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Study on the Retention Characteristics and Separation Mechanism of Resolution of Naproxen Enantiomers with Hydroxypropyl-β-cyclodextrin as Chiral Additive

[1,2]Feipeng Jiao, [1]Kelong Huang, [2]Xia Yuan, [1]Fengrong Ning and [1]Weiguo Hu
[1]School of Chemistry and Chemical Engineering, Central South University, Changsha 410083, People's Republic of China
[2]School of Chemical Engineering, Xiangtan University, Xiangtan 411105, People's Republic of China

Abstract: Resolution of naproxen by reversed phase High Performance Liquid Chromatography (HPLC) has been studied using hydroxypropyl-β-cyclodextrin (HP-β-CD) as chiral additive. The effects of mobile phase composition including the concentration of HP-β-CD and ethanol, pH of aqueous phase, were researched in detail. The retention characteristics and separation mechanism will be discussed emphatically, graphs of the reciprocal of capacity factor versus concentration of 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 plots of the natural logarithm of separation factor versus reciprocal of temperature, it was found that the enantioseparation was enthalpy driven and the inclusion process was exothermic.

Key words: Resolution, HPLC, naproxen enantiomers, HP-β-CD, retention characteristics, thermodynamic parameter

INTRODUCTION

Chiral separations are a topic of great interest because of their importance in several fields, including biomedical research. High Performance Liquid Chromatography (HPLC) is the primary chiral separation technique used for pharmaceutical applications. Three approaches are generally used to achieve chiral separation by HPLC: indirect separations used chiral reagents to form permanent diastereomers, direct separation using a chiral stationary (CSP) and direct separation using Chiral Mobile Phase Additives (CMPA). Several reports demonstrate the utility of β-cyclodextrins (CDS) as a CMPA for the direct separation of enantiomers using reversed-phase HPLC (Sekhar and Shoukry, 1996; Healy *et al*., 2001; Cheristian and Anita, 1995).

β-CD is inherently chiral and undergo chiral interactions with analytes. *β*-CD separates enantiomers utilizing the phenomenon of host-guest complexation, where a transient diastereomeric complex is formed between the CD and the analyte. In a derivatized HP-*β*-CD, 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 (Emmanuel and James, 1998; Han *et al*., 2005).

The goal of our research was to investigate the chromatographic behavior of a system comprised of HP-*β*-CD. A non-steroidal anti-inflammatory agent naproxen were used as a model compound for the study (Fillet *et al*., 1998). The effects of mobile-phase composition are studied, in order to investigate retention characteristics and chiral recognition mechanism, the stoichiometry of complexation of naproxen with HP-β-CD and column temperature on enantioseparation are considered and the thermodynamic parameters also be calculated.

MATERIALS AND METHODS

Materials: The present research were started one year ago and were completed and concluded in School of Chemical engineering and Chemistry of Central South University in 2005. Racemic naproxen and *S*-naproxen were obtained from Zhejian Xianju Pharmaceutic Plant in China; β-CD was purchased from Beijing Abxing Biological Technology Co. Ltd. in China, Me-β-CD was purchased from Shandong Xinda Fine Chemical Co. Ltd. in China and HP-β-CD was obtained from Jiangsu Yiming Fine Chemical Co. Ltd. in China. TEA, methanol, ethanol, glacial acetic acid and other reagents utilized were all of analytical grade. Water was deionized and bidistilled.

Corresponding Author: Feipeng Jiao, School of Chemistry and Chemical Engineering, Central South University, Changsha 410083, People's Republic of China

Apparatus and operating conditions: Chromatographic studies were performed using a LC-10AD pump (Shimadzu, Japan), an SIL-10A injection valve with 20 μL loop, an SPD-10A UV/VIS spectrophotometer detector (Shimadzu, Japan) at 254 nm, an AT-130 temperature controller (Autoscience, Tianjin, China) was used to control column temperature, a Lichrospher column (150×4.6 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, China).

The mobile phase consisted of ethanol and aqueous containing HP-*β*-CD, TEA was added to mobile phase (Robert *et al*., 1995) and finally, pH was adjusted to the appropriate value by adding glacial acetic acid. Mobile phase was filtered through a 0.45 μm filter and sonicated prior to use. Column was operated at ambient temperature 25°C and flow-rate set at 1.0 mL min^{-1}. Racemic naproxen and *S*-naproxen sample were dissolved in ethanol at a concentration of 2.0 mg mL^{-1}. The first eluate from chiral chromatography was found to be *S*-enantiomer based on *S*-naproxen sample and the previous report (Healy *et al*., 2001).

