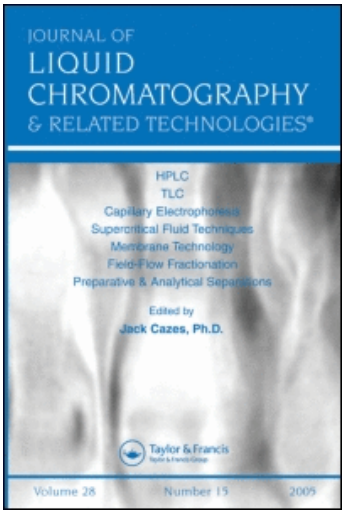


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# HPLC RETENTION AND INCLUSION OF IMIDAZOLE DERIVATIVES USING HYDROXYPROPYL-β-CYCLODEXTRIN AS A MOBILE PHASE ADDITIVE

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## ABSTRACT

Using the results of high performance liquid chromatography (HPLC), this paper investigates the separation and inclusion of a series of weakly polar imidazole derivatives, with hydroxypropyl-β-cyclodextrin (HP-β-CD) in the mobile phase over a wide range of column temperatures. These compounds are used for the treatment of onychomycosis. Gibbs Helmholtz parameters ($\Delta(\Delta H)$ and $\Delta(\Delta S)$) of two adjacent imidazole peak on a chromatogram were determined from the graph of the logarithm of the separation factor, $\alpha$, against the reciprocal of the temperature. A temperature dependent reversal of the elution order between a pair of imidazole derivatives was studied.

The results revealed that the main parameter determining interactions between imidazole derivatives and HP-β-CD increased as

follows: HP-β-CD ⇔ solute hydrogen bonding interaction > HP-β-CD ⇔ solute complexation.

## INTRODUCTION

The α-, β-, and γ- cyclodextrins (CDs) are torus-shaped cyclic oligosaccharides consisting of six, seven, and eight α-(1,4)-linked D-glucopyrannose units, respectively.[1] Although they have found many applications,[2] such as the ability of forming an inclusion complex with a wide variety of organic molecules, they have some undesirable properties. The limited application of natural cyclodextrins in the pharmaceutical field seems to be related to their relatively low aqueous solubility.[1]

The chemically modified cyclodextrins, particularly hydroxyalkyl CDs, have received considerable attention because their pharmaceutical properties and inclusion behaviors are different from those of natural CDs. For example, the hydroxypropyl-β-cyclodextrin (HP-β-CD), a product obtained by condensation of β-CD with propylene oxide, is known for its good solubilizing power on various drugs.[3]

Bassani et al.[4] have demonstrated that the combination of albendazole and HP-β-CD in a molar ratio of 1/10 resulted in a significant increase in the aqueous solubility of the drug, up to 3500 times. Thus, albendazole/HP-β-CD complexes could be recommended as a parentally administrated formulation because of its good solubility properties and the safety of the CD used. Molecular recognition by substituted CDs as host compounds has been applied to the studies of enzyme analogues[5] and in chromatographic separations.[6] (S)-2- and (R, S)-2- HP-β-CD have been bonded to silica gel and extensively investigated as a new type of stationary phase for reversed phase high-pressure liquid chromatography.[7] Twenty racemates have successfully been resolved on this chiral stationary phase. The resolution of some of these was previously not possible by columns using single unsubstituted cyclodextrins as stationary phases.

Imidazole and triazole derivatives have been used for the treatment of onychomycosis.[8-10] Nevertheless, these hydrophobic compounds had a weak penetration into hydrophilic human nail matrices. Their inclusion in the apolar cyclodextrin cavity could improve this penetration, considering the hydrophilic character of the cyclodextrin rim, in which the primary hydroxy groups are on the narrower base and the secondary hydroxy groups are on the wider base of the toroid. Morin et al.[11] have already studied the inclusion complex formation of six imidazole derivatives with β-CD.

The aim of this paper was to examine the separation and inclusion of a series of six imidazole compounds using high performance liquid chromatography (HPLC) and to present the respective mechanistic aspects of the interactions between solutes and HP-β-CD.

