, 50 mbar for 10 s.

Table 1. Migration times and enantiomeric resolutions for econazole and sulconazole using different buffer concentrations and different values of applied voltage.

	Chiral selectors	Buffer concentration	Voltage								
			20 kV *			25 kV			30 kV **		
			t_1 (min)	Rs	Current (μA)	t_1 (min)	Rs	Current (μA)	t_1 (min)	Rs	Current (μA)
Econazole	5 mM HP-β-CD + 30 mM [TBA][L-Lys]	50 mM	51.2	5.4	50	32.8	4.8	70	16.8	4.3	105
		75 mM	57.6	-	60	26.3	4.7	80	15.5	4.1	115
		100 mM	> 60	-	80	19.0	4.2	165	HC	HC	190
	5 mM HP-β-CD + 30 mM [TBA][L-Glu]	50 mM	50.5	5.4	54	31.4	5.0	70	18.4	4.3	80
		75 mM	57.0	-	50	31.1	4.8	77	16.0	3.9	113
		100 mM	> 60	-	80	22.2	4.3	130	HC	HC	180
Sulconazole	2 mM HP-β-CD + 30 mM [TBA][L-Lys]	50 mM	54.6	3.1	50	30.7	2.8	70	14.8	2.8	105
		75 mM	> 60	-	60	33.6	3.3	80	15.9	2.7	115
		100 mM	> 60	-	80	21.3	2.5	165	HC	HC	190
	2 mM HP-β-CD + 30 mM [TBA][L-Glu]	50 mM	44.6	3.7	54	23.9	2.8	70	19.7	3.1	80
		75 mM	53.7	4.9	50	37.3	4.9	77	19.9	2.8	113
		100 mM	> 60	-	80	26.0	2.7	130	HC	HC	180

CE conditions: phosphate buffer (pH 2.5); uncoated fused-silica capillary, 50 μM ID x 48.5 cm (40 cm of effective length); UV detection at 200 nm; temperature 15ºC; injection by pressure, 50 mbar for 4 s.
Rs: Resolution; t1: time of the first-migrating enantiomer (min).
*Analyses were stopped at 60 min. The second-migrating enantiomer of econazole using a buffer of 75 mM was not detected in 60 min. In all these cases, Rs values were not determined.
** HC: High current level (~200 μA).

Table 2. Analytical characteristic of two CE methodologies for the quantification of econazole and sulconazole enantiomers with dual systems.

	Econazole		**Sulconazole**	
	First enantiomer	Second enantiomer	First enantiomer	Second enantiomer
External standard calibration method [a]				
Range	5-50 mg/L	5-50 mg/L	7-50 mg/L	7-50 mg/L
Slope ± t · S_a	4.1 ± 0.6	4.4 ± 0.7	9.6 ± 1.1	9.8 ± 1.2
Intercept ± t · S_b	-12.3 ± 16.8	-14.0 ± 19.7	-28.9 ± 32.1	-30.5 ± 34.8
r	0.992	0.990	0.994	0.994
p-value (ANOVA) [b]	0.0814	0.0632	0.136	0.163
Matrix interferences [c]				
Slope ± t · S_a	5.9 ± 1.8	6.1 ± 2.4	10.1 ± 2.5	10.7 ± 2.3
Accuracy [d]				
Recovery	96 ± 7 %	98 ± 4 %	101 ± 5 %	101 ± 4 %
Precision				
Instrumental repeatability [e]				
t, RSD (%)	1.2	1.3	2.0	2.1
A, RSD (%)	4.9	4.5	3.1	3.2
Method repeatability [f]				
t, RSD (%)	0.7	2.4	2.5	2.5
A, RSD (%)	2.9	4.3	4.9	5.8
Intermediate precision [g]				
t, RSD (%)	3.9	3.9	2.7	2.7
A, RSD (%)	9.1	8.8	7.2	6.2
LOD [h]	1.3 mg/L	1.3 mg/L	1.6 mg/L	1.5 mg/L
LOQ [h]	4.3 mg/L	4.3 mg/L	5.3 mg/L	5.0 mg/L

[a] Six standard solutions at different concentration levels injected in triplicate for two days.
[b] p-value for ANOVA to confirm that experimental data fit properly to linear models.
[c] Comparison of the confidence intervals for the slopes corresponding to the standard addition and the external standard calibration methods.
[d] Accuracy was evaluated as the recovery obtained from pharmaceutical cream samples solutions containing 40 mg/L of racemic drug (as labeled amount) with 40 mg/L (100%) of racemic drug.
[e] Six consecutive injections (n = 6) of a standard solution containing 40 mg/L of chiral drug.
[f] Three standard solutions containing 40 mg/L of racemic drug injected in triplicate (n = 9) on the same day.
[g] Three standard solutions containing 40 mg/L of racemic drug injected in triplicate on three different days (n = 9).
[h] LOD and LOQ obtained experimentally for a S/N = 3 or S/N = 10, respectively.

