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## Research Article

# Enantioselective separation of azole compounds by EKC. Reversal of migration order of enantiomers with CD concentration

The enantioselective separation of a group of six weak base azole compounds was achieved in this work using EKC with three neutral  $\beta$ -CDs as chiral selectors. The native  $\beta$ -CD and two other  $\beta$ -CD derivatives with different types and positions of the substituents on the CD rim ((2-hydroxy)propyl- $\beta$ -CD (HP- $\beta$ -CD) and heptakis-2,3,6-tri-*O*-methyl- $\beta$ -CD (TM- $\beta$ -CD)) were employed. Apparent binding constants for each pair compound-CD were determined in order to study analyte-CD interactions. The best enantiomeric resolutions for miconazole, econazole, and sulconazole were observed with HP- $\beta$ -CD whereas for the separation of the enantiomers of ketoconazole, terconazole, and bifonazole, TM- $\beta$ -CD was the best chiral selector. The enantioseparations obtained were discussed on the basis of the structure of the compounds taking into account that inclusion into the hydrophobic CD cavity occurred through the phenyl ring closer to the azole group. In addition, a change in the migration order for the enantiomers of two of the compounds studied (ketoconazole and terconazole) with the concentration of HP- $\beta$ -CD was observed for the first time.

### Keywords:

Apparent binding constants / Azole compounds / CD / EKC / Enantioseparation

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## 1 Introduction

Enantioseparations by CE rely on the same principle as in chromatographic techniques, that is, in the enantioselective interaction between a chiral selector and the chiral analyte using mainly the CE mode named EKC [1–7]. CE has a great potential for chiral separations because it offers interesting advantages (fast screening, high peak efficiency, and flexibility) for the detection of very fine enantioselective effects. Moreover, this technique is very useful in order to study analyte-CD binding interactions, since it enables the measurement of binding constants exactly under the same conditions under which enantioseparations are performed without adaptations or approximations [5, 8, 9]. Another interesting

aspect of CE is the possibility of changing the migration order of the enantiomers of a chiral compound. This aspect is crucial in several instances such as when the optical purity of one of the enantiomers has to be determined.

There are different ways to change the enantiomeric migration order by CE when CDs are used as chiral selectors [4, 10–13]: (i) modifying the CD cavity size [14]; (ii) changing the nature of CD substituents [15, 16]; (iii) varying the location of substituents on the CD rim [17]; (iv) employing charged CDs in the BGE and changing the polarity of the applied voltage [18, 19]; (v) modifying the pH of the BGE [18, 20]; (vi) changing the EOF, *i.e.*, eliminating or reversing it [19, 20]; (vii) using achiral additives (*i.e.*, micelles or ligand-exchange compounds as metals) in chiral systems [21, 22], and finally, (viii) varying the concentration of the CD in the separation buffer. However, this last phenomenon has scarcely been observed. Thus, reversal in the migration order of enantiomers with the CD concentration was only described for (2-hydroxy)propyl- $\beta$ -CD (HP- $\beta$ -CD) [23–25], heptakis(2,3-dimethyl-6-sulfato)- $\beta$ -CD [26], and  $\gamma$ -CD [27]. The variation of the concentration of HP- $\beta$ -CD enabled a reversal in the migration order for the two enantiomers of dansylated phenylalanine at pH 6 [23, 24] and pH 2.5 [25] and also for the two enantiomers of dansylated tryptophan at pH 2.5 [25].

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**Abbreviations:** HP- $\beta$ -CD, (2-hydroxy)propyl- $\beta$ -CD; TM- $\beta$ -CD, heptakis-2,3,6-tri-*O*-methyl- $\beta$ -CD

With respect to heptakis(2,3-dimethyl-6-sulfato)- $\beta$ -CD, this CD enabled a change in the migration order for the enantiomers of phenyl compounds (1-phenyl-1-butanol, 1-phenyl-2-butanol, 1-phenyl-1-pentanol, 2-phenyl-2-pentanol) when phosphate buffer at pH 2.5 was used [26]. More recently, the reversal in the migration order for the enantiomers of promethazine, ethopropazine, and trimeprazine with the  $\gamma$ -CD concentration was reported in citrate buffer at pH 3.0 [27].

In this work, the enantioseparation of six weak baseazole compounds by EKC using three neutral  $\beta$ -CDs is studied on the basis of the determination of the apparent analyte-CD binding constants. The compounds studied (ketoconazole, terconazole, bifonazole, miconazole, econazole, and sulconazole) have at least one chiral center and are characterized by their antifungal activity [28]. The inversion in the migration order of the enantiomers of some of the compounds studied with the HP- $\beta$ -CD concentration observed for the first time for these kind of compounds is also discussed.