## EXPERIMENTAL

### Apparatus

The HPLC system consisted of a Waters HPLC 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 x 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 (30°C ≤ T ≤ 55°C) and an Osi Julabo FT 200 cryoimmerser (Elancourt, France) for low temperatures (0°C ≤ T ≤ 25°C)). The mobile phase rate was fixed at 1 mL/mn and the wavelength at 230 nm.

### Solvents and Samples

HPLC grade methanol (Carlo Erba, Val de Reuil, France) was 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 (73/27 v/v) mixture containing various HP-β-CD concentrations (0, 2, 4, 6, 12, and 20 mM). 2-HP-β-CD (DS = 0.6) was a gift from the Roquette Laboratories (Lestrem, France). The phosphate buffer was composed of 0.01 M $(NH_4)_2HPO_4$/ 0.02 M $NH_4H_2PO_4$ and was adjusted to pH 3 with 1% phosphoric acid. Bifonazole (1), clotrimazole (2), econazole (3), sulconazole (4), miconazole (5), and oxiconazole (6), purchased from Sigma (Saint Quentin Fallavier, France), were dissolved in mobile phase to obtain a concentration of 1 mg/L. The chemical structure of these compounds is given in Figure 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).

### Temperature Studies

Compound retention factors were determined at the temperature values of 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, and 55°C. The chromatographic system was allowed to equilibrate at each temperature for at least 1 h prior to each

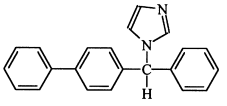
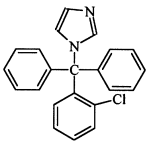
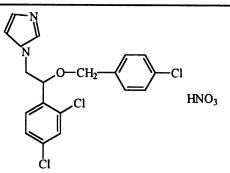
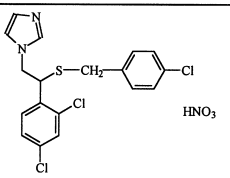
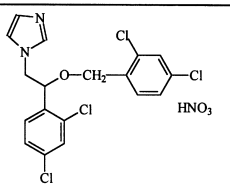
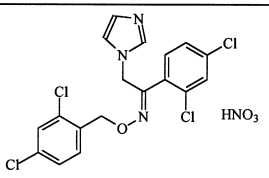
| Compound N° | Nomenclature | Chemical structure |
|---|---|---|
| (1) | Bifonazole |  |
| (2) | Clotrimazole |  |
| (3) | Econazole |  |
| (4) | Sulconazole |  |
| (5) | Miconazole |  |
| (6) | Oxiconazole |  |

**Figure 1**. Imidazole derivatives.

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 0.7%, making the chromatographic system sufficiently equilibrated for use after 1 h. All the solutes and mixtures of these were injected in triplicate at each temperature and HP-β-CD concentration.

## Method

### *Inclusion Complex Formation Constant*

In HPLC, the values of the formation constant of complexation between the solute and the cyclodextrin, $K_f$, are obtained from the slope-to-intercept ratio of a plot of the reciprocal of the retention factor k' of each eluting solute, versus the concentration of cyclodextrin incorporated in the mobile phase:[12]

$$\frac{1}{k'} = \frac{1}{k'_o} + \frac{K_f[HP-\beta-CD]}{k'_o} \quad (1)$$

where $k'_o$ is retention factor of free solute and [HP-β-CD] is the concentration of HP-β-CD in mobile phase, neglecting the adsorption of cyclodextrin itself or its complex with the solute, on the stationary phase.[11,12]

### *Separation Factor*

Solute retention is usually expressed in terms of the retention factor k', by the well known equations:[13,14]

$$\ln k' = \frac{-\Delta H^\circ}{RT} + \Delta S^{\circ *} \quad (2)$$

$$\Delta S^{\circ *} = \frac{\Delta S^\circ}{R} + \ln\phi \quad (3)$$

where ΔH° and ΔS° are, respectively, the corresponding standard enthalpy and entropy of transfer of the solute from the mobile to the stationary phases. T is the absolute temperature, R the gas constant and ϕ the phase ratio (volume of stationary phase divided by volume of mobile phase). Applying Eq. (2) to a pair of two adjacent peaks on a chromatogram, the following equations were obtained:

$$\ln k'_{i(i+1)} = \frac{-\Delta H^\circ_{i(i+1)}}{RT} + \Delta S^{\circ *}_{i(i+1)} \quad (4)$$

$$\Delta S^{\circ *}_{i(i+1)} = \frac{\Delta S^{\circ}_{i(i+1)}}{R} + \ln\phi \quad (5)$$

where $\Delta H°_i$ and $\Delta S°_i$ ($\Delta H°_{i+1}$ and $\Delta S°_{i+1}$) are, respectively, the standard enthalpy and entropy of transfer of the i compound (i+1 compound) from the mobile to the stationary phases.

