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Chiral separation of econazole using micellar electrokinetic chromatography with hydroxypropyl-γ-cyclodextrin

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ABSTRACT

A cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) method with hydroxypropyl-γ-cyclodextrin (HP-γ-CD) as chiral selector for the enantiomeric separation of econazole is reported. Enantioseparation of econazole was successfully achieved by the optimized CD-MEKC system containing 40 mM HP-γ-CD, 50 mM SDS and 20 mM phosphate buffer (pH 8) solution with an analysis time of less than 9 min. Calibration curves were linear for the two stereoisomers of econazole ($r^2 > 0.998$). Good repeatabilities in the migration time, peak area and peak height were obtained in terms of RSD% ranging from 0.30 to 7.67%. Combination of solid-phase extraction (SPE) procedure using diol column and the CD-MEKC method was successfully applied to the determination of econazole in a formulated cream sample.

1. Introduction

Chiral separation is one of the important applications of chromatography, especially in the agrochemical and pharmaceutical fields, since it is well known that a pair of enantiomers (two stereoisomers) can display different biological activities. Analytical methods are required for the separation and quantification of enantiomers possessing good resolution, good efficiency and good reproducibility. Recently, many studies on enantiomeric separations by chromatographic techniques have appeared, mainly using liquid chromatography [1].

In recent years, capillary electrophoresis (CE) has been developed as a separation analysis method suitable for routine applications. Its popularity may be attributed to its extremely high efficiency, short analysis time and wide application range. Capillary electrophoresis has shown to be a powerful separation technique for enantiomers compared to conventional chromatographic techniques [2–4]. It has no need for expensive chiral stationary phases, since the chiral selector is simply added to the buffer. Cyclodextrin-modified micellar electrokinetic chromatography (CD-MEKC) has been established as a versatile and robust capillary electrophoresis (CE) method for the separation of enantiomers [5–11]. The advantages of CD-MEKC for enantiomer separations are rapid method development, low consumption of analyte and minimal use of expensive chiral reagents.

Econazole is a chiral imidazole drug (Fig. 1). It has a chiral center and consists of two stereoisomers (a pair of enantiomers). It is widely used for the treatment of topical fungal infection and is used as nitrate salt in a variety of pharmaceutical formulations. Several papers have reported the chiral separation of econazole and other antifungal compounds by HPLC [12,13], SFC [14] and CE [15–17]. The enantiomeric separation of econazole was obtained using CE with sulfobutyl ether-β-cyclodextrin (SBE-β-CD) as chiral buffer modifier [17]. In their work, resolution (R_s) of 2.46 and a migration time of ~13 min was achieved with the use of 0.1 mM SBE-β-CD and 20% methanol. The use of a 1 mM SBE-β-CD in the buffer solution offers no resolution for econazole. They also used a 20 mM HP-β-CD chiral selector with 10% methanol in the buffer solution and a R_s 1.6 was achieved with a separation time of ~16 min. However, the chiral surfactant used i.e. SBE-β-CD, is more expensive compared to the one used in the current study, 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD). Our separation time in the current CD-MEKC method is ~8.5 min (~40% significant time saving) and with no methanol being used as modifier.

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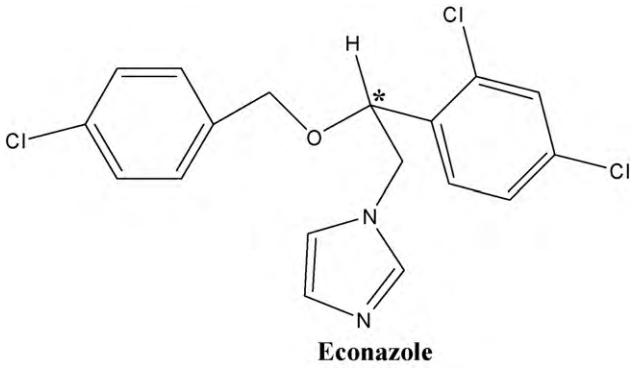

Fig. 1. Chemical structures of azole drugs used in this study (* indicates the position of the chiral center).

