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Peculiarities of an imidazole derivative retention mechanism in reversed-phase liquid chromatography: β-cyclodextrin concentration and temperature considerations

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Abstract

The retention mechanism of a series of imidazole derivatives in reversed-phase liquid chromatography (RPLC) was investigated over a range of column temperatures and β-cyclodextrin concentrations. The changes in van't Hoff plots as a function of the β-cyclodextrin concentration were examined. Enthalpy, entropy and the Gibbs free energy were determined for the two physicochemical processes: (i) the solute transfer from the mobile to the stationary phases, (ii) the solute complexation by β-cyclodextrin. These thermodynamic data showed that the solute retention mechanism was dependent on the β-cyclodextrin concentration in the mobile phase. Enthalpy–entropy compensation revealed that the main parameter determining retention increased as follow: β-cyclodextrin ⇄ solute complexation > RP_{18} stationary phase ⇄ solute interaction. This fact confirms that the main parameter determining retention in RPLC is the distribution of the solute molecule in the mobile phase, whereas the interactions with the stationary phase play a minor role.

Keywords: Retention mechanism; Cyclodextrin; Imidazole

1. Introduction

Cyclodextrins (CDs), which are torus-shaped cyclic oligosaccharides consisting of six or more α-(1,4)-linked D-glucopyrannose units are one of the well known host molecules capable of forming an inclusion complex (host–guest complex) with a wide variety of organic molecules or so-called guest molecules [1]. CDs are extensively used as stationary phase components in gas chromatography as well as stationary or mobile phase additives in liquid chromatography [2]. The investigation and utilization of secondary chemical equilibria (SCE) such as complexation have been much studied. Rozbeh and Hurtubise [3] investigated the effects of β-cyclodextrin (β-CD) as an additive in three types of hydroorganic mobile phases, methanol–water, acetonitrile–water and methanol–acetonitrile–water on the reversed-phase liquid chromatography (RPLC) retention characteristics of metabolites of benzo[*a*]pyrene. Rozbeh and Hurtubise [4] later investigated the effect of γ-cyclodextrin in a methanol–water mobile phase on the retention of some metabolites of benzo[*a*]pyrene. Generally, more effective

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than β-CD, γ-CD also significantly improved the separation of mono-hydroxylbenzo[*a*]pyrene isomers that were previously very difficult to separate. Landau and Grushka [5] observed that the addition of an organic modifier, such as methanol, to the mobile phase displaced the included water molecules from the cyclodextrin cavity and facilitated entrance of solute molecules in their place. Higher concentrations of methanol resulted in a less polar mobile phase in which the nonpolar solutes became more soluble, thus decreasing the stability of the inclusion complex. Husain et al. [6] examined the effect of two comodifiers containing *tert*.-butyl moities, *tert*.-butyl-(N-hydroxy)-carbamate and *tert*.-butylcarbamate, on the retention behavior of six polycyclic aromatic hydrocarbons (PAHs) using β- and γ-CD modified mobile phases. Both modifiers resulted in shorter retention times for all PAHs, attributed to the formation of a ternary PAH–CD-modifier complex. Nawakowski et al. [7] found that the behavior of α-CD differed strongly from that of the larger β- and γ-CD oligomers and that γ-CD showed an unexpectedly high hydrophobic surface for solvent interaction and a reduced interaction with the surface of C_{18}-bonded silica. Shimada et al. [8,9] found β-CD to be a useful additive for the separation of two types of neurosteroids and vitamin D, its metabolites, and related compounds. They also examined the retention behavior of vitamin D_2-D_5 and provitamin D_2-D_5 on the addition of heptakis (2,6-di-O-methyl)-β-CD to the mobile phase [10]. Letellier et al. [11] confirmed via fluorescence and RPLC data the inclusion of floctafenine, an analgesic drug, within the cavity of methyl-β-CD in an aqueous solution. Binding constants (1:1) were calculated, and fluorescence enhancement was observed upon inclusion of floctafenine. Letellier et al. [12] measured the formation constant for the 1:1 inclusion complex of rutin and methyl-β-CD in aqueous solution by UV and fluorescence spectroscopy, RPLC, and capillary electrophoresis. The Hummel Dreyer method was used to determine the association constants of inclusion complexes of steroid hormones with cyclodextrins [13]. Loukas et al. [14] used high-performance liquid chromatography to determine the stability constant of a cyclodextrin inclusion complex of an organophosphorus insecticide. Lamparczyk and Zarzycki [15] measured the retention of 17-α-estradiol and equilin in relation to β-CD concentration (0–16 m*M*) and temperature (5–80°C) and observed very unusual temperature effects: consistently nonlinear van't Hoff plots (ln k' versus $1/T$) with a fixed acetonitrile–water mobile phase, implied, at subambient temperature, a decrease in retention with a decrease in temperature.