## 2 Materials and methods

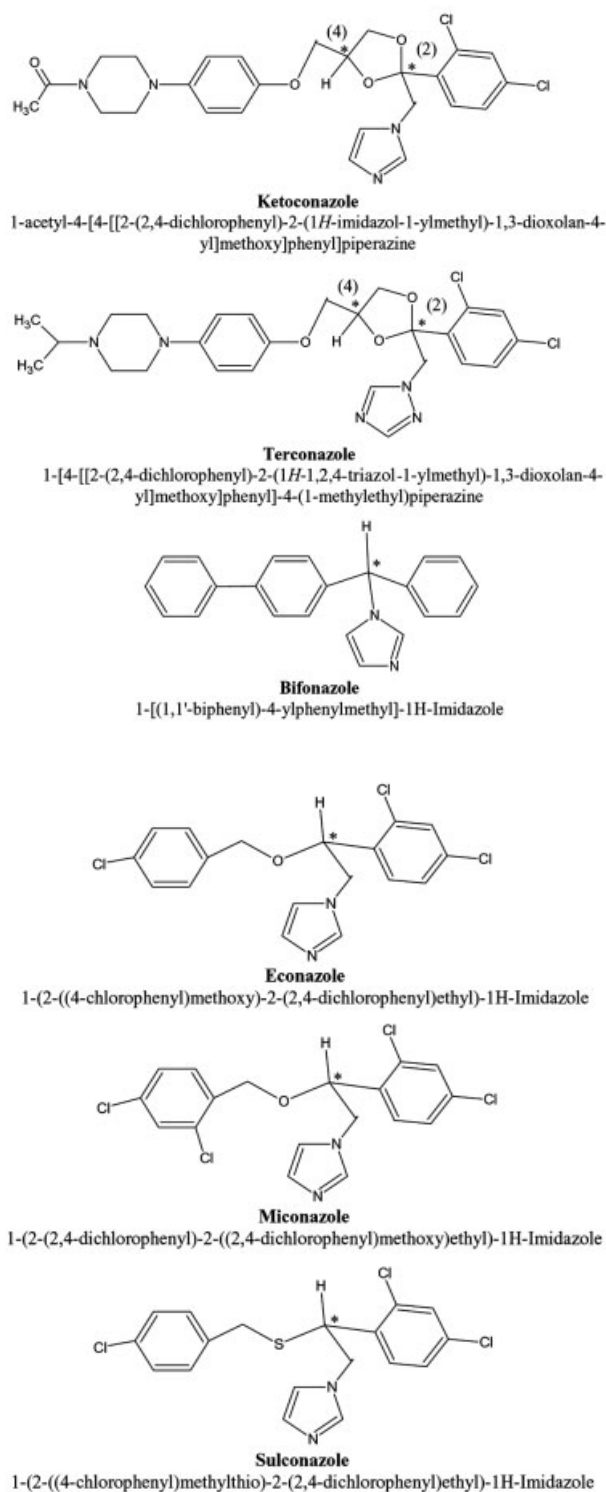
### 2.1 Reagents and samples

A racemic mixture of ketoconazole, miconazole, econazole, sulconazole, and bifonazole was supplied from Sigma (St. Louis, MO, USA). Terconazole was kindly provided as racemate and as pure 2*R*,4*S*-enantiomer by Johnson & Johnson (Beerse, Belgium). The structures of these compounds are shown in Fig. 1. It is important to remark that although ketoconazole and terconazole have two chiral centers, only two enantiomers are present in the racemic mixture of each one because the *cis*-configuration is present in both enantiomers (*i.e.*, the hydrogen and the 2,4-dichlorophenyl group at the two chiral centers are on the same side of the dioxolane ring, see Fig. 1).

All reagents employed for the preparation of the BGE were of analysis grade. Orthophosphoric acid, sodium hydroxide, and hydrochloric acid were supplied from Merck (Darmstadt, Germany).  $\beta$ -CD, HP- $\beta$ -CD, heptakis-2,3,6-tri-O-methyl- $\beta$ -CD (TM- $\beta$ -CD), and DMSO were purchased from Fluka (Buchs, Switzerland). Water used to prepare solutions was purified through a Milli-Q system from Millipore (Bedford, MA, USA).

### 2.2 Apparatus

An HP<sup>3D</sup> CE system from Agilent Technologies (Palo Alto, CA, USA) equipped with an on-column DAD was employed. Instrument control and data acquisitions were performed with the HP<sup>3D</sup>-CE ChemStation software. Separations were performed on uncoated fused-silica capillaries of 50  $\mu$ m id (375  $\mu$ m od) with a total length of 48.5 cm (40 cm to the detector) or with a total length of 56 cm (64.5 cm to the detector) purchased from Composite Metal Services (Worces-



**Figure 1.** Structures of theazole compounds studied.

ter, England). A 744 pH-meter from Metrohm (Herisau, Switzerland) was employed to adjust the pH of the separation buffers.

### 2.3 CE conditions

Before its first use, a new capillary was rinsed with 1 M NaOH for 30 min, followed by 5 min with water, 5 min 0.1 M HCl, and conditioned with phosphate buffer (pH 3.0) for 60 and 30 min, the BGE used for separation. When the BGE was changed, the capillary was conditioned with the new BGE for 30 min. Between runs, the capillary was rinsed with BGE for 2 min.