Additionally, the separation factor, α, between the i and i+1 adjacent peaks on the chromatogram is given by the two following equations:

$$\alpha = \frac{k_{i+1}}{k_i} \quad (6)$$

$$\ln\alpha = \frac{-\Delta(\Delta H)}{RT} + \frac{\Delta(\Delta S)}{R} \quad (7)$$

where $k_i$ and $k_{i+1}$ are the retention factors of the two compounds i and i+1. Δ(ΔH) and Δ(ΔS) are, respectively, the difference in the dissolution enthalpy and entropy between the i and i+1 compounds. From a plot of ln α versus the reciprocal of the absolute temperature (called a van't Hoff plot) and using Eq. (7), Δ(ΔH) and Δ(ΔS) can be calculated from the slope and intercept respectively for each adjacent compound pair on a chromatogram noted (i, i+1).

## RESULTS AND DISCUSSION

### Values of Complex Formation Constants Between Solute and HP-β-CD

In according with Eq. (1), linear plots were obtained for all the solutes. The correlation coefficient values, r, for the linear fit were over 0.978. For example, Figure 2 represents 1/k' versus HP-β-CD concentration at T = 283 K for bifonazole. Complex formation constants were calculated at different temperatures. The $K_f$ values decreased with increasing temperature (Table I).

These values could be compared with those reported in the literature by Morin et al.,[11] concerning the inclusion of bifonazole, clotrimazole, econazole, sulconazole, miconazole, and oxiconazole with β-CD.

It is interesting to note that at T = 20°C, the inclusion of the imidazole derivatives in the HP-β-CD cavity instead of the β-CD cavity resulted in a significant decrease in the complex formation constant, up to 9, 3, 15, 16, 5, and 5 times for bifonazole, clotrimazole, econazole, sulconazole, miconazole, and oxiconazole, respectively. At T = 50°C, the six imidazole derivatives had nil $K_f$ values when studying their inclusion with the HP-β-CD molecule, whereas the $K_f$ values were in the range 21.5-185 $M^{-1}$ when studying their inclusion with the

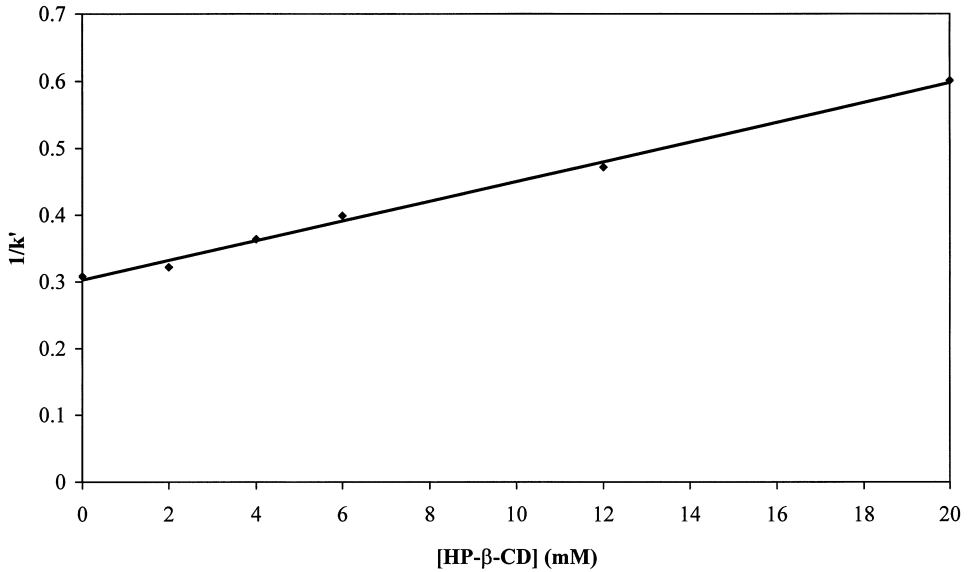



**Figure 2**. Representation of 1/k' versus [HP-β-CD] at T = 283 K for bifonazole.



β-CD molecule. These results firstly suggest that the β-CD cavity size (internal diameter and depth) is more appropriate for imidazole inclusion than the HP-β-CD cavity size.

