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Chromatographic Separation of Naproxen Enantiomers using Hydroxypropyl-β-Cyclodextrin as Chiral Mobile Phase Additive

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Abstract: Chromatographic separation of naproxen has been studied using hydroxypropyl-β-cyclodextrin as chiral mobile phase additive. The effects of mobile-phase composition were researched in detail. The appropriate composition of mobile phase was 85:15 (v/v) aqueous with 0.5% TEA at pH 3.5/ ethanol containing 25 mM HP-β-CD, and the column temperature was set of 25°C. Graphs of $1/k$ versus [HP-β-CD] gave good linear relationships, indicating the stoichiometry ratio of naproxen with HP-β-CD of 1:1. Apparent thermodynamic parameters were also calculated from the plots of $\ln\alpha$ versus 1/T, it was found that the enantioseparation was enthalpy driven and the inclusion process was exothermic.

Keywords: Chromatographic separation, naproxen enantiomers, hydroxypropyl-β-cyclodextrin

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INTRODUCTION

Naproxen, 6-methoxy-α-methyl-2-naphthalene-acetic acid, belongs to an important group of medicines called non-steroidal anti-inflammatory agents and is widely used in the treatment of rheumatic and other inflammatory diseases and for the relief of mild to moderate pain. Naproxen has a chiral center and the pharmacological activity resides mainly in the *S*-naproxen. Therefore, enantiomeric separation is vital in assuring good quality in the pharmaceutical production of naproxen (1).

Cyclodextrins [CDs; cyclic oligosaccharides composed of six, seven, or eight α-*D*-glucopyranose units (α-, β-, γ-CD, respectively)] form a family of excellent chiral selectors in HPLC. They are inherently chiral and undergo chiral interactions with analytes. CDs separate enantiomers utilizing the phenomenon of host–guest complexation, where a transient diastereomeric complex is formed between the CD and the analyte. Derivatization of the hydroxyl groups increases solubility and selectivity compared to the native β-CD and the hydroxyl groups also undergo additional interactions with the analytes, thereby enhancing chiral recognition (2).

HPLC method for direct enantioseparation of underivatized naproxen can be obtained either with chiral stationary phase (CSP) (3–5) or chiral mobile phase additive (CMPA). Since CSP is usually quite expensive and difficult to synthesize, use of CMPA can be a cheap, convenient, yet effective alternative. In the last years, chromatographic separation of *R*,*S*-naproxen mixtures has also been reported with two different CDs as CMPA, methyl-β-CD (Me-β-CD) and hydroxypropyl-β-CD (HP-β-CD), the latter showing less enantioselectivity and resolution (6). Depite this, we find that use of HP-β-CD as CMPA in a new mobile phase system, to separation of naproxen enantiomers, is a successful method of chromatographic separation, which includes a few of the merits.

This paper describes the enantioseparation of naproxen enantiomers by reversed phase HPLC using HP-β-CD as CMPA on an achiral column (C_{18}). The effects of mobile-phase composition are extensively studied. In order to investigate chiral recognition mechanism, the stoichiometry of complexation of naproxen with HP-β-CD and column temperature on enantioseparation are studied and the thermodynamic parameters also calculated.

EXPERIMENTAL

Chemicals and Reagents

Racemic naproxen and *S*-naproxen were obtained from Xianju Pharmaceutic Plant (Zhejian, PR, China); β-CD was purchased from Abxing Biological Technology Co. Ltd. (Beijing, PR, China), Me-β-CD was purchased from Xinda Fine Chemical Co. Ltd. (Shandong, PR, China), and HP-β-CD was

obtained from Yiming Fine Chemical Co. Ltd. (Taixing, PR, China). Triethylamine (TEA), methanol, acetonitrile, ethanol, glacial acetic acid and other reagents utilized were all of analytical grade. Water was deionized and bidistilled.

Apparatus and Operating Conditions

Chromatographic studies were performed using a LC-10 AD pump (Shimadzu, Japan), an SIL-10 A injection valve with 20 μl loop, an SPD-10 A UV/VIS spectrophotometer detector (Shimadzu, Japan) at 254 nm, an AT-130 temperature controller (Autoscience, Tianjin, PR, China) was used to control column temperature, a Lichrospher column (150 mm × 4.6 mm i. d.) packed with RP C_{18} was used for analysis, The pH measurement was performed on a pH meter (Orion, model 818, Shanghai, PR, China).