The selected instrumental conditions were: capillary temperature 15°C, injection by pressure, 50 mbar for 4–6 s, applied voltage 30 kV, and UV detection at 200 nm with a bandwidth of 10 nm, and a response time of 0.1 s.

Buffer solutions were prepared diluting the appropriate volume of orthophosphoric acid with Milli-Q water, adjusting the pH to the desired value (pH 3.0) with 0.1 M sodium hydroxide solution before completing the volume with water to get the desired buffer concentration (0.1 M). Finally, BGEs were prepared dissolving the appropriate amount of different CD in the buffer solution. Thus, CD concentrations ranged from 0.1 to 15 mM for  $\beta$ -CD; from 0.1 to 120 mM for HP- $\beta$ -CD, and from 0.1 to 50 mM for TM- $\beta$ -CD.

The stock standard solutions of differentazole compounds were prepared by dissolving them in DMSO up to a final concentration of 2000 mg/L. These solutions were stored at 5°C. From these stock solutions, dilutions in phosphate buffer (pH 3.0) were made to obtain a concentration of 200 mg/L for each compound, except for ketoconazole, which was prepared with a concentration of 100 mg/L in a mixture with terconazole. In addition, enantiomer migration order was studied in solutions of racemic ketoconazole (20 mg/L) spiked with one of the enantiomers (2*R*,4*S*-form at 10 mg/L).

All these solutions (buffers, BGEs, and standards) were filtered prior to use through 0.45  $\mu$ m pore size disposable nylon filters from Titan (Eatontown, NJ, USA).

### 2.4 Data treatment

The values of resolution between adjacent peaks for the enantiomers were obtained from the migration times of the enantiomers and their peak widths at half height using the ChemStation software.

From the experimental raw data, the electrophoretic mobility for each enantiomer ( $\mu_i$ ) was determined by the following equation [29]:

$$\mu_i = \frac{l_d l_T}{V} \left( \frac{1}{t_i} - \frac{1}{t_0} \right) \quad (1)$$

where  $l_T$  and  $l_d$  are the total capillary length and the length to the detector, respectively,  $V$  is the run voltage,  $t_i$  the enantiomer migration time, and  $t_0$  the migration time of DMSO, used as a neutral marker to correct changes in solution viscosity caused by variations in CD concentration. The electrophoretic mobilities of the free solutes were calculated at a zero concentration of CD, being equal for both enantiomers.

Assuming a 1:1 stoichiometry, apparent binding constants (calculated considering concentrations instead activities) can be determined using the following expression [29, 30]:

$$K[L] = \left( \frac{\mu_f - \mu_i}{\mu_i - \mu_c} \right) \quad (2)$$

where  $K$  is the apparent binding constant,  $[L]$  the equilibrium concentration of uncomplexed ligand,  $\mu_f$  and  $\mu_c$  are the electrophoretic mobilities of the free and complexed solute, respectively, and  $\mu_i$  is the solute mobility at the ligand concentration  $[L]$ .  $L$  is the chiral selector, *i.e.*, the CD. Thus, given that the CD concentration is considerably higher than the analyte concentration (more than 1000 times), the equilibrium concentration of CD is considered to be approximately the CD concentration added to the separation media.

In order to avoid the measurement of the mobility of the complex,  $\mu_c$ , required in Eq. (2), this equation can be rearranged in many plotting forms [29, 30]. In this work, the  $\gamma$ -reciprocal approach was used:

$$\frac{[L]}{\mu_i - \mu_f} = \frac{1}{\mu_c - \mu_f} [L] + \frac{1}{(\mu_c - \mu_f)K} \quad (3)$$

The uncertainties in the binding constants listed in Table 1 were calculated by error propagation methods using the errors in the slopes and intercepts obtained by least squares methods [31].

Finally, from the binding constant values for each enantiomer, the optimal concentrations of the chiral selector ( $C_{opt}$ ) [29], and the enantioselectivities of complexation ( $\alpha$ ) [30] were calculated using the following equations:

$$C_{opt} = \frac{1}{(K_1 K_2)^{1/2}} \quad (4)$$

$$\alpha = \frac{K_i}{K_j} \quad (5)$$

where  $K_1$  is the binding constant for the first-migrating enantiomer and  $K_2$  for the second-migrating enantiomer, and  $K_i$  and  $K_j$  could be either  $K_1$  or  $K_2$  but always with  $K_i > K_j$ .

Experimental data analysis and parameters were calculated using Excel Microsoft XP® and Origin® version 7.0 software. Graphs with different electropherograms were composed in Origin version 7.0 software.