For evaluation of enantioseparation, the following parameters were measured: k_S, 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, capacity factor of the second eluted *R*-enantiomer, was calculated in the same way. β 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 peak (Anna *et al*., 2001; Küsters and Spondlin, 1996).

RESULTS AND DISCUSSION

Influence of the concentration of hp-β-cd and study on retention characteristics: In principle, separation efficiencies can be affected by increasing the concentration of CMPA because the interaction between CMPA and solute is very important for chiral recognition. Consequently, the concentration of HP-β-CD was studied during optimation, addition of HP-β-CD (10-30 mmol L^{-1}) to mobile phase composed of 80: 20 (v/v) aqueous, 1% TEA, pH 4.4/ethanol. The results were shown in Table 1. An increase in HP-β-CD concentration yield a corresponding increase in chiral enantioselectivity (*α*) and a rapid decrease in capacity factor (k), due to the formation of the analyte-HP-β-CD complexes in mobile phase. The optimal concentration was achieved with a 25 mmol L^{-1} HP-β-CD in mobile phase, considering the influence of solubility of HP-β-CD.

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

Concentration (mmol L^{-1})	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

Naproxen is a weak acid, in the solution can dissociate as follows:

$$Hnp \Leftrightarrow H^+ + Np^- \quad (1)$$

$$K_a = [H^+][Np^-]/[HNp] \quad (2)$$

Therefore interactions of naproxen molecular with stationary phase can be described as follows:

$$Hnp+G \Leftrightarrow GHNp \quad (3)$$

$$K_1 = [GHNp]/[HNp][G] \quad (4)$$

$$Np^- + G \Leftrightarrow Gnp^- \quad (5)$$

$$K_2 = [Gnp^-]/[Np^-][G] \quad (6)$$

where, HNp represents naproxen molecular, [] is the concentration symbol. K_a is the dissociation constant, K_1 and K_2 are equilibrium constants and G is a stationary phase absorption site, [CD] is the concentration of HP-β-CD.

According to Chen's report (2004), the resolved analyte and naproxen molecular only form inclusion with the 1: 1 stoichiometry when CDS as chiral additive. In case of HP-β-CD and HP-β-CD-analyte are not reserved by stationary phase, the equilibrium of resolution process can be described as follows:

$$CD+HNp \Leftrightarrow CD\text{-}HNp \quad (7)$$

$$K_{CD\text{-}HNp} = [CD\text{-}HNp]/[CD][HNp] \quad (8)$$

$$CD+Np^- \Leftrightarrow CD\text{-}Np^- \quad (9)$$

$$K_{CD\text{-}HNp} = [CD\text{-}Np^-]/[CD][Np^-] \quad (10)$$

where [CD-HNp] or [CD-Np^-] represents the concentration of inclusion formed by HP-β-CD with HNp or Np^-, $K_{CD\text{-}HNp}$ and $K_{CD\text{-}Np^-}$ are the corresponding inclusion constants of equilibrium, respectively. The total analyte resolved is given by:

$$S_{tot} = HNp+Np^-+G\text{-}HNp+G\text{-}Np^-+CD\text{-}HNp+CD\text{-}Np^- \quad (11)$$

The capacity factor is defined by:

$$k = \frac{\phi([GHNp]+[GNp^-])}{[Hnp]+[Np^-]+[CD\text{-}HNp]+[CD\text{-}Np^-]} \quad (12)$$

Where ϕ is the phase ratio. Substituting Eq. 2, 4, 6, 8, 10 and 11 into and rearranging Eq. 12:

$$1/k = \frac{[H^+]+K_a}{[H^+][G]\,K_1\phi\pounds\ll K_2K_a\phi[G]} + \frac{(K_{CD:HNa}+K_{CD:Na}-K_a)[CD]}{[H^+][G]\,K_1\phi\pounds\ll K_2K_a\phi[G]} \quad (13)$$

from Eq. 13, we can see that capacity factor is the function of [CD] and pH, so the relationship between capacity factor (k) and HP-β-CD concentration is of 1: 1 stoichiometry under the same pH. A plot of 1/k versus [CD] resulted in a linear plot at moderate to high concentrations of HP-β-CD, this suggests that HP-β-CD might form 1: 1 complexes with naproxen in resolution process (Fig. 1).