The mobile phase consisted of different mixtures of organic modifier and aqueous containing CMPA, TEA was added to the mobile phase (7), and finally, pH was adjusted to the appropriate value by adding glacial acetic acid. The mobile phase was filtered through a 0.45 μm filter and sonicated prior to use. The column was operated at ambient temperature 25°C. The flow-rate was set at 1.0 ml min^{-1}. The test racemic naproxen and *S*-naproxen sample were dissolved in ethanol at a concentration of 2.0 mg ml^{-1}, the solutions were stored at 4°C. The first eluate from chiral chromatography was found to be the *S*-enantiomer based on the *S*-naproxen sample and the previous report (6).

For the evaluation of the enantioseparation, the following parameters were measured: k_S is the capacity factor of the first eluted *S*-enantiomer, was calculated using the formula $(t_S - t_0)/t_0$, where t_0 is the time at which the first baseline disturbance by the solvent peak occurred, k_R the retention factor of the second eluted *R*-enantiomer, was calculated in the same way, α the selectivity factor: k_R/k_S, while R_s the resolution: $2(t_R - t_S)/(w_R + w_S)$, where w is the base width of the peak (8, 9).

RESULTS AND DISCUSSION

Influence of the Concentration of HP-β-CD

In principle, the separation efficiencies can be affected by increasing the concentration of CMPA because the interaction between CMPA and solute is very important for the chiral recognition. Consequently, the concentration of HP-β-CD was studied during optimation, addition of HP-β-CD (10–30 mM) to the mobile phase composed of 80:20 (v/v) aqueous, 1% TEA, pH 4.4/ ethanol. The results are shown in Table 1. An increase in HP-β-CD concentration yield a corresponding increase in

Table 1. Effect of the concentration of HP-β-CD on resolution

Concentration / mM	Capacity factor k_S	Capacity factor k_R	Selectivity (α)
10	36.85	39.27	1.07
15	24.61	26.89	1.09
20	20.15	22.28	1.11
25	17.14	19.84	1.16
30	14.95	12.89	1.16

Chromatographic conditions: 80:20 (v/v) aqueous with 1% TEA at pH 4.4/ ethanol.

chiral enantioselectivity (α), and a rapid decrease in capacity factor (k), due to the formation of the analyte-CD complexes in the mobile phase. The optimal concentration was achieved with a 25 mM HP-β-CD in the mobile phase. It should be noted that the optimum CMPA concentration depends on the percentage of the organic modifier in the mobile phase

In most cases it is assumed that complexation between HP-β-CD and solute molecule is 1:1. In some cases two or more CD molecule can bind to a single solute molecule. De Ying C. et al. (10) derived the following equation describing the relationship between the capacity factor and HP-β-CD concentration in the case of 1:1 stoichiometry.

$$\frac{1}{k} = \frac{1}{\phi K[A]} + \frac{K_1[CD]}{\phi K[A]} \quad (1)$$

where K is the corresponding equilibrium constant for the interact of solute molecule with stationary phase absorption site, while K_1 the corresponding equilibrium constant for the interact of solute molecule with HP-β-CD. ϕ is the phase ratio, A the stationary phase absorption site, and [CD] the concentration of HP-β-CD. A plot of $1/k$ versus [CD], with the correlation coefficient of 0.995, resulted in a linear plot at moderate to high concentrations of HP-β-CD, this suggests that HP-β-CD forms 1:1 complexes with naproxen in the separation process (Figure 1).

Influence of the Type and Content of Organic Modifier

The use of amounts of organic modifier is especially important because the addition of organic modifier to the mobile phase is known to greatly decrease the solubility of CDs. Acetonitrile, methanol, and ethanol were tried as the organic modifiers, the mobile phase composition was 20 mM HP-β-CD with 1% TEA at pH 4.4. Addition of acetonitrile or methanol to mobile phase permitted limited solubility of HP-β-CD in the mobile phase, addition of ethanol to the mobile phase allowed the use of high concentration