## 3 Results and discussion

### 3.1 Study of the enantioselectivity through apparent analyte–CD binding constants

Assuming a 1:1 stoichiometry, apparent binding constants for the six azole compounds were determined by using the  $\gamma$ -reciprocal approach (see Section 2.4). This supposition was supported by previous works where the relation between the

**Table 1.** Apparent binding constants ( $K_1$  for the first-migrating enantiomer and  $K_2$  for the second-migrating enantiomer), enantioselectivities of complexation ( $\alpha$ ), and optimal CD concentrations ( $C_{\text{opt}}$ ) calculated using the  $\gamma$ -reciprocal approach (see equations in Section 2.4), and experimental values of the CD concentration for maximum enantiomeric resolution ( $C_{R_s}$ ) and values for the maximum enantiomeric resolution ( $R_s$ ) obtained for the sixazole compounds studied in this work with the three CDs employed as chiral selectors

Compound	Concentration range (mM)	$K_1$ ( $M^{-1}$ )	$K_2$ ( $M^{-1}$ )	$\alpha$	$C_{\text{opt}}$ (mM)	$C_{R_s}$ (mM)	$R_s$
<b><math>\beta</math>-CD</b>							
Ketoconazole	1–15 ( $r = 0.998$ ; $n = 5$ )	$566 \pm 105$	$566 \pm 105$	1.00	–	–	0.0
Terconazole	1–15 ( $r = 0.998$ ; $n = 5$ )	$610 \pm 110$	$610 \pm 110$	1.00	–	–	0.0
Bifonazole	1–15 ( $r = 0.999$ ; $n = 5$ )	$2767 \pm 552$	$2767 \pm 552$	1.00	–	–	0.0
Miconazole	0.5–15 ( $r = 0.999$ ; $n = 6$ )	$749 \pm 91$	$932 \pm 120$	1.24	1.2	1	1.8
Econazole	0.5–15 ( $r = 0.998$ ; $n = 6$ )	$622 \pm 97$	$737 \pm 121$	1.18	1.5	2	1.3
Sulconazole	0.1–15 ( $r = 0.999$ ; $n = 7$ )	$2357 \pm 611$	$2394 \pm 640$	1.02	0.4	0.5	0.5
<b>HP-<math>\beta</math>-CD</b>							
Ketoconazole	0.1–2 ( $r = 0.998$ ; $n = 4$ )	$2477 \pm 421$	$2495 \pm 347$	1.01	0.4	1	0.9
	10–120 ( $r = 0.999$ ; $n = 6$ )	$142 \pm 23$	$146 \pm 22$	1.03	7.0	25	1.9
Terconazole	0.1–2 ( $r = 0.999$ ; $n = 4$ )	$1488 \pm 84$	$1504 \pm 38$	1.01	0.7	1	0.5
	40–120 ( $r = 0.999$ ; $n = 5$ )	$47.4 \pm 3.2$	$46.8 \pm 3.2$	1.01	21.2	60	0.9
Bifonazole	0.1–20 ( $r = 0.999$ ; $n = 8$ )	$2487 \pm 557$	$2487 \pm 557$	1.00	–	–	0.0
Miconazole	0.1–20 ( $r = 0.999$ ; $n = 8$ )	$855 \pm 62$	$1012 \pm 78$	1.18	1.1	1	2.2
Econazole	0.1–20 ( $r = 0.999$ ; $n = 8$ )	$584 \pm 32$	$719 \pm 42$	1.23	1.5	2	2.0
Sulconazole	0.1–20 ( $r = 0.999$ ; $n = 8$ )	$2161 \pm 306$	$2406 \pm 389$	1.11	0.4	0.5	1.2
<b>TM-<math>\beta</math>-CD</b>							
Ketoconazole	5–30 ( $r = 0.996$ ; $n = 6$ )	$53.8 \pm 3.7$	$62.9 \pm 3.9$	1.17	17.2	20	5.2
Terconazole	2–30 ( $r = 0.992$ ; $n = 7$ )	$89 \pm 10$	$104 \pm 11$	1.16	10.4	10	1.7
Bifonazole	0.1–10 ( $r = 0.999$ ; $n = 6$ )	$1590 \pm 239$	$1885 \pm 252$	1.19	0.6	2	1.4
Miconazole	2–30 ( $r = 0.997$ ; $n = 7$ )	$113 \pm 10$	$122 \pm 10$	1.09	8.5	10	1.1
Econazole	20–50 ( $r = 0.999$ ; $n = 6$ )	$91.6 \pm 2.9$	$90.4 \pm 3.3$	1.01	11.0	50	0.5
Sulconazole	2–30 ( $r = 0.998$ ; $n = 7$ )	$198 \pm 19$	$215 \pm 21$	1.09	4.8	5	0.9

structure and enantioseparation of different *N*-imidazole derivatives was studied by using neutral CDs or anionic highly sulfated CDs [32–34]. In order to calculate the apparent analyte–CD binding constants for the six compounds studied in this work with three neutral  $\beta$ -CDs ( $\beta$ -CD, HP- $\beta$ -CD, and TM- $\beta$ -CD), the mobilities of the enantiomers of each compound were measured at different concentrations of chiral selector. To reach a reasonable precision by computer fitting of data ( $[L]/(\mu_i - \mu_f)$  vs.  $[L]$ ; see Eq. 3), from four to eight pairs of raw data were considered for each compound and each one of the CDs employed (see Table 1). In all cases, good linear fitting was obtained with correlation coefficients higher than 0.99.