Imidazole and triazole derivatives were used for the treatment of onychomycosis [16–18]. Nevertheless, these hydrophobic compounds had a weak penetration into hydrophilic human nails. Their inclusion in the apolar cyclodextrin cavity could improve this penetration, considering the hydrophilic character of the exterior of the cyclodextrin, constituted of a great number of hydroxyl groups.

In this paper, the thermodynamic behavior of a series of six imidazole derivatives was investigated over a wide range of column temperatures, between −8 and 70°C and β-CD concentrations. The shapes of van't Hoff plots were used to assess changes in the chromatographic process in relation to temperature and β-CD concentration. A thermodynamic study was then carried out to evaluate both the complexation and retention mechanism.

2. Experimental

2.1. Apparatus

The RPLC system consisted of a Waters RPLC pump 501 (Saint-Quentin, Yvelines, France), an Interchim Rheodyne injection valve, model 7125 (Montluçon, France), fitted with a 20-μl sample loop, a Shimadzu SPD-10A (Touzart-Matignon, Vitry sur Seine, France) variable wavelength UV spectrophotometer detector (Nogent sur Marne, France). A Lichrocart® 125 mm×4 mm I.D. RP_{18} column (5 μm particle size) (Merck, Darmstadt, Germany) was used with a controlled temperature (in an Interchim oven, TM No. 701 for high temperatures and an Osi Julabo FT 200 cryoimmerser (Elancourt, France) for low temperatures). Mobile phase flow-rate was fixed at 1 ml/min and the wavelength at 230 nm.

2.2. Solvents and samples

RPLC grade methanol (Carlo Erba, Val de Reuil, France) and acetone (Prolabo, Paris, France) were

used without further purification. Water was obtained from an Elgastat option I water purification system (Odil, Talant, France), fitted with a reverse osmosis cartridge. The mobile phase used for these studies was a methanol–phosphate buffer (75:25, v/v) adjusted to pH 3 with 1% phosphoric acid, with various CD concentrations (0, 0.5, 1, 1.5, 2, 2.5 and 3 m*M*). β-CD was a gift from the Roquette Laboratories (Lestrem, France). The phosphate buffer was composed of 0.01 *M* diammonium hydrogen phosphate–0.02 *M* ammonium dihydrogen phosphate and 0.005 *M* *n*-nonylamine to avoid peak tailing. Bifonazole (1), clotrimazole (2), econazole (3), sulconazole (4), miconazole (5) and oxiconazole (6), purchased from Sigma (Saint Quentin, Fallavier, France), were dissolved in pure acetone to obtain a concentration of 1 mg/l. The chemical structure of these compounds is given in Fig. 1. Each solute, or mixture of these, was injected and the retention times were measured using a Merck D2500 chromatointegrator. Sodium nitrate was used as a dead time marker (Merck, Nogent-sur-Marne, France).

2.3. Temperature studies

Compound retention factors were determined at the temperature values of −8, −2, 3, 10, 15, 20, 30, 40, 50, 60 and 70°C. The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each experiment. To study this equilibration, the compound retention time of the bifonazole was measured every hour for 7 h and again after 22, 23 and 24 h. The maximum relative difference in the retention times of this compound between these different measurements was always 0.8%, making the chromatographic system sufficiently equilibrated for use after 1 h. All the solutes were injected in triplicate at each temperature and β-CD concentration.

3. Methods

3.1. Thermodynamic relationships

Solute retention is usually expressed in terms of the retention factor k' by the well-known equation [19–22]:

$$\ln k' = \frac{-\Delta H^{\circ}_{\mathrm{M.Ls}}}{RT} + \Delta S^{\circ *}_{\mathrm{M.Ls}} \quad (1)$$

$$\Delta S^{\circ *}_{\mathrm{M.Ls}} = \frac{\Delta S^{\circ}_{\mathrm{M.Ls}}}{R} + \ln \phi \quad (2)$$

where $\Delta H^{\circ}_{\mathrm{M.Ls}}$ and $\Delta S^{\circ}_{\mathrm{M.Ls}}$ are respectively the enthalpy and entropy of transfer of the solute molecule M, from the mobile phase to the stationary phase ligand Ls, T, the temperature, R, the gas constant and ϕ, the phase ratio of the column (volume of the stationary phase divided by the volume of the mobile phase). Ln k' versus $1/T$ is called a van't Hoff plot. For a linear plot, the slope and intercept are respectively $-\Delta H^{\circ}_{\mathrm{M.Ls}}/R$ and $\Delta S^{\circ *}_{\mathrm{M.Ls}}$. For a nonlinear van't Hoff plot [23], these thermodynamic data can be calculated using the following method. If an equation can be obtained for the best fit of a curved van't Hoff plot, then the partial derivative of ln k' with respect to $1/T$ will yield a second equation which represents the negative enthalpy divided by R in relation to temperature. Using Eqs. (1) and (2), $\Delta S^{\circ *}_{\mathrm{M.Ls}}$ can be determined at a particular temperature.