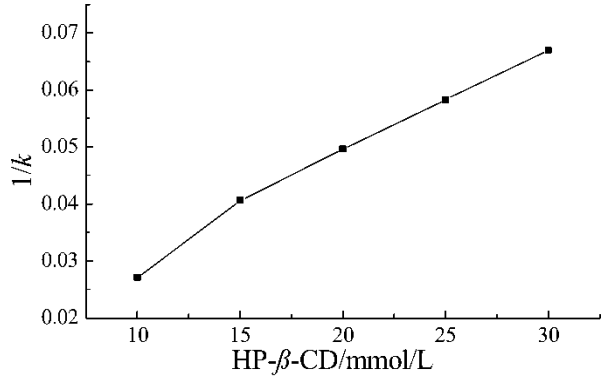

Figure 1. Plot of $1/k$ vs. HP-β-CD concentration.

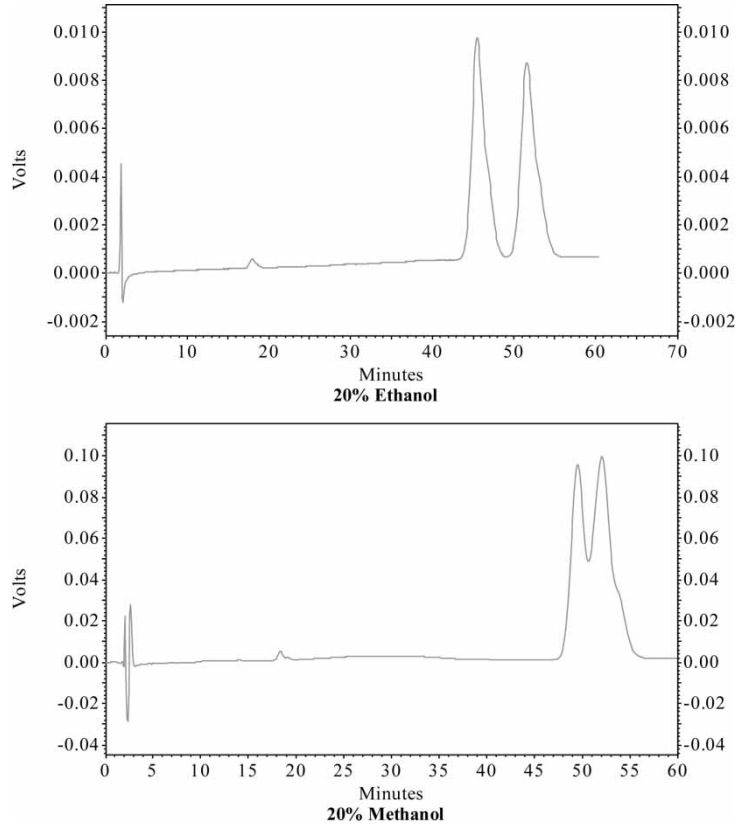

Figure 2. Chromatograms of resolution of naproxen under different organic modifier chromatographic conditions: 20 mM HP-β-CD with 1% TEA at pH 4.4.

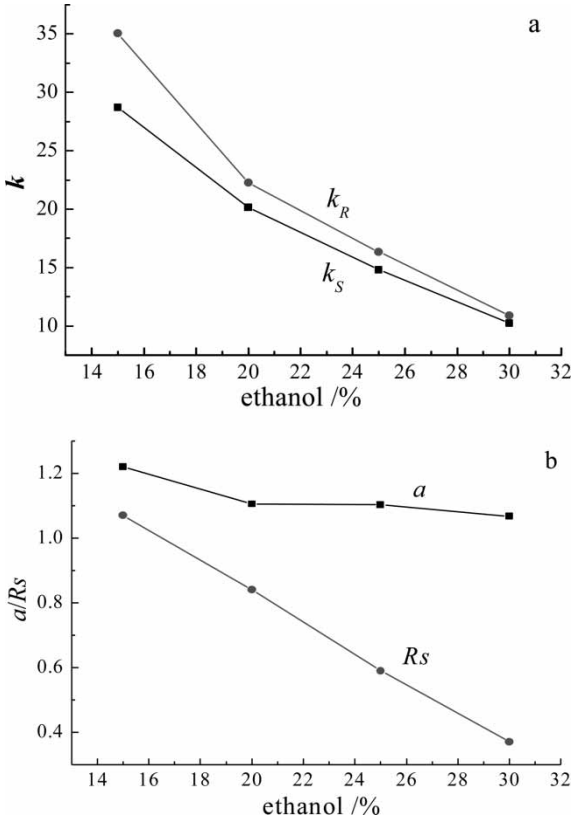

Figure 3. Influence of the ethanol concentration on (a) capacity factor and (b) selectivity and resolution chromatographic conditions: 20 mM HP-β-CD with 1% TEA at pH 4.4.