Nevertheless, as reported by Szente,[13] the results of the substitution for parent cyclodextrins by the 2-hydroxypropyl groups imply that the attached hydroxypropyl groups not only provide additional sites for hydrogen bonding through their OH moiety with solutes, but can also cause steric interactions. Thus, the hydroxypropyl groups occlude the entrance for the solute of the cyclodextrin cavity and sterically influence the complex formation process, suggesting, as reported by Buvári-Barcza et al.,[14] that imidazole interacts with HP-β-CD molecule more through hydrogen bond with hydroxyl groups situated in the cyclodextrin rim than through hydrophobic effects with cyclodextrin cavity, justifying the lowest $K_f$ values when HP-β-CD is used.

## Gibbs Helmholtz Parameters

Δ(ΔH) and Δ(ΔS) were calculated at different HP-β-CD concentrations. In accordance with Eq. (7), linear plots were obtained for all solutes. The corre-

**Table 1**

**Inclusion Complex Formation Constant, $K_f(M^{-1})$ at Different Temperatures for all Imidazole Derivatives**

| | T = O°C | T = 5°C | T = 10°C | T = 15°C | T = 20°C | T = 25°C | T = 30°C | 35°C≤T≤55°C |
|---|---|---|---|---|---|---|---|---|
| Bifonazole (1) | 73.4 | 63.2 | 53.9 | 42.2 | 34.7 | 17.6 | 15.5 | 0.0 |
| Clotrimazole (2) | 31.7 | 26.4 | 19.0 | 18.8 | 10.3 | 2.4 | 0.0 | 0.0 |
| Econazole (3) | 27.2 | 23.3 | 20.0 | 14.7 | 13.1 | 6.2 | 5.1 | 0.0 |
| Sulconazole (4) | 43.8 | 36.2 | 27.8 | 21.0 | 17.5 | 0.4 | 0.0 | 0.0 |
| Miconazole (5) | 24.2 | 20.0 | 15.8 | 10.5 | 10.6 | 0.0 | 0.0 | 0.0 |
| Oxiconazole (6) | 28.0 | 23.1 | 16.2 | 13.7 | 12.6 | 0.0 | 0.0 | 0.0 |