3.2. Secondary chemical equilibria

The retention behavior of imidazole derivatives in RPLC is based on the partitioning of the samples between the mobile and stationary phases. The solute retention is split into two main physicochemical processes, i.e, solute complexation by β-CD and free solute (or noncomplexed) transfer from the hydro-organic mobile phase to the stationary phase [24]. In RPLC, the values of the formation constant of complexation, K_f, are obtained from the slope to intercept ratio of a plot of the reciprocal of the capacity factor, k', of each eluting solute versus the concentration of cyclodextrin incorporated in the mobile phases [12,24]:

$$\frac{1}{k'} = \frac{1}{k'_0} + \frac{K_f \times [\beta\text{- CD}]}{k'_0} \quad (3)$$

where k'_0 is the capacity factor of the free solute, and [β-CD] is the concentration of cyclodextrin in the mobile phase.

Enthalpy and entropy of inclusion complex formation between the solute molecule, M, and the cyclodextrin ($\Delta H^{\circ}_{\mathrm{M.CD}}$ and $\Delta S^{\circ}_{\mathrm{M.CD}}$ respectively) are

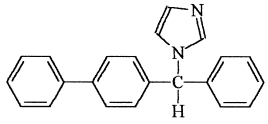
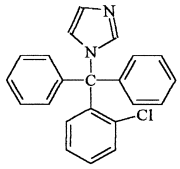
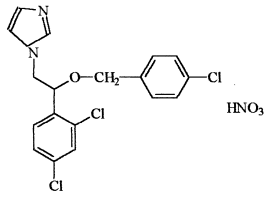
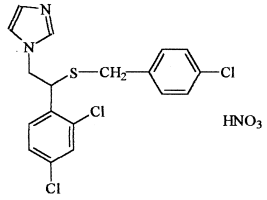
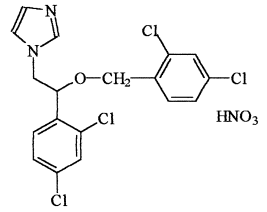
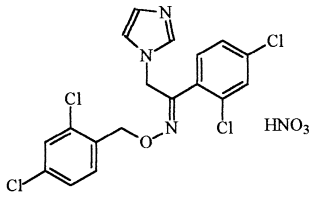
Compound N°	Nomenclature	Chemical structure
(1)	Bifonazole	
(2)	Clotrimazole	
(3)	Econazole	
(4)	Sulconazole	
(5)	Miconazole	
(6)	Oxiconazole	

Fig. 1. Six imidazole derivatives.

determined by plotting the logarithm of the complex formation constant values as a function of the temperature reciprocal:

$$\ln K_f = \frac{-\Delta H^\circ_{M.CD}}{RT} + \frac{\Delta S^\circ_{M.CD}}{R} \quad (4)$$

For a linear plot, the slope and intercept are respectively $-\Delta H^\circ_{M.CD}/R$ and $\Delta S^\circ_{M.CD}/R$. The Gibbs free energy is determined at a particular temperature using the following equations:

$$Ln\ K_f = \frac{-\Delta G^\circ_{M.CD}}{RT} \quad (5)$$

$$\Delta G^\circ_{M.CD} = \Delta H^\circ_{M.CD} - T\Delta S^\circ_{M.CD} \quad (6)$$

4. Results and discussion

4.1. Complex formation constant values between solute and β-cyclodextrin

According to Eq. (3), linear plots were obtained for all solutes. The *r* value for the linear fit was in excess of 0.980. The complex formation constants were calculated for different temperatures. Their values decreased with increasing temperatures. The values for K_f again agreed with values reported in the literature [25,26], showing the high reliability of the RPLC method for the formation constant evaluation. For example, the K_f values are given for each compound, at $T=20$ and 50°C, in Table 1. Two groups of compounds were distinguished according to the K_f values. Bifonazole, econazole and sulconazole which constitute the first group had the highest formation constant values in comparison with that of the second group composed of miconazole, oxiconazole and clotrimazole. This result demonstrates that the β-CD size is more appropriate for bifonazole, econazole and sulconazole inclusion than for miconazole, oxiconazole and clotrimazole.