of HP-β-CD (11). The chromatograms are described in Fig. 2. The baseline separation could be achieved in the presence of acetonitrile or ethanol. However, complexes of HP-β-CD and naproxen eluted after 80 min in the use of acetonitrile. Methanol only caused partial resolution. As a result ethanol was chosen as the organic modifier in this study. The effect of ethanol concentration is given in Fig. 3. The capacity factor (k) significantly decreased as the concentration of ethanol increased because of decreased interaction between the inclusion complexes and the stationary phase. As a result, the peak shape improved. However, increasing the ethanol concentration decreased the enantioselectivity (α) and resolution (R_s) between the two enantiomers diminished. The changes in enantioselectivity maybe due to two different phenomena. First of all, both the alcohol and solute compete in occupying the HP-β-CD cavity and restrain the inclusion of CD to naproxen. On the other hand, the hydrophobic action of CD cavity decreased with the

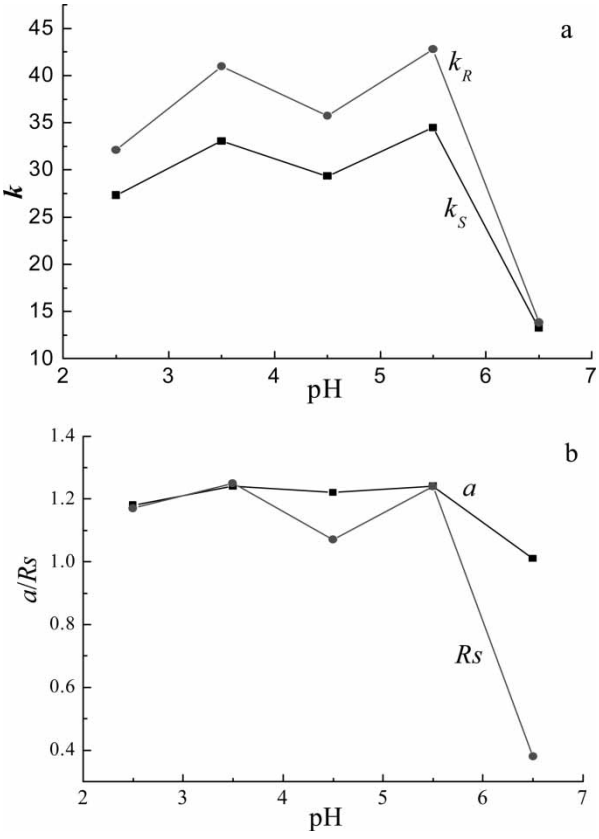

Figure 4. Effect of pH on (a) capacity factor and (b) selectivity and resolution chromatographic conditions: 85:15 (v/v) aqueous, 1% TEA, pH 4.4/ ethanol contaning 20 mM HP-β-CD.

increasing the concentration of polar alcohol, thereby debasing the enantiosectivity (12). But then, the retention times increase with the decrease of ethanol concentration. So the content of ethanol was better about 15%.

Influence of the pH of Aqueous Solution

pH has been reported to be a parameter affecting the resolution when using β-CDs (13). Consequently, the effect of pH of the mobile phase was investigated using 85: 15 (v/v) aqueous with 1% TEA/ ethanol containing 20 mM HP-β-CD. The results obtained are shown in Fig. 4. When the pH was 3.5 or 5.5, better baseline separation was obtained and the corresponding enantiosselectivity was 1.25 or 1.24. When the pH was or above 6.5, there provided partial or entirely no resolution. Since naproxen has a carboxylic acid

functionality ($pK = 4.26$), the molecule dissociates in a aqueous solution releasing a proton. Ionization suppression by pH control results in longer retention, as expected, and improves the likelihood of chiral discrimination (14). Although pH had some influences on the degree of separation, the separation results were not significantly affected in the pH range of 2.5–5.5. It was a appropriate choice of pH such as 3.5.