Figure 1.

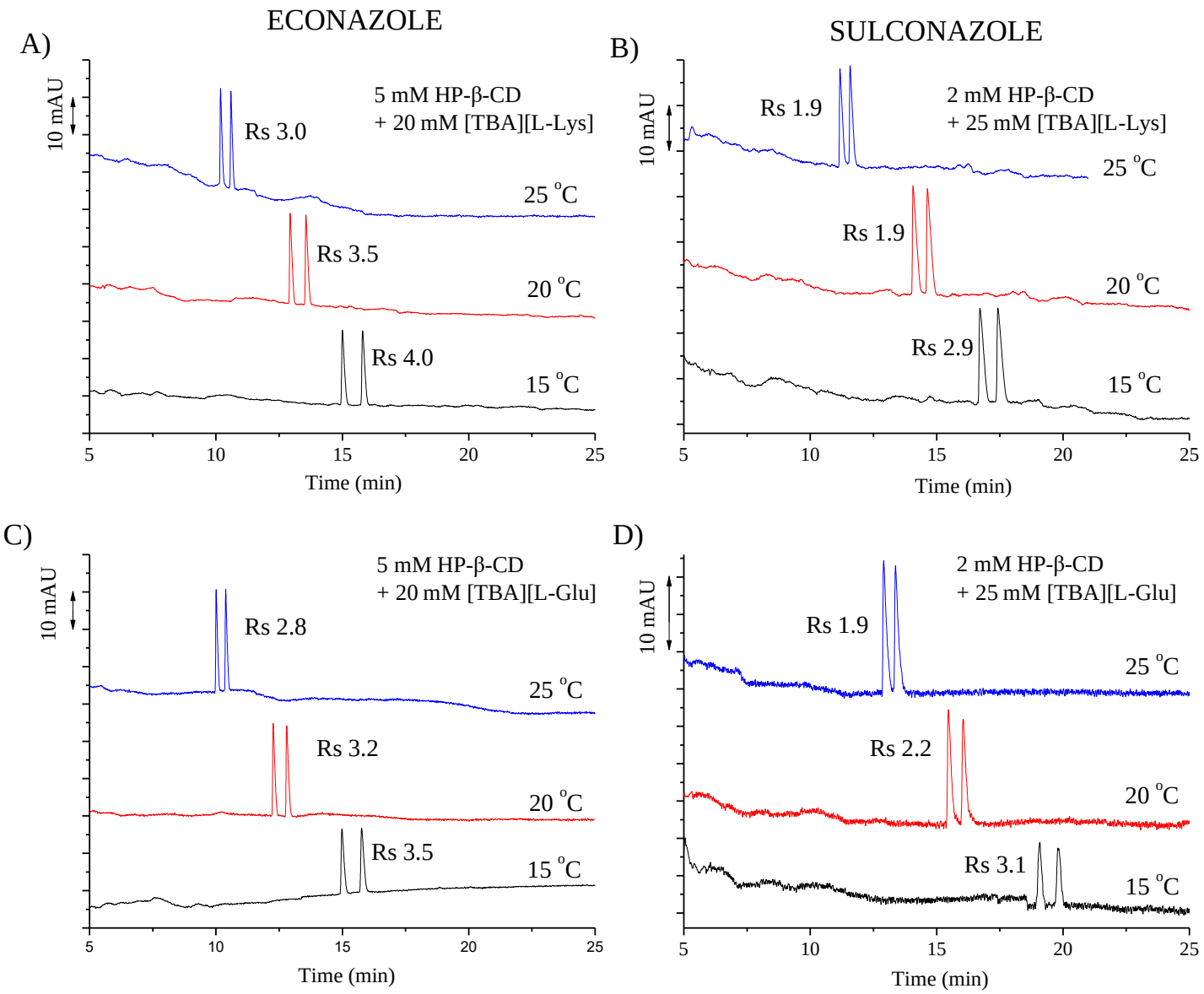
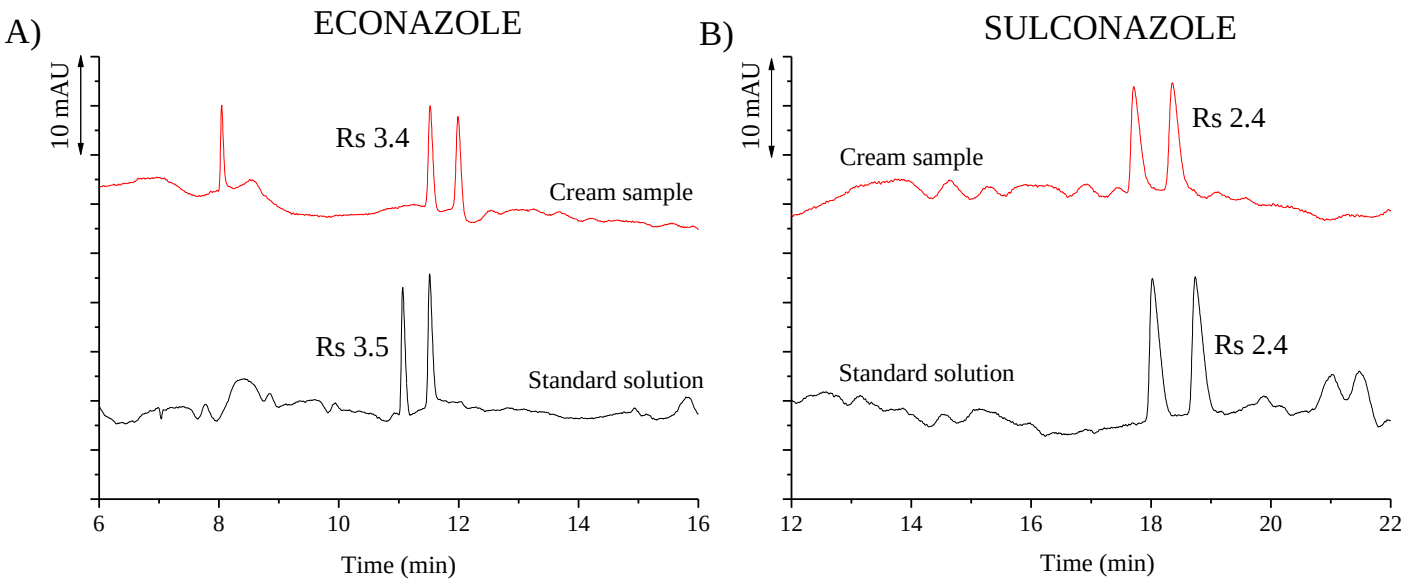