4.2. Enthalpy, entropy and Gibbs free energy changes for the solute inclusion in the cyclodextrin cavity

The van't Hoff plots of Eq. (4) ($\ln K_f$ versus $1/T$) for all samples were linear. The correlation coefficient, *r*, for all the fits was over 0.992. $\Delta H^\circ_{M.CD}$, $\Delta S^\circ_{M.CD}$ and $\Delta G^\circ_{M.CD}$ (calculated with Eq. (6) at 25°C) values are listed in Table 2. $\Delta H^\circ_{M.CD}$ was negative, and this indicates that it is energetically favorable for the weak polar solute to be included in the CD cavity. Obviously, the largest changes in enthalpy would be for the imidazole derivatives with the highest formation constant. For the entropy change, the values obtained were negative proving the apparent lower degrees of freedom of the solute included in the CD cavity. It is interesting to note that for every solute evaluated, when $\Delta H^\circ_{M.CD}$ was compared to $T\Delta S^\circ_{M.CD}$ (Table 2) over the temperature range studied, the magnitude of $\Delta H^\circ_{M.CD}$ was always greater than that of $T\Delta S^\circ_{M.CD}$. This indicated that enthalpy played a greater role in the complexation process, and therefore in the retention process than entropy did.

Table 1
K_f (M^{-1}) values at two temperatures for the six imidazole derivatives

	K_f	
	$T=20$°C	$T=50$°C
Bifonazole (1)	321.2	184.8
Clotrimazole (2)	29.5	21.5
Econazole (3)	192.9	116.6
Sulconazole (4)	275.8	161.1
Miconazole (5)	47.6	32.9
Oxiconazole (6)	55.5	38.0

Table 2
Thermodynamic parameters $\Delta H^\circ_{M.CD}$, $\Delta S^\circ_{M.CD}$, $T\Delta S^\circ_{M.CD}$ at 25°C and $\Delta G^\circ_{M.CD}$ at 25°C (solute inclusion) for the six imidazole derivatives

	$\Delta H^\circ_{M.CD}$ (kJ/mol)	$\Delta S^\circ_{M.CD}$ (J/mol K)	$T\Delta S^\circ_{M.CD}$ (kJ/mol)	$\Delta G^\circ_{M.CD}$ (kJ/mol)
(1)[a]	−14.5	−1.5	−0.5	−14.1
(2)	−8.3	−0.2	−0.1	−8.2
(3)	−13.2	−1.3	−0.4	−12.8
(4)	−14.1	−1.4	−0.4	−13.7
(5)	−9.7	−1.0	−0.3	−9.4
(6)	−9.9	−0.4	−0.1	−9.8

[a] See the corresponding compound in Table 1.

4.3. Enthalpy, entropy changes for the solute transfer from the mobile to the stationary phase

4.3.1. For 0≤[CD]≤0.5 mM

The van't Hoff plots were all linear for the six imidazole derivatives. The capacity factor increased when the temperature decreased. The correlation coefficient, *r*, for all the fits was over 0.996. Fig. 2A shows the van't Hoff plot for miconazole, at β-CD concentration equal to 0 m*M*. A complete list of $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ values, for all solutes, is shown in Table 3 at β-CD concentrations equal to 0 m*M* and 0.5 m*M*. Both $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ were negative for these two β-CD concentrations. Negative $\Delta H^{\circ}_{M.Ls}$ indicated that it was energetically more favorable for the solute to be in the stationary phase. Negative $\Delta S^{\circ *}_{M.Ls}$ also indicated an increase in the order of the chromatographic system as the solute was transferred from the mobile to the stationary phase. At this CD concentration range, 0≤[CD]≤ 0.5 m*M*, the equilibrium of complexation between the free solute and the CD was displaced in the direction of the free solute (or noncomplexed with the CD) because of the low quantity of CD in the mobile phase. The retention mechanism of the compound involved the classical transfer of a solute from the bulk methanol–water mobile phase to the stationary phase. Retention factors decreased with increasing temperature and $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ were negative values (Table 3). Thus, the transfer of the solute, from the mobile to the stationary phase, was enthalpically driven.