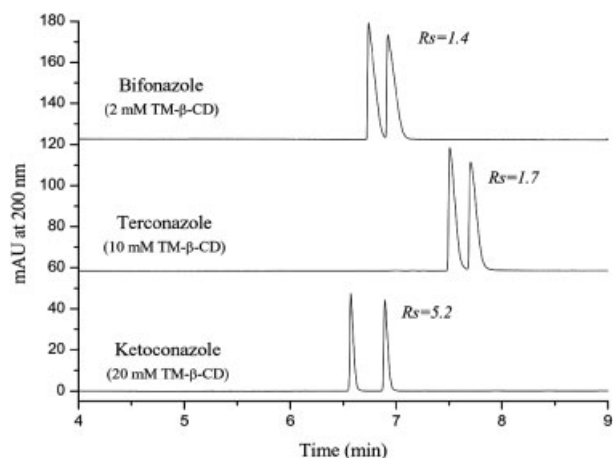
Table 1 groups the apparent binding constants obtained for the three neutral  $\beta$ -CDs and the six compounds studied (see Eq. 3), the enantioselectivities of complexation (see Eq. 5), and the optimal CD concentrations calculated from the binding constants (see Eq. 4). This table also shows the experimental CD concentrations for maximum enantiomeric resolution and the experimental values obtained for this resolution. For all the compounds studied, stronger interactions were observed with the native  $\beta$ -CD than with TM- $\beta$ -CD. In the case of HP- $\beta$ -CD, two different ranges of CD concentration showed enantioresolution for some com-

pounds such as ketoconazole and terconazole being possible the determination of two binding constants for each compound (see Table 1). In the low mM range, higher enantiomer–HP- $\beta$ -CD interactions than at high HP- $\beta$ -CD concentrations were observed. Moreover, interactions of bifonazole, econazole, and sulconazole with  $\beta$ -CD were higher than with HP- $\beta$ -CD while for miconazole, similar interactions were established with  $\beta$ -CD and HP- $\beta$ -CD.

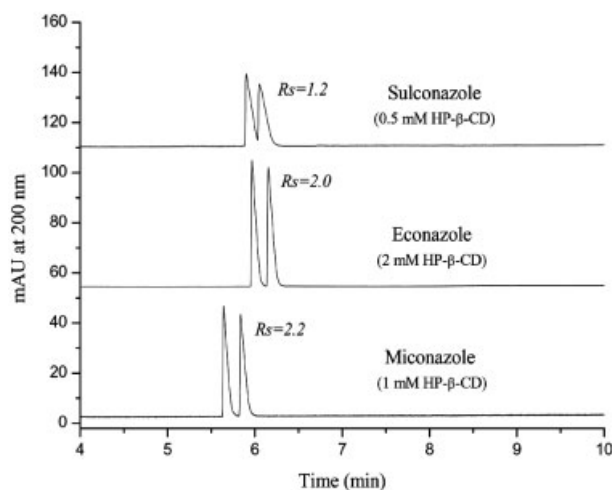
As it can be observed in Table 1, the highest analyte–CD interactions (*i.e.*, the highest apparent binding constant values) were not related to the highest enantioselectivities, fact that has already been reported for otherazole derivatives with neutral and highly sulfated CDs [32, 33]. Thus, TM- $\beta$ -CD was the best chiral selector for the separation of the enantiomers of ketoconazole, terconazole, and bifonazole. However, in this case, TM- $\beta$ -CD–analyte binding constants were lower than those obtained for  $\beta$ -CD and HP- $\beta$ -CD. On the other hand, the best enantiomeric resolutions for miconazole, econazole, and sulconazole were obtained with HP- $\beta$ -CD and only miconazole presented the highest binding constant compared with  $\beta$ -CD and TM- $\beta$ -CD. In the case of econazole and miconazole, binding constants with HP- $\beta$ -CD have already been reported in the literature using another model [35]. These reported binding constant values were

similar to those obtained in this work in the case of econazole. However, for miconazole slightly higher values than those reported previously were observed in this work, which could be due to the fact that EOF was neglected in the referenced article [35]. Although this assumption was performed for both compounds, miconazole was more affected because its interaction with HP- $\beta$ -CD was higher and its mobility was closer to the EOF mobility.