Influence of the TEA Content

TEA is commonly used in reversed phase separations to improve peak symmetry and minish tailing through hydrogen bonding, ionic interaction or adsorption to the C_{18} stationary phase surface (15), which was studied using 85:15 (v/v) aqueous at pH 3.5 / ethanol containing 25 mM HP-β-CD. Chromatograms and results are described in Fig. 5 and Fig. 6. Although the peak shape narrowed and improved with the increase of the TEA concentration, the changes were not distinct. The capacity factor was decreased as the increase of the TEA content, which might be related to the adsorption between the solute and stationary (16). The enantioselectivity and resolution were also decreased with the increasing TEA concentration. The retention times slightly increase with the decrease of TEA concentration. The appropriate TEA content was about 0.5%.

Influence of the Different CMPA

Chemical modification of β-CDs has been shown to "stretch" the cavity mouth and therefore change the hydrophobicity of the molecule and the

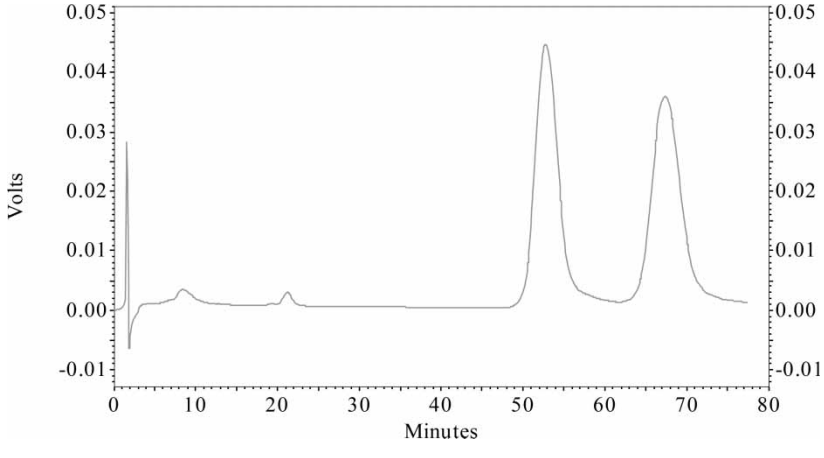

Figure 5. Chromatograms of resolution of naproxen under 0.5% TEA concentration chromatographic conditions: 85:15 (v/v) aqueous at pH 3.5/ ethanol containing 25 mM HP-β-CD.

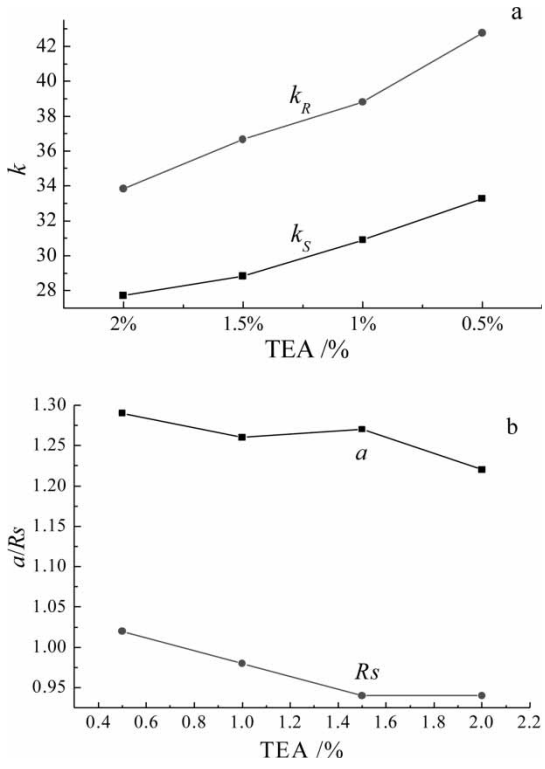

Figure 6. Effect of TEA on (a) capacity factor and (b) selectivity and resolution chromatographic conditions: 85:15 (v/v) aqueous at pH 3.5/ethanol containing 25 mM HP-β-CD.