Table 3
Thermodynamic parameters $\Delta H^{\circ}_{M.Ls}$ (kJ/mol) and $\Delta S^{\circ a}_{M.Ls}$ (transfer from the mobile to the stationary phase), at different β-CD concentrations for the six imidazole derivatives

	A: [CD]=0 m*M*		B: [CD]=0.5 m*M*	
	$\Delta H^{\circ}_{M.Ls}$	$\Delta S^{\circ *}_{M.Ls}$	$\Delta H^{\circ}_{M.Ls}$	$\Delta S^{\circ *}_{M.Ls}$
(1)[a]	−0.2	−0.2	−3.4	−1.1
(2)	−7.6	−2.7	−5.6	−1.8
(3)	−2.2	−0.3	−3.8	−0.7
(4)	−3.4	−0.5	−4.3	−0.8
(5)	−4.6	−0.7	−9.4	−2.6
(6)	−6.1	−1.2	−6.4	−1.2

[a] See the corresponding compound in Table 1.

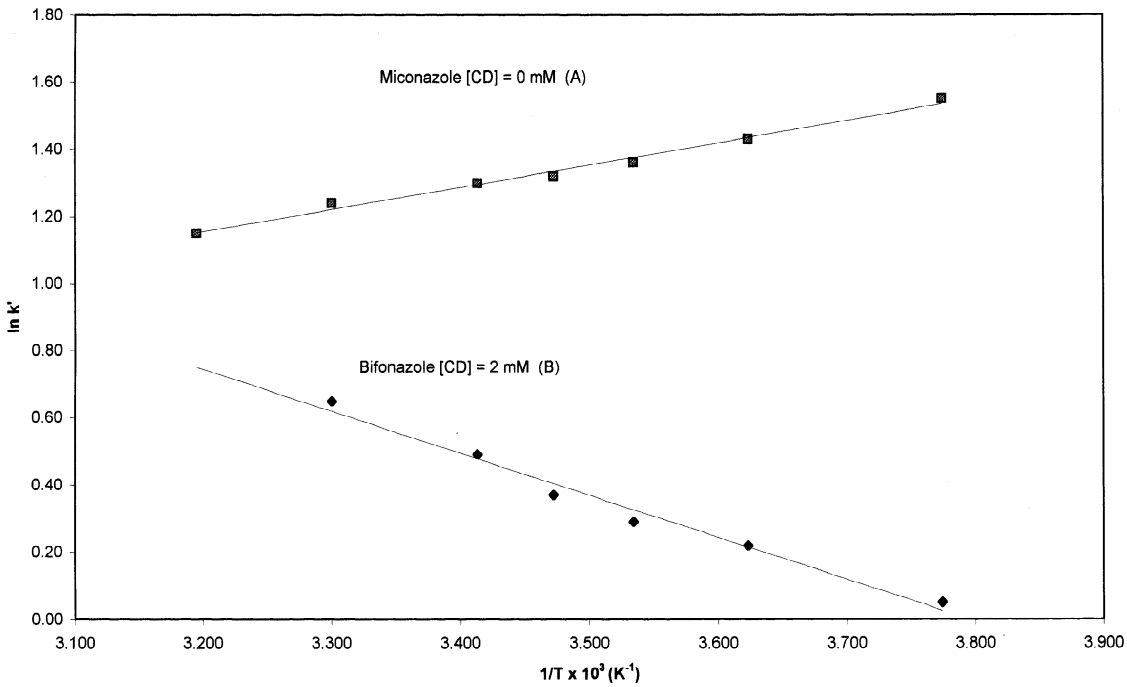


Fig. 2. Van't Hoff plot for miconazole at a 0 m*M* β-CD concentration (A) and for bifonazole at a 2 m*M* β-CD concentration (B).

4.3.2. For 2≤[CD]≤3 mM

The van't Hoff plots were all linear for the six imidazole derivatives. The capacity factor increased when the temperature increased. Lamparczyk and Zarzycki [15] observed the same behavior, measuring the retention of different estradiols, in relation to β-CD concentration and temperature with an acetonitrile–water mobile phase. The correlation coefficient, *r*, for all the fits was over 0.994. Fig. 2B shows the van't Hoff plot for bifonazole, at a β-CD concentration of 2 m*M*. A complete list of $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ values, for all solutes, is shown in Table 4 at β-CD concentrations equal to 2, 2.5 and 3 m*M*. Both $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ were positive for these three β-CD concentrations. Positive $\Delta H^{\circ}_{M.Ls}$ indicated that it was energetically more favorable for the solute to be in the mobile phase. Positive $\Delta S^{\circ *}_{M.Ls}$ values indicated a decrease in the order of the chromatographic system as the solute was transferred from the mobile phase to the stationary phase. The quantity of CD in the mobile phase, between 2 and 3 m*M*, was sufficient to displace the equilibrium of complexation between the free solute and the CD in the direction of the inclusion compound. Therefore, the weak polar solute was included in a hydrophobic cavity of the β-CD. Retention factors increased with increasing temperature and $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ became positive values (Table 4). Thus, the transfer of the solute, from the mobile to the stationary phase was entropically driven.