A)
ECONAZOLE
10 mAU
5 mM HP-β-CD
+ 20 mM [TBA][L-Lys]
Rs 3.0
25 °C
Rs 3.5
20 °C
Rs 4.0
15 °C
5
10
15
20
25
Time (min)
B)
SULCONAZOLE
10 mAU
2 mM HP-β-CD
+ 25 mM [TBA][L-Lys]
Rs 1.9
25 °C
Rs 1.9
20 °C
Rs 2.9
15 °C
5
10
15
20
25
Time (min)
C)
10 mAU
5 mM HP-β-CD
+ 20 mM [TBA][L-Glu]
Rs 2.8
25 °C
Rs 3.2
20 °C
Rs 3.5
15 °C
5
10
15
20
25
Time (min)
D)
10 mAU
2 mM HP-β-CD
+ 25 mM [TBA][L-Glu]
Rs 1.9
25 °C
Rs 2.2
20 °C
Rs 3.1
15 °C
5
10
15
20
25
Time (min)

Figure 2.


A)
ECONAZOLE
10 mAU
Rs 3.4
Cream sample
Rs 3.5
Standard solution
6
8
10
12
14
16
Time (min)
B)
SULCONAZOLE
10 mAU
Cream sample
Rs 2.4
Standard solution
Rs 2.4
12
14
16
18
20
22
Time (min)