Chiral separation of econazole was also achieved with the use of 5 mM HP-β-CD in 50 mM pH 2.5 triethanolamine phosphate buffer with R_s 2.0 but no quantitative study of econozale was performed [16]. Enantioselective separation of econazole was also achieved using EKC (R_s 2.0) with 2 mM HP-β-CD in 100 mM phosphate buffer at pH 3.0 [15]. The high concentration of phosphate buffer used (100 mM) is not favorable as it generates high current and possible Joule heating. The analysis time obtained was ~6.5 min. However, the LODs and the analysis of econazole enantiomers on pharmaceutical samples were not discussed in their works [15–17]. Quantitative determination of econazole nitrate (pK_a = 6.6) in cream formulation by CZE using 20 mM phosphate buffer adjusted to pH 2.5 with phosphoric acid has also been reported with an analysis time of 1.2 min. However, no chiral separation was performed [18]. Quantitative determination of econazole in pessaries using 75 mM acetic acid–acetate buffer in methanol:water (20:80, v/v) (pH 5.18) was also performed and no enantiomeric separation was achieved [19]. The separation time achieved was ~4.3 min. Even though both the CZE analysis time was short, the method would not be suitable for enantiomeric separation as ordered by the US Food and Drug Administration for pharmaceutical, agrochemical and other chemical-based industries to specify the enantiomeric purity of the chiral compound [20]. Thus, there is an increasing demand for the direct method of chiral resolution of enantiomers of the chiral compound. It is thus of interest in the present study to develop a CD-MEKC method for the chiral analysis of econazole using HP-γ-CD, a cheaper chiral surfactant than the one previously used i.e. heptakis (2,3,6-tri-O-methyl)-β-CD (TM-β-CD) [16] (no chiral recognition of econazole was obtained) and SBE-β-CD [17]. It is also of interest to compare the performance of HP-γ-CD compared to HP-β-CD as previously used [16,17] and finally the quantification of econazole in cream formulation of econazole nitrate. Our work focused on the optimization process to achieve the best separation of the two enantiomers of econazole by the developed CD-MEKC method and applying the optimum CD-MEKC conditions to the analysis of econazole in formulated pharmaceutical cream sample.

Derivatized cyclodextrins is one of the most widely used chiral selectors due to their water solubility and low UV absorbance. The HP-γ-CD is a nonionic cyclic oligosaccharides consisting of eight glucose units and has numerous chiral recognition centers. It has been used as chiral selector in the CD-MEKC methods [5–10]. The choice of HP-γ-CD as chiral selector is based on our previous successful triazole fungicides separation [5–8]. The triazole fungicides has a 1,2,3-triazole ring (or a 1,2,4 triazole ring) while econazole, an imidazole fungicide has a 1,2-diazole ring. The similar structure between the two prompted us to use our previous successful CD-MEKC conditions with HP-γ-CD as the chiral selector in the current work since there is no report about the chiral separation of econazole by CD-MEKC method. Effects of HP-γ-CD concentration, SDS concentration, buffer pH and buffer concentration on the enantioresolution of econazole are investigated. Combination of solid-phase extraction (SPE) procedure using a diol column and the CD-MEKC method is then applied to the analysis of commercial formulation (cream) of econazole.

2. Experimental

2.1. Chemicals and reagents

Econazole nitrate and 2-hydroxypropyl-γ-cyclodextrin (HP-γ-CD) were obtained from Sigma (St. Louis, MO, USA), sodium dodecyl sulfate (SDS) was obtained from Fisher Scientific (Loughborough, UK), and disodium hydrogen phosphate 12-hydrate was obtained from Riedel-de Haen (Seelze, Germany). All other chemicals and solvents were common brands of analytical-reagent grade or better, and were used as received. Water (18 MΩ cm) was collected from a Millipore Water Purification System (Molsheim, France). Cartridges containing 500 mg/6 mL of DSC-diol sorbent were obtained from Supelco (Bellefonte, PA, USA). Formulated cream sample (econazole nitrate 1%, w/w) was purchased from a local drug store in Johor, Malaysia.

The stock solutions of the econazole were prepared in methanol in the concentration of 2000 mg L^{-1}. Final dilutions (concentrations in the figures) were prepared by diluting the stock solution with methanol. The separation solutions for CD-MEKC were prepared by dissolving appropriate amounts of SDS and HP-γ-CD in phosphate buffers and adjusting the pH of the buffer with phosphoric acid solution. All running buffers were filtered through a 0.45 μm nylon syringe filter from Whatman (Clifton, NJ, USA).