4.3.3. For 1≤[CD]≤1.5 mM

The van't Hoff plots for all imidazole derivatives showed distinct changes in slope which were indicative of a modification in the solute retention mechanism. With decreasing temperatures, the plots were first of all constant, within the experimental errors, and then became parabolic. All the parabolic plots were a good fit using a second order polynomial. The correlation coefficients of these fits were in excess of 0.988. Fig. 3 shows the van't Hoff plots for econazole at β-CD concentrations equal to 1 m*M*. The constant linear part of the plot finishes at a temperature T^{*}. The value of T^{*} is equal to approximately 20°C. For the curved part of the plot, the change in slope appears at a temperature T^{**}, between 3 and 10°C. Table 5 shows $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ values at different temperatures ($-8°C \leq T \leq T^{*}$) for all solutes with these two β-CD concentration values (Table 5A: [β-CD]=1 m*M*; B: [β-CD]=1.5 m*M*). When T was above the T^{*} value, within the experimental error, $\Delta H^{\circ}_{M.Ls}$ was approximately equal to 0.0 kJ/mol for all solutes. When $T<T^{**}$, $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ were positive and when $T^{**}<T<T^{*}$, $\Delta H^{\circ}_{M.Ls}$ and $\Delta S^{\circ *}_{M.Ls}$ were negative. For $T<T^{**}$, as the temperature increased, the equilibrium of complexation between the free solute and the cyclodextrin was completely displaced in the direction of the inclusion compound. This behavior is identical to that of CD concentrations equal to 2, 2.5 and 3 m*M*. Thus, the transfer of the solute, from the mobile to the stationary phase, was entropically driven. For $T \geq T^{**}$, as the temperature increased, the chemical equilibrium between the free solute and the cyclodextrin was progressively displaced in the direction of the complex dissociation. The solute was completely free for $T \geq T^{*}$. Thus, the retention process corresponded to a simple chromatographic system in reversed-phase as shown for CD concentrations equal to 0 and 0.5 m*M*: the transfer of

Table 4
Thermodynamic parameters $\Delta H^{\circ}_{M.Ls}$ (kJ/mol) and $\Delta S^{\circ *}_{M.Ls}$ (transfer from the mobile to the stationary phase), at different β-CD concentrations for the six imidazole derivatives

	A: [CD]=2 m*M*		B: [CD]=2.5 m*M*		C: [CD]=3 m*M*	
	$\Delta H^{\circ}_{M.Ls}$	$\Delta S^{\circ *}_{M.Ls}$	$\Delta H^{\circ}_{M.Ls}$	$\Delta S^{\circ *}_{M.Ls}$	$\Delta H^{\circ}_{M.Ls}$	$\Delta S^{\circ *}_{M.Ls}$
(1)[a]	11.4	5.0	15.6	7.1	16.2	8.0
(2)	7.8	3.7	9.7	4.6	10.3	5.1
(3)	0.4	1.2	1.5	2.3	3.2	2.4
(4)	1.2	1.8	2.3	2.0	3.1	2.1
(5)	3.4	3.0	5.6	4.5	5.9	5.0
(6)	0.9	2.3	2.0	3.1	2.4	3.3

[a] See the corresponding compound in Table 1.