stereoselectivity of the inclusion process. Different CDs, native β-CD, HP-β-CD and Me-β-CD were compared as chiral additives for the enantioresolution of naproxen. In all instances, a ethanol–aqueous with 1% TEA at pH 4.4 (20:80, v/v) containing 25 mM chiral additive, was used for elution. The results obtained are depicted in Fig. 7. HP-β-CD was the only selector that provided the entire resolution at tested concentration with the enantioselectivity and resolution were 1.14 and 1.01, respectively. Because HP-β-CD's some hydroxyl groups are substituted with hydroxypropyl functional groups, this modification allows for a more stereospecific and stronger interaction between the hydroxyl groups and hydrogen-bonding moiety present in the naproxen structure. In addition to the two aromatic rings which form inclusion complexes with the HP-β-CD, naproxen has a carboxylic acid group at the chiral centre that could participate in additional interactions with the rim hydroxypropyl groups of the HP-β-CD (17), the results also suggest that the hydrogen-bond interact between chiral additive and solute might be the major chiral recognition mechanism for HP-β-CD.

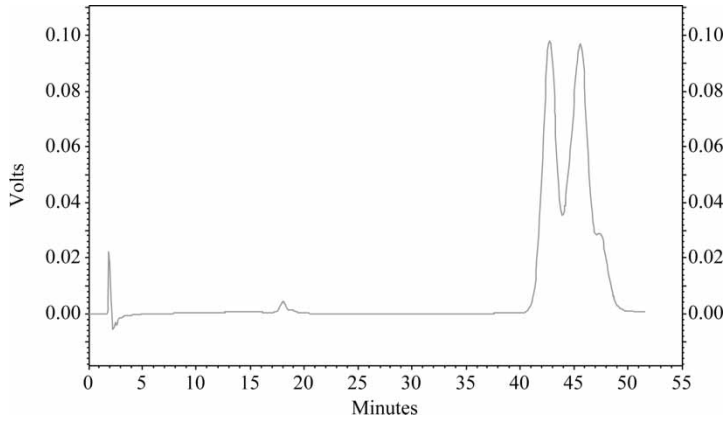

Figure 7. The chromatograms of Me-β-CD as chiral additive chromatographic conditions: ethanol–aqueous with 1% TEA at pH 4.4 (20:80, v/v).

Influence of the Column Temperature

Temperature is an important factor in controlling chiral recognition processes, assuming there are no changes in the interaction property among the solute, CMPA, and the stationary phase with the variation of temperature. Conventionally, we consider that the relationships of column temperature with thermodynamic parameters are as follows (18).

$$-\Delta\Delta G^0 = RT \ln \alpha = RT \ln \frac{k_R}{k_S} \tag{2}$$

$$\Delta_{R,S}\Delta G = \Delta_{R,S}\Delta H - T\Delta_{R,S}\Delta S \tag{3}$$

$$\ln \alpha = \frac{\Delta_{R,S}\Delta H^0}{RT} + \frac{\Delta_{R,S}\Delta S^0}{R} \tag{4}$$

where $\Delta_{R,S}\Delta G$, $\Delta_{R,S}\Delta H$ and $\Delta_{R,S}\Delta S$ represent the differences of free energy, enthalpy and entropy for a given pair of enantiomers, respectively. And R is the gas constant, T the absolute temperature.

In order to study the influence of temperature on retention and selectivity, the same experiments were carried out at the range of 25–45°C using 25 mM HP-β-CD with 0.5% TEA at pH 4.0 containing 15% ethanol. We can see from Fig. 8, the values of k, α, and R_s decreased when the column temperature was increased. According to $\ln\alpha$ versus $1/T$ (from Fig. 9), the plot was highly linear ($r^2 > 0.996$), suggesting that the conformation of the stationary phase was rigid over the temperature range of 25–45°C. The chiral discrimination mechanism remains unchanged, and corresponding thermodynamic parameters are temperature-independent. If the van't Hoff plots of ln α against $1/T$ are linear within a temperature range, the correlative thermodynamic