2.2. Instrumentation

All electropherograms were obtained with the Agilent capillary electrophoresis system from Agilent Technologies (Waldbronn, Germany), equipped with temperature control and diode array detection (DAD). Separations were performed using an untreated fused silica capillary of 64.5 cm × 50 μm i.d. (with an effective length of 56 cm) obtained from Polymicro Technologies (Phoenix, AZ, USA). Sample injection was performed electrokinetically at 3 kV for 3 s. The detection wavelength used was 200 nm. Separation voltage used was 30 kV and separation temperature used was maintained at 25 °C. Data were collected and processed on computer using ChemStation software (Agilent Technologies). The new capillary was conditioned with 1 M NaOH solution for 5 min followed by deionized water for 10 min and finally with an appropriate running buffer for 10 min. Between runs, the capillary was rinsed with 0.1 M NaOH, water and run buffer for 2 min each.

2.3. Extraction procedure

The SPE procedure used in this study was adopted from a previous study [21] with some modifications. A sample equivalent to about 2 mg of econazole nitrate (200 mg cream sample) was treated with 5 mL of dichloromethane and, after a 2 min sonication, the volume was adjusted to 10 mL with the same solvent. The resulting opalescent solution was filtered and 2 mL aliquot of the clarified solution was passed under vacuum through a diol solid-phase extraction column, which was preconditioned with 6 mL of dichloromethane. After application of the sample, the column was washed with two 3-mL portions of *n*-hexane-dichloromethane (4:1, v/v) and aspirated to dryness. The retained analyte was then eluted with three 1-mL portions of methanol under aspiration and

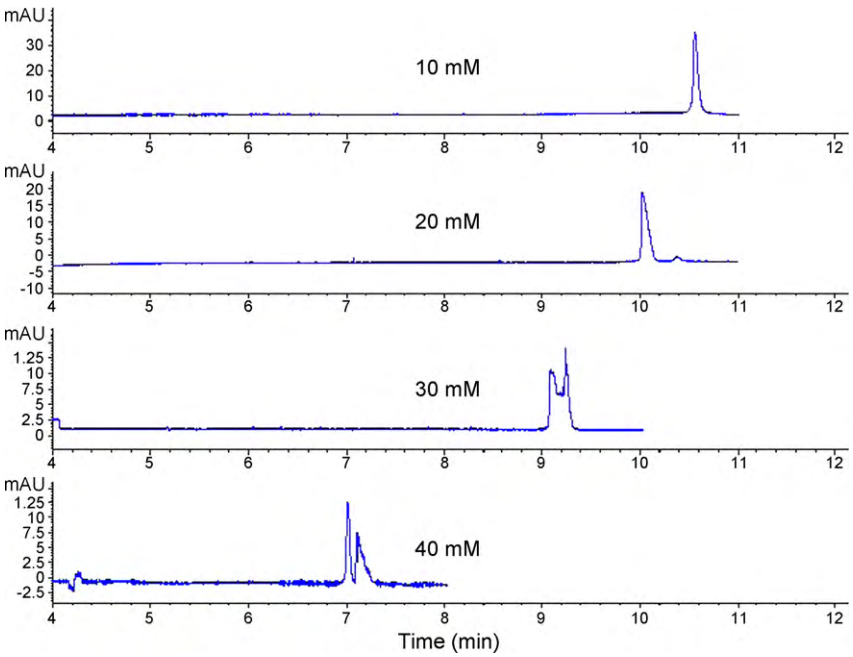

Fig. 2. Enantiomeric separation of econazole using CD-MEKC at different HP-γ-CD concentrations. Separation solution: 10–40 mM HP-γ-CD, 40 mM SDS in 20 mM phosphate buffer (pH 7); capillary, 64.5 cm × 50 μm I.D. (effective length, 56 cm); applied voltage, 30 kV; temperature, 25 °C; detection wavelength, 200 nm; injection, 3 kV per 3 s; analyte concentration, 200 mg L^{-1}.

the combined eluates were diluted to 5 mL with methanol. The resulting solution was used for the CE analysis.

2.4. Analytical characteristics of the method

The performance of the method was examined in terms of the linearity, repeatability, limit of detection (LOD) and limit of quantification (LOQ). Linearity of the optimized method was assessed by constructing the calibration curve of average peak areas ($n = 3$) against the concentration of standards (at the linear range). The repeatabilities in the migration time, peak area and peak height were recognized in terms of the relative standard deviation (RSD%, $n = 3$). The LOD was determined by the calibration curve along with the signal-to-noise ratio (S/N) as 3 and the limit of quantification as S/N = 10.