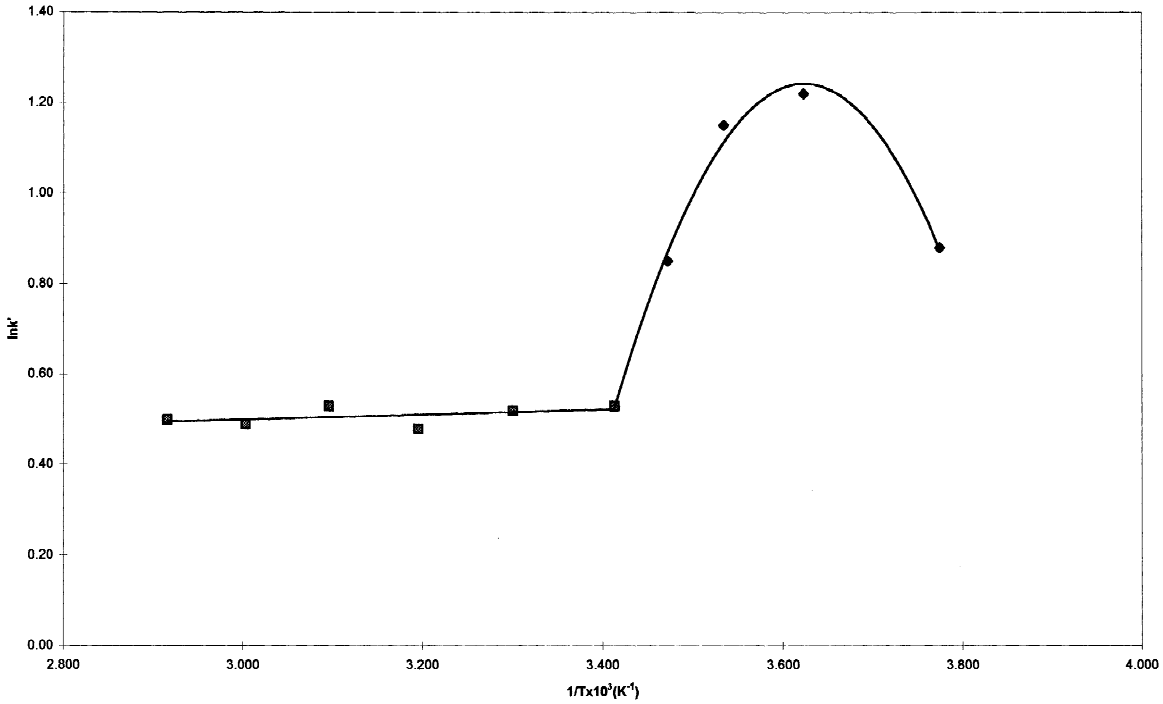


Fig. 3. Van't Hoff plot for econazole at a 1 m*M* β-CD concentration.

Table 5
Thermodynamic parameters $\Delta H^{\circ}_{M.Ls}$ (kJ/mol) and $\Delta S^{\circ *}_{M.Ls}$ (transfer from the mobile to the stationary phase), at different β-CD concentrations for the six imidazole derivatives, at different temperatures

T (K)	$\Delta H^{\circ}_{M.Ls}$						$\Delta S^{\circ *}_{M.Ls}$					
	(1)[a]	(2)	(3)	(4)	(5)	(6)	(1)	(2)	(3)	(4)	(5)	(6)
A: [CD]=1 m*M*												
265	51.6	87.2	44.2	94.5	61.9	79.4	23.4	39.2	29.4	43.0	29.4	36.9
271	32.6	56.3	21.5	56.9	38.7	51.5	15.0	25.3	10.8	27.3	18.6	24.4
276	17.5	31.7	3.0	30.1	20.3	29.3	8.0	14.4	2.8	14.2	10.5	14.5
283	−3.2	−1.8	−18.3	−8.3	−4.9	−1.1	−1.2	0.3	−7.7	−2.2	−0.4	1.5
288	−17.2	−23.2	−37.6	−37.4	−21.4	−21.6	−6.8	−9.5	−14.9	−13.3	−7.3	−7.3
293	−31.3	−46.5	−53.7	−59.7	−37.8	−40.1	−12.9	−15.6	−21.4	−23.7	−14.1	−15.2
B: [CD]=1.5 m*M*												
265	65.7	58.2	62.0	62.0	62.3	59.5	29.7	26.3	29.0	29.2	29.0	28.7
271	36.0	34.3	30.4	30.7	29.8	30.0	16.8	16.4	14.8	15.2	15.2	15.3
276	13.6	15.4	5.5	5.7	4.5	6.3	6.6	7.3	3.7	4.2	4.3	4.8
283	−17.2	−7.4	−28.3	−28.4	−30.2	−25.4	−6.8	−4.7	−10.9	−10.3	−10.9	−9.6
288	−38.5	−23.9	−51.9	−50.7	−53.7	−47.2	−15.7	−9.5	−20.7	−19.9	−20.2	−17.8
293	−59.0	−39.8	−71.3	−72.9	−77.1	−68.1	−24.2	−18.3	−29.9	−29.1	−28.7	−26.5

[a] See the corresponding compound in Table 1.

the solute from the mobile to the stationary phase was enthalpically driven.