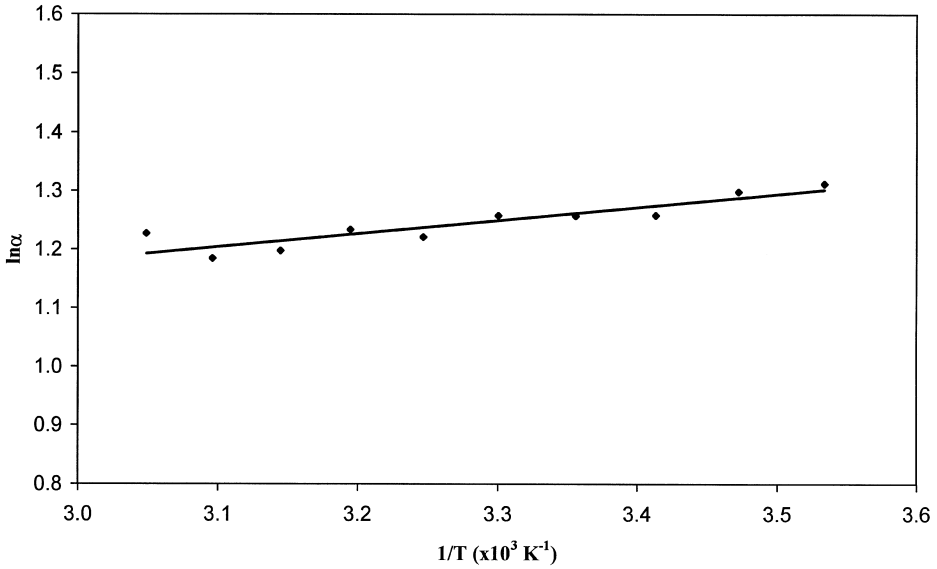



**Figure 3.** Van't Hoff plot for (3,4) imidazole pair; [HP-β-CD] = 0 mM.



lation coefficient values, r, for the linear fit were in excess of 0.980. The typical standard deviations of the slope and intercept were, respectively, over 0.002 and 0.04. For example, Figure 3 represents the van't Hoff plot for the (3,4) imidazole pair at a HP-β-CD concentration equal to 0 mM.

A complete list of Δ(ΔH) and Δ(ΔS) values determined for all the adjacent compound pairs at HP-β-CD concentration of 0, 2, 4, 6, 12, and 20 mM is shown in Table 2, from which the following conclusions can be drawn.

As shown in Figure 4 for the (1,2) pair for example, the Δ(ΔH) values became increasingly negative as the HP-β-CD concentration increased for the five imidazole pairs. These data indicate an increase in imidazole pair separation. Δ(ΔH) negative values imply that the separation was enthalpically controlled.

The results suggest that the solute pair interacted more favorably with the hydroxypropyl groups at the HP-β-CD cavity rim through strong hydrogen bonding or steric interactions in relation to the compound structure than with the hydrophobic HP-β-CD cavity through van der Waals or hydrophobic inter-

## Table 2

### $\Delta(\Delta H)$ (kJ.mol$^{-1}$) and $\Delta(\Delta S)$ (J.(mol.K)$^{-1}$) Values Determined for the Five Pairs of Peaks for Different HP-β-CD Concentrations

| Peak Pair[a] | [HP-β-CD][b] = 0 | r[c] | [HP-β-CD]=2 | r | [HP-β-CD] = 4 | r | [HP-β-CD] =6 | r | [HP-β-CD] = 12 | r | [HP-β-CD] = 20 | r |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (1,2) $\Delta(\Delta H)$ | -0.123 | | -0.237 | | -0.237 | | -0.321 | | -2.976 | | -4.278 | |
| | | 0.992 | | 0.993 | | 0.980 | | 0.983 | | 0.991 | | 0.994 |
| $\Delta(\Delta S)$ | 9.727 | | 9.811 | | 9.977 | | 9.727 | | 2.577 | | -0.582 | |
| (2,3) $\Delta(\Delta H)$ | -1.483 | | -2.125 | | -2.921 | | -3.327 | | -3.809 | | -6.043 | |
| | | 0.989 | | 0.994 | | 0.996 | | 0.993 | | 0.992 | | 0.997 |
| $\Delta(\Delta S)$ | 4.905 | | 2.744 | | 0.250 | | -0.915 | | -3.409 | | -11.473 | |
| (3,4) $\Delta(\Delta H)$ | -1.051 | | -1.158 | | -1.431 | | -1.952 | | -2.035 | | -2.186 | |
| | | 0.991 | | 0.992 | | 0.995 | | 0.995 | | 0.988 | | 0.997 |
| $\Delta(\Delta S)$ | 6.651 | | 6.485 | | 5.238 | | 3.492 | | 2.744 | | 2.411 | |
| (4,5) $\Delta(\Delta H)$ | -0.133 | | -0.399 | | -0.582 | | -1.265 | | -1.490 | | -2.091 | |
| | | 0.985 | | 0.985 | | 0.993 | | 0.996 | | 0.987 | | 0.999 |
| $\Delta(\Delta S)$ | 12.139 | | 11.390 | | 10.808 | | 9.395 | | 8.564 | | 7.233 | |
| (5,6) $\Delta(\Delta H)$ | -0.197 | | -0.575 | | -0.940 | | -1.532 | | -1.933 | | -2.022 | |
| | | 0.987 | | 0.990 | | 0.981 | | 0.996 | | 0.988 | | 0.995 |
| $\Delta(\Delta S)$ | 9.062 | | 7.815 | | 6.402 | | 4.157 | | 3.575 | | 2.993 | |

[a] See the corresponding compound in Table 1. [b] Expressed in mM. [c]Coefficient correlation.