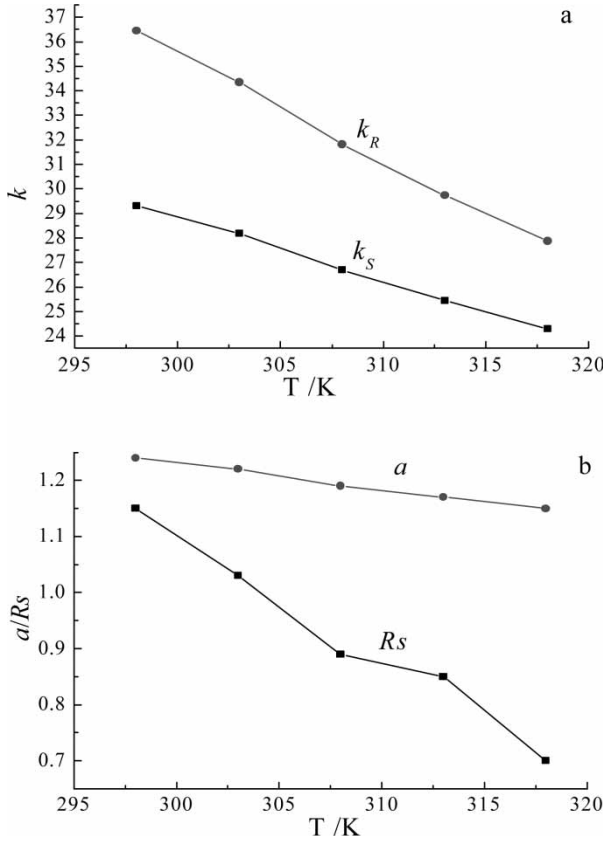

Figure 8. Effect of column temperature on (a) capacity factor and (b) selectivity and resolution Chromatographic conditions: 85:15 (v/v) aqueous with 0.5% TEA at pH 4.0/ ethanol containing 25 mM HP-β-CD.

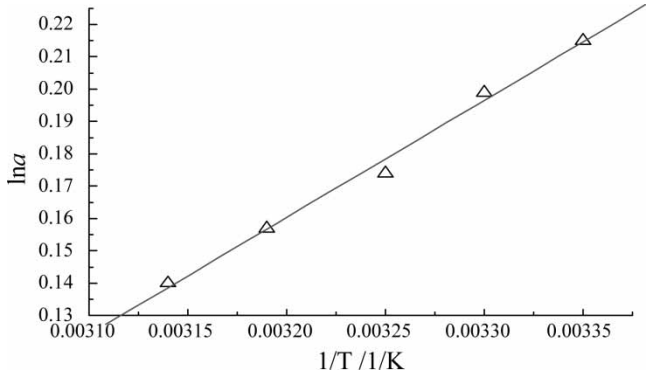

Figure 9. The plots of lnα vs. 1/T for enantioseparation of naproxen.

Table 2. Thermodynamics parameters of the enantioseparation of naproxen enantiomers

Regression eluation	r^2	$-\Delta_{R,S}\Delta H$	$-\Delta_{R,S}\Delta S$	$\Delta_{R,S}\Delta G$	R_S	α
		J/mol	J/(mol*K)	298K/(J/mol)	298K	298K
Y = −0.0.9970 + 361.7x	0.996	3007	8.289	−535.6	1.15	1.24

parameters (Table 2) can be obtained from the slope or intercept of the straight lines. The Gibbs–Helmholtz parameters, $\Delta_{R,S}\Delta H$ and $\Delta_{R,S}\Delta S$ can be calculated from the plots, they are apparent, the negative value indicates that the separation is enthalpy driven and the inclusion process is exothermic. The negative entropy of chiral separation is unfavorable to the happening of the process of chiral recognition and must be compensated by the release of enthalpy during the action of chiral recognition of HP-β-CD to naproxen molecule.

CONCLUSIONS

Chromatographic separation of *R*,*S*-naproxen was achieved on an achiral C_{18} column with HP-β-CD as CMPA, the composition of appropriate chiral mobile was 15% ethanol, 25 mM HP-β-CD with 0.5% TEA at pH 3.5. The column temperature was set at 25°C and the flow rate of 1 ml min^{-1}. A wider range of pH in the aqueous phase offers a moderate and simple method for the confection of the mobile phase, and the mobile phase without using any buffer salt is in favor of the maintenance of the good state of the HPLC column system. However, considering the longer retention times obtained for the target compound is an important task to have to be solved at once.

It appears from the studies that HP-β-CD forms 1:1 complexes with naproxen in the separation process. Apparent thermodynamic parameters were also calculated from the plots of $\ln\alpha$ versus $1/T$. It was found that the enantioseparation is enthalpy driven and the inclusion process is exothermic.

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