4.4. Enthalpy–entropy compensation

Investigation of the enthalpy–entropy compensation temperature is a thermodynamic approach to the analysis of physicochemical data. Mathematically, enthalpy–entropy compensation can be expressed by the formula [22]

$$\Delta H^\circ = \beta \Delta S^\circ + \Delta G^\circ{}_\beta \quad (7)$$

where $\Delta G^\circ{}_\beta$ is the Gibbs free energy of a physicochemical interaction at a compensation temperature β (β and $\Delta G^\circ{}_\beta$ are constant). ΔH° and ΔS° are, respectively, the corresponding standard enthalpy and entropy. According to Eq. (7), when enthalpy–entropy compensation is observed with a group of compounds in a particular chemical interaction, all of the compounds have the same free energy ($\Delta G^\circ{}_\beta$) at temperature β. Therefore, if, enthalpy–entropy compensation is observed for the six compounds, all will have the same net retention at the compensation temperature β, although their temperature dependencies may differ. Applying Eq. (7) to the two chemical processes, i.e, solute transfer from the mobile phase to the stationary phase and solute complexation by β-CD, the following was obtained

$$\Delta H^\circ{}_{\mathrm{M.Ls}} = \beta \Delta S^\circ{}_{\mathrm{M.Ls}} + \Delta (G^\circ{}_{\mathrm{M.Ls}})_\beta \quad (8)$$

$$\Delta H^\circ{}_{\mathrm{M.CD}} = \beta \Delta S^\circ{}_{\mathrm{M.CD}} + \Delta (G^\circ{}_{\mathrm{M.CD}})_\beta \quad (9)$$

Rewriting Eq. (8) using Eq. (1),

$$\ln (k')_T = \ln k'_0 - \frac{\Delta H^\circ{}_{M.Ls}}{R} \times \left(\frac{1}{T} - \frac{1}{\beta} \right) \quad (10)$$

where

$$\ln k'_0 = -\frac{\Delta (G^\circ{}_{M.Ls})_\beta}{R\beta} + \ln \phi \quad (11)$$

Rewriting Eq. (9) using Eq. (4),

$$\ln (K_f)_T = \ln K'_0 - \frac{\Delta H^\circ{}_{M.CD}}{R} \times \left(\frac{1}{T} - \frac{1}{\beta} \right) \quad (12)$$

where

$$\ln K'_0 = -\frac{\Delta (G^\circ{}_{\mathrm{M.CD}})_\beta}{R\beta} \quad (13)$$

Eq. (10) (respectively Eq. (12)) shows that, if a plot of $\ln (k')_T$ (respectively $\ln (K_f)_T$) against $-\Delta H^\circ{}_{\mathrm{M.Ls}}$ (respectively $-\Delta H^\circ{}_{\mathrm{M.CD}}$) is linear, then the six solutes are retained by an essentially identical interaction mechanism.

A plot of $(\ln k')_T$ (for $T=303$ K), calculated for each of the six compounds, against $-\Delta H^\circ{}_{\mathrm{M.Ls}}$, was drawn without β-CD in the mobile phase and for example for a β-CD concentration equal to 2 m*M*. The *r* values, for the fits, were respectively 0.606 and 0.912. This can be considered adequate to verify enthalpy–entropy compensation [23]. Nevertheless, if clotrimazole is not taken into account, these linear fits are better ($r>0.997$). Therefore, the retention mechanism can be thought to be independent of the molecular structure, with or without β-CD in the mobile phase. A plot of $(\ln K_f)_T$, calculated at $T=303$ K for each of the six compounds against $-\Delta H^\circ{}_{\mathrm{M.CD}}$ was drawn. The *r* value obtained when all the solutes were plotted, was 0.999. This high degree of correlation indicates that the six compounds have the same inclusion mechanism. The compensation temperature β was determined for each solute for the two chemical processes, solute transfer from the mobile phase to the stationary phase and solute complexation by β-CD. The β value, for a particular solute, for the complexation process was always greater than the β value for the solute transfer from the mobile phase to the stationary phase. This result shows that the solute complexation by β-CD in the mobile phase contributed to the retention mechanism more significantly than the solute interactions with the stationary phase.

5. Conclusions

In this paper, the retention mechanism in RPLC and the inclusion complex formation with β-CD were studied for six imidazole derivatives. The complex formation constants were measured in relation to temperature. The thermodynamic parameter trends were determined over a wide range of column temperatures. The variations observed can be explained in terms of a split into two main physico-

chemical processes, solute inclusion in the CD cavity and solute transfer from the mobile phase to the stationary phase. The thermodynamic results obtained show that the imidazole complexation by β-CD must be considered in order to accurately describe the retention process. Enthalpy–entropy compensation revealed that the imidazole derivative retention was due primarily to the solute complexation in the mobile phase and less to the interactions in the stationary phase. This result is substantiated by the fact that generally, the main parameter determining retention in RPLC is the distribution of the solute molecule in the mobile phase (to cluster the organic modifier–water in a hydroorganic mobile phase [27,28]), whereas the interactions with the stationary phase play a minor role. This suggests general rules that may be of use in the understanding of the solute retention mechanism.

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