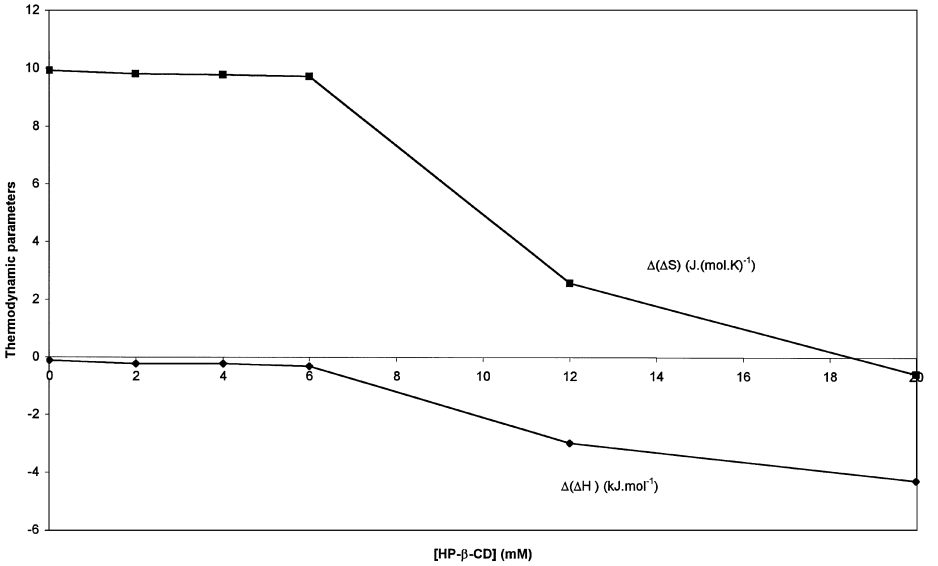



**Figure 4**. Thermodynamic parameters Δ(ΔH) (kJ.mol$^{-1}$) and Δ(ΔS) (J.(mol.K)$^{-1}$) values for the (1,2) pair versus HP-β-CD concentration.



actions. This indicates that imidazole pair peak separation was principally governed by hydrogen bonding (or steric repulsion) whereas the inclusion of solute in the HP-β-CD cavity was not the preponderant factor.

As shown in Figure 4 for the (1,2) pair for example, the Δ(ΔS) values were always positive except for the (1,2) peak pair at [HP-β-CD] = 20 mM and for (2,3) peak pair at [HP-β-CD] = 6, 12, and 20 mM. The Δ(ΔS) values decreased progressively (Figure 4) as the HP-β-CD concentration increased, for the five imidazole pairs. These data indicate a decrease in the order of all the five imidazole pairs when the HP-β-CD concentration increased. At a high HP-β-CD concentration, the HP-β-CD molecules tended, as shown by Mcalpine et al.,[15] to self association, decreasing the possibility for solutes to form hydrogen bonding with them. The particular temperature-dependant reversal of the elution order, θ, at which there was no resolution between the two adjacent compounds on the chromatogram was expressed by:

$$\theta = \frac{\Delta(\Delta H)}{\Delta(\Delta S)} \qquad (8)$$

The values of Table 2 permitted the verification that an elution reversal, within the temperature range, was not detected for 0, 2, 4, 6, 12, and 20 mM HP-β-CD concentrations, for all the peak pairs.

## CONCLUSION

In this paper, the retention mechanism in HPLC and the inclusion complex formation with HP-β-CD were studied for six imidazole derivatives. The complex formation constants were measured in relation to temperature. The Gibbs Helmholtz parameter trends were determined over a range of HP-β-CD concentrations. The results observed (low $K_f$ values, $\Delta(\Delta H)$ negative values and $\Delta(\Delta S)$ positive values) can be explained in terms of hydrogen bonding and steric repulsion between the imidazole and hydroxypropyl groups of cyclodextrin molecules whereas the solute inclusion in the HP-β-CD cavity played a minor role.



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