3. Results and discussion

Initially, CD-MEKC method used in this present study was adopted from our previous studies on the chiral separation of triazole compounds [5–8] with some modifications. The HP-γ-CD concentration was first varied in order to achieve the best separation of econazole enantiomers using CD-MEKC. The HP-γ-CD concentration range was optimized from 10 to 40 mM in 10 mM increment (with 40 mM SDS in 20 mM phosphate buffer solution, pH 7.0). Typical enantiomeric separation of econazole by CD-MEKC at different HP-γ-CD concentrations is shown in Fig. 2. It can be seen that there was no chiral resolution for econazole enantiomers at 10 and 20 mM HP-γ-CD. While at 30 mM HP-γ-CD, econazole enantiomers were only partially separated. Enantioseparation of econazole was successfully achieved at 40 mM HP-γ-CD as chiral selector ($R_s = 1.02$). This concentration was employed in all subsequent investigations.

The migration time of econazole enantiomers were gradually shortened with stepwise increase in the concentration of HP-γ-CD. A reason behind this observation is expected since with increasing HP-γ-CD concentration more of the econazole enantiomers will become included in the HP-γ-CD phase and will be transported quickly towards the detector. The addition of HP-γ-CD to the buffer displace the distribution of the enantiomers from the micellar to the water phase as a function of the possible interaction between the water soluble HP-γ-CD and the enantiomers. The results obtained in this work are similar with those obtained by other authors on the chiral separation of polychlorinated biphenyls [10] and pemoline enantiomers [11] by CD-MEKC methods.

Chiral recognition should be co-contributed by several interactions, such as electrostatic force, steric effects, hydrophobicity and hydrogen-bonding interaction. Econazole contains the diazole ring, chlorophenyl group and alkyl group near the chiral center (Fig. 1). It is probable that diazole ring was the structural moiety that participated in the electrostatic interactions with HP-γ-CD. While the chlorophenyl group and alkyl group were the structural moiety that participated in the hydrophobic interaction with the cavity of the HP-γ-CD. In addition, the hydroxyl group nears the chiral center enabled the hydrogen-bonding of this compound with the rim of the HP-γ-CD cavity. In summary, this suggests that electrostatic interaction, hydrophobic and hydrogen-bonding interactions between the azole drug and HP-γ-CD play a critical role in this chiral recognition mechanism.

The effect of SDS concentration was then investigated in this work, ranging from 30 to 60 mM (with 40 mM HP-γ-CD in 20 mM phosphate buffer solution, pH 7.0). Typical enantiomeric separation of econazole by CD-MEKC at different SDS concentrations is shown in Fig. 3. An increase in the concentration of SDS from 30 to 50 mM caused a significant increase in the enantioresolution ($R_s = 1.46$). However, further increase in the concentration of SDS up to 60 mM caused decrease in enantioresolution ($R_s = 0.85$). In addition, an increase in the concentration of SDS caused a significant increase in the migration time of econazole enantiomers due to the increasing viscosity of the solution at increasing SDS concentration. This observation has also been reported by other authors [8,17,18]. In light of these aspects, optimal concentration chosen for SDS was 50 mM.

The effects of phosphate buffer pH and buffer concentration on the enantioresolution of econazole were also investigated. Chi-