Figure 2 shows the electropherograms corresponding to the enantiomeric separation of bifonazole, terconazole, and ketoconazole with TM- $\beta$ -CD using CD concentrations that gave rise to the highest enantioselective recognition for these three analytes. Although the two latter compounds had been separated previously by our research group using this neutral CD [36], it is the first time that the enantiomers of bifonazole have been separated using the neutral TM- $\beta$ -CD. In fact, bifonazole had enantiomerically been separated with a partial resolution ( $R_s$  1.0) using the anionic sulfobutyl ether- $\beta$ -CD (approximately three times more expensive than TM- $\beta$ -CD) in a buffer solution containing methanol which originated a higher analysis time (double than in this work) [37]. On the other hand, Fig. 3 shows the electropherograms corresponding to the enantiomeric separation of sulconazole, econazole, and miconazole with HP- $\beta$ -CD using the CD concentrations that enabled the best enantiomeric resolutions for these compounds. Although the baseline enantiomeric separation of miconazole [37] and econazole [35, 38] had previously been performed using this neutral CD, it is the first time that the separation of the enantiomers of sulconazole is achieved using a neutral CD as chiral selector. In fact, in spite of the similitude of its chemical structure with the chemical structure of econazole, the enantiomers of sulconazole had only been separated when a dextrin was used as chiral selector ( $R_s$  1.9) [39].



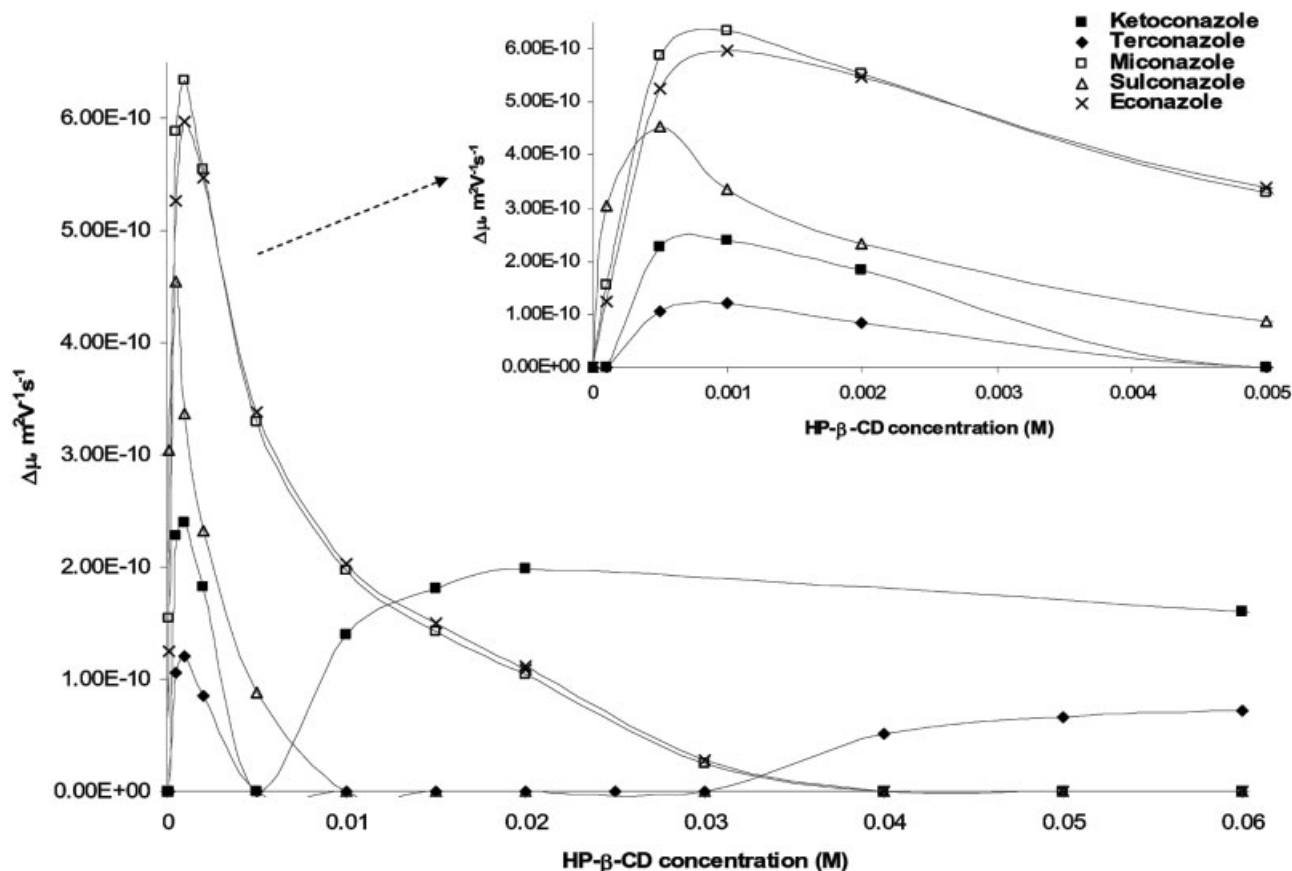
**Figure 2.** Electropherograms showing the best enantiomeric separations for ketoconazole, terconazole, and bifonazole. BGE: 0.1 M phosphate buffer at pH 3.0 containing TM- $\beta$ -CD (concentrations indicated in parentheses). Capillary, 50  $\mu$ m  $\times$  48.5 cm uncoated fused-silica; injection, 50 mbar for 4 s; run voltage, 30 kV; temperature, 15°C; detection wavelength, 200 nm.