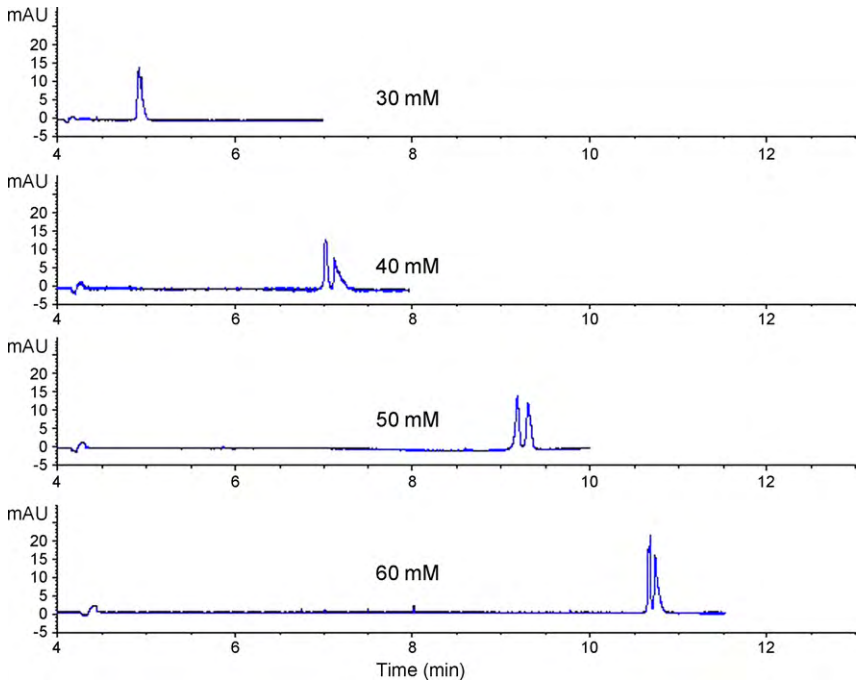

Fig. 3. Enantiomeric separation of econazole using CD-MEKC at different SDS concentrations. Separation solution: 40 mM HP-γ-CD, 30–60 mM SDS in 20 mM phosphate buffer (pH 7); other conditions are as in Fig. 2.

ral separation of econazole was investigated in the pH range from 7 to 9 (with 40 mM HP-γ-CD + 50 mM SDS in 20 mM phosphate buffer). The best enantioresolution was obtained at pH 8 (R_s = 2.19) (Fig. 4). In addition, increasing phosphate buffer concentration from 15 to 30 mM resulted in increase of migration time of enantiomers while resolutions were not significantly increased (data not shown). Optimal concentration chosen for phosphate buffer (pH 8) was 20 mM, since it yielded very good resolution (R_s = 2.19) with relatively short migration time (less than 9 min).

Linearity, repeatability, limit of detections (S/N = 3) and limit of quantification of the optimized CD-MEKC method are summarized in Table 1. Calibration curves were linear for all enantiomers with r^2 greater than 0.998. Good repeatabilities were obtained in terms of RSD% (n = 3) for migration time, peak area and peak height ranging from 0.30 to 7.67%. The limit of detection was from 3.6 to 4.3 μg mL^{-1} and the limit of quantification was from 12.1 to 14.3 μg mL^{-1}.

Combination of the modified solid-phase extraction (SPE) procedure using diol column [21] and the CD-MEKC method was

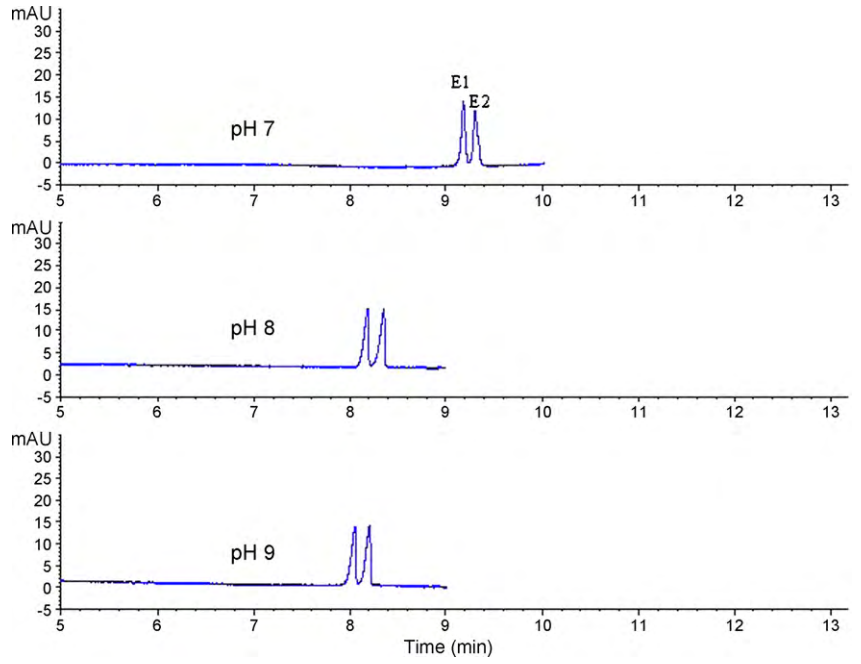

Fig. 4. Enantiomeric separation of econazole using CD-MEKC at different buffer pH. Separation solution: 40 mM HP-γ-CD, 50 mM SDS in 20 mM phosphate buffer; other conditions are as in Fig. 2.

Table 1
Linearity, repeatability, LOD (S/N = 3) and LOQ (S/N = 10) for separation of econazole enantiomers in the optimized CD-MEKC method.