**Figure 3.** Electropherograms showing the best enantiomeric separations for miconazole, econazole, and sulconazole. BGE: 0.1 M phosphate buffer at pH 3.0 containing HP- $\beta$ -CD (concentrations indicated in parentheses). Other experimental conditions as in Fig. 2.

With respect to the effect of the CD concentration on the enantioselectivity, there is an optimum concentration ( $C_{opt}$ ) which enables the maximum difference between the mobilities of the enantiomers. The optimal concentrations calculated from the binding constant values obtained for the six compounds studied in this work are grouped in Table 1. Good agreement between the calculated CD optimum concentration and the experimental one was obtained with few exceptions. One exception is econazole with TM- $\beta$ -CD, where calculated and experimental optimal concentrations were quite different (see Table 1), but in this case very low enantioselective recognition was observed ( $R_s \sim 0.5$ ). Other case is bifonazole with TM- $\beta$ -CD, where the difference between the calculated concentration (0.6 mM) and the experimental concentration (2 mM) was not too high, enabling both concentrations to obtain a good resolution between bifonazole enantiomers (1.0 and 1.4, respectively). Finally, another interesting case is that of ketoconazole and terconazole with HP- $\beta$ -CD where in the low concentration range the calculated and experimental optimal CD concentrations were similar, whereas in the high concentration range the calculated CD concentration is estimated low for both compounds. This could be explained taking into account the results shown in Fig. 4 where the difference between the mobilities of the enantiomers as a function of HP- $\beta$ -CD concentration is depicted. It can be observed that at high CD concentrations the differences between the mobilities of the enantiomers of ketoconazole and terconazole increase but do not reach a maximum value as at low concentrations. The absence of a maximum in the difference between the mobilities of the enantiomers could be the reason for the discrepancy between the experimental and calculated optimal concentrations.





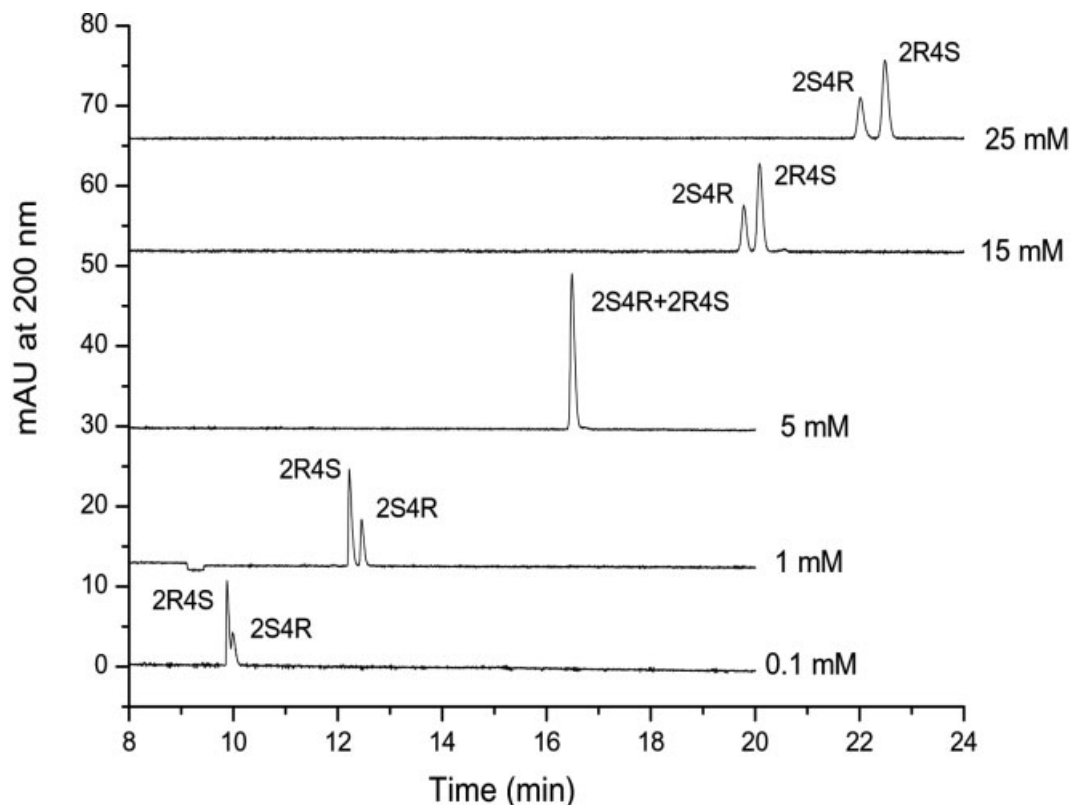
**Figure 4.** Variation of the differences between the electrophoretic mobilities of the enantiomers for ketoconazole, terconazole, miconazole, sulconazole, and econazole with the HP- $\beta$ -CD concentration. Other experimental conditions as in Fig. 2.