Parameter	Peak-1[a]	Peak-2[a]
Concentration range (μg/mL)	50–200	50–200
Regression equation[b]	$y = 0.3059x - 2.7050$	$y = 0.3081x - 2.4100$
r^2	0.9997	0.9996
RSD (%, $n = 3$); (intra-day/inter-day)		
Migration time	0.30/1.89	0.36/1.88
Peak area	1.15/2.12	1.66/6.43
Peak height	1.23/7.67	2.16/5.29
LOD (μg/mL)	3.6	4.3
LOQ (μg/mL)	12.1	14.3

Conditions as in Fig. 4 (pH 8).
[a] 1 and 2 are first and second migrating econazole enantiomers, respectively.
[b] Linearity range: 50–200 μg/mL; y = peak area; x = concentration (μg/mL).

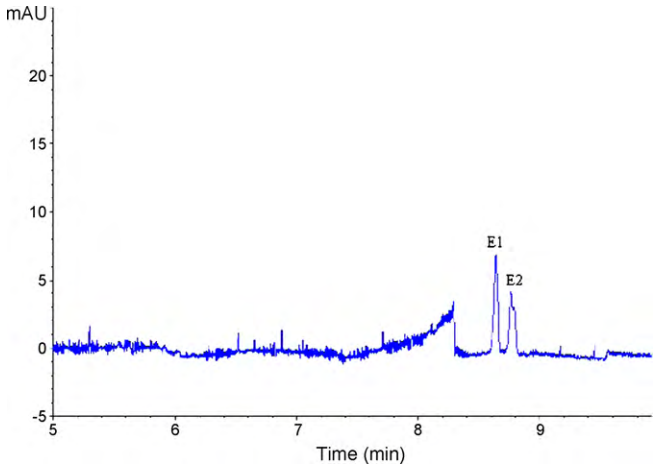

Fig. 5. Electropherogram of econazole cream sample after SPE pretreatment. The CD-MEKC conditions are as in Fig. 4 (pH 8).

Table 2
Results obtained in the analysis of commercial cream sample containing econazole nitrate using the proposed CD-MEKC method.

Results	Econazole nitrate in sample
Amount labelled (%)	1
Amount found[a] (%)	0.93
Percentage found (%)	93.00
RSD (%, $n = 3$)	8.05

[a] Average of three determinations

also successfully applied to the analysis of econazole in the formulated cream sample (labeled amount of econazole nitrate = 1%, w/w). It can be seen in, that no interference peak was observed in the electropherogram (Fig. 5). As can be seen in Table 2, result shows a relatively good accuracy with amount of econazole nitrate analyzed in the formulated cream sample = 93% (RSD% = 8.05, $n = 3$).

The proposed method showed better R_s (2.19) and significant reduction in the analysis time (~40% reduction) compared to the work which used 20 mM HP-β-CD with 10% methanol [17] and a ~30% significant reduction in analysis time compared to the one which used 0.1 mM SBE-β-CD [17]. Comparable performance (R_s and analysis time) with the analysis which used 15 mM HP-β-CD in 50 mM pH 2.5 triethanolamine phosphate buffer [16] was obtained with the current work. Even though the work which used 100 mM phosphate buffer at pH 3.0 containing 2 mM HP-β-CD produce a shorter analysis time, the concentration of the phosphate buffer used is rather high which is not favourable as it may generate high current and possible Joule heating effect.

4. Conclusion

The enantioseparation of econazole was successfully performed using a mixture of 40 mM HP-γ-CD, 50 mM SDS in 20 mM phosphate buffer solution (pH 8). The method was able to separate the two stereoisomers of econazole with good resolution (R_s) (>2.0) in a relatively short analysis time (<9 min). This is the first CD-MEKC separation of econazole enantiomers achieved using a neutral CD (HP-γ-CD) as chiral selector. The proposed method offers enantioseparation of econazole at a reasonable analysis time using an easily available and cheap CD. Even though there are CZE methods that offer shorter analysis, no enantioseparation of econazole was obtained and thus does not lend the method for enantiomeric purity check. Calibration curves were linear for all enantiomers ($r^2 > 0.998$). The repeatabilities in the CD-MEKC method concerning the RSD% ($n = 3$) for migration time, peak area and peak height were generally good ranging from 0.30 to 7.67%. The present CD-MEKC method is applicable for the determination of econazole in formulated cream sample and can be used as a preliminary study for further research on the chiral analysis of azole drugs on other samples. Excellent recovery of econazole (93% with RSD 8.05%) was obtained and with the presented CD-MEKC method using electrokinetic injection, it is possible to detect econazole down to 3.6–4.3 mg L^{-1}.

Conflict of interest

The authors declared that they do not have any conflict of interest.

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