### 3.2 Reversal of migration order of enantiomers with CD concentration

As shown in Fig. 4, the mobility difference between enantiomers for miconazole, sulconazole, and econazole increased with the HP- $\beta$ -CD concentration reaching a maximum value and then, dropping off at higher values reaching a 0 value (no enantioselectivity) at 10 mM for sulconazole and at 40 mM for econazole and miconazole. Moreover, miconazole and econazole showed the highest maximum value of mobility difference with the HP- $\beta$ -CD concentration giving rise to the best enantiomeric separations as previously stated. However, an interesting phenomenon was observed for ketoconazole and terconazole. In fact, after reaching a maximum in the difference of the mobilities for the enantiomers of these compounds at low HP- $\beta$ -CD concentrations, this difference became zero at a 5 mM HP- $\beta$ -CD concentration and increased again for concentrations higher than 5 mM (for ketoconazole) or 30 mM (for terconazole). This behavior could be explained if a reversal in the migration order for the two enantiomers of these compounds is assumed when varying the CD concentration.

Only for ketoconazole it was possible to confirm this supposition due to the availability of one of the enantiomers, the pure 2*R*,4*S*-enantiomer. The reversal in the migration order for ketoconazole enantiomers when varying the HP- $\beta$ -CD concentration can be clearly observed in Fig. 5, where the identification of enantiomers was performed by spiking with the 2*R*,4*S*-form a racemic mixture of ketoconazole. The first-migrating enantiomer at CD concentrations lower than 5 mM is the 2*R*,4*S*-enantiomer whereas at CD concentrations higher than 5 mM is the 2*S*,4*R*-form which migrates in the first place.

This behavior has scarcely been observed to date with HP- $\beta$ -CD and even with other CDs (see Section 1). Thus, only the two dansylated amino acids (dns-amino acids) phenylalanine (Phe) and tryptophan (Trp) have shown a reversal in the migration order for their enantiomers with the variation of the HP- $\beta$ -CD concentration. For dns-amino acids, the inversion in the migration order for the enantiomers with the CD concentration was observed at a pH 2.5, which is near the  $\text{p}K_{\text{a}}$  value for these compounds ( $\text{p}K_{\text{aDns-Phe}}$  1.8 and  $\text{p}K_{\text{aDns-Trp}}$  2.4) and took place when the complex mobility was lower than that of the free analyte [12]. Accord-



**Figure 5.** Electropherograms corresponding to the separation of ketoconazole enantiomers in 0.1 M phosphate buffer at pH 3.0 containing different concentrations of HP- $\beta$ -CD (indicated in the figure). Capillary, 50  $\mu\text{m} \times 56$  (64.5) cm uncoated fused-silica; injection, 50 mbar for 6 s. Other experimental conditions as in Fig. 2.

ing to these results, the reversal migration order with the HP- $\beta$ -CD concentration was observed in this work for ketoconazole at pH 3.0 which is very close to its  $pK_a$  ( $pK_{a1}$  2.94). In addition, at this acid pH the ketoconazole-HP- $\beta$ -CD complex possesses a mobility that is lower than that of the free imidazole due to the neutral nature of the CD.

#### 4 Concluding remarks

The enantioselective recognition of a group of six weak baseazole compounds with three neutral  $\beta$ -CDs ( $\beta$ -CD, HP- $\beta$ -CD, and TM- $\beta$ -CD) has been discussed in this work on the basis of the determination of the apparent binding constants for each pair compound-CD. The best enantiomeric resolutions for miconazole, econazole, and sulconazole were observed with HP- $\beta$ -CD whereas for ketoconazole, terconazole, and bifonazole were obtained with TM- $\beta$ -CD. Concentrations in the mM range (from 2 to 20 mM) were optimal for TM- $\beta$ -CD whereas for HP- $\beta$ -CD concentrations in the very low mM range (from 0.5 to 2 mM) gave rise to the best enantiomeric resolutions. Using these CDs as

chiral selectors, analysis times lower than 8 min were obtained for the separation of the enantiomers of all theazole compounds studied.

A reversal in the migration order for the enantiomers of ketoconazole was confirmed varying the concentration of HP- $\beta$ -CD as chiral selector in phosphate buffer at pH 3.0. A similar phenomenon was observed for terconazole, which possesses a similar structure to ketoconazole, although in this case the unavailability of a pure enantiomer did not enable to confirm the opposite migration order of enantiomers when varying the HP- $\beta$ -CD concentration. This phenomenon has scarcely been reported previously and it is the first time that it has been observed for imidazole compounds.

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