Influence of content of ethanol: The effect of ethanol concentration was investigated with mobile phase composition of 25 mmol/L HP-β-CD with 1% TEA at pH

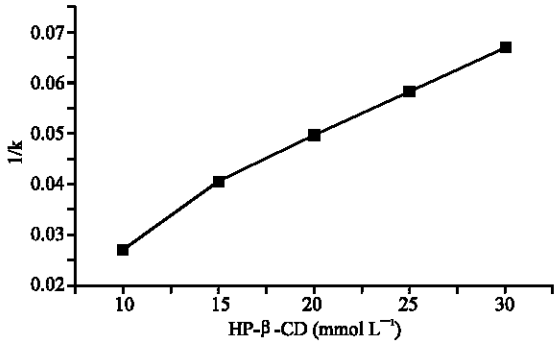

Fig. 1: Plot of 1/k vs. HP-β-CD concentration

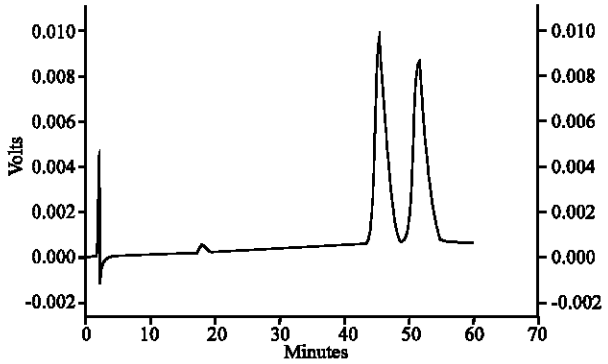

Fig. 2: The chromatogram of naproxen with 25 mmol L^{-1} HP-β-CD Chromatographic conditions: A ethanol-aqueous with 1% TEA at pH 4.4 (20:80, v/v) containing 25 mmol L^{-1} HP-β-CD

4.4. Capacity factor (k) significantly decreased as the concentration of ethanol increased because of decreased interaction between the inclusion complexes and stationary phase, as a result, the peak shape improved. Increasing the ethanol concentration decreased enantioselectivity (α) and resolution (R_s) between the two enantiomers diminished, while decreasing the ethanol concentration increased retention time (Fig. 2). 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 HP-β-CD to naproxen. On the other hand, the hydrophobic action of HP-β-CD cavity decreased with the increasing the concentration of polar alcohol, thereby debasing the enantiosectivity. So the content of ethanol was better kept within the range of 15-20%.

Influence of the pH of aqueous solution: pH was found to be important for improvement of enantioselectivity, which was investigated for naproxen enantiomers using 85: 15 (v/v) aqueous with 1% TEA/ethanol containing 20 mmol L HP-β-CD, the results are described in Fig. 3, pH had a profound effect on the degree of separation without particular rule, which is consistent with the relationships of k with $[H^+]$ described by Eq. 13. When pH were 3.5 and 5.5, baseline separations were obtained and the enantioselectivitys were 1.25 and 1.24, respectively. When pH was above 6.5, the selectivity rapidly decreased. Since naproxen has a carboxylic acid functionality (pH = 4.26), the molecule dissociates in a aqueous solution releasing a proton. Ionisation suppression by pH control results in longer retention, as expected and improves the likelihood of chiral discrimination (Healy *et al*., 2001). Therefore, appropriate pH was over the range of 4-5.5.

Influence of the column temperature and study on mechanism of chiral recognition: Temperature is an important factor in controlling chiral recognition processes, assuming there is no change in the interaction property between solute and stationary phase with the variation of temperature. Conventionally, in Table 2 we consider that the relationships of column temperature with thermodynamic parameters are as follows (Chen *et al*., 2004; Küsters and Spondlin, 1996):

$$-\Delta\Delta G^0 = RT\ln\alpha = RT\ln\frac{k_R}{k_S} \quad (14)$$

$$\Delta_{R,S}\Delta G = \Delta_{R,S}\Delta S - T\Delta_{R,S}\Delta S \quad (15)$$

$$\ln\alpha = \frac{\Delta_{R,S}\Delta H^0}{RT} + \frac{\Delta_{R,S}\Delta S^0}{R} \quad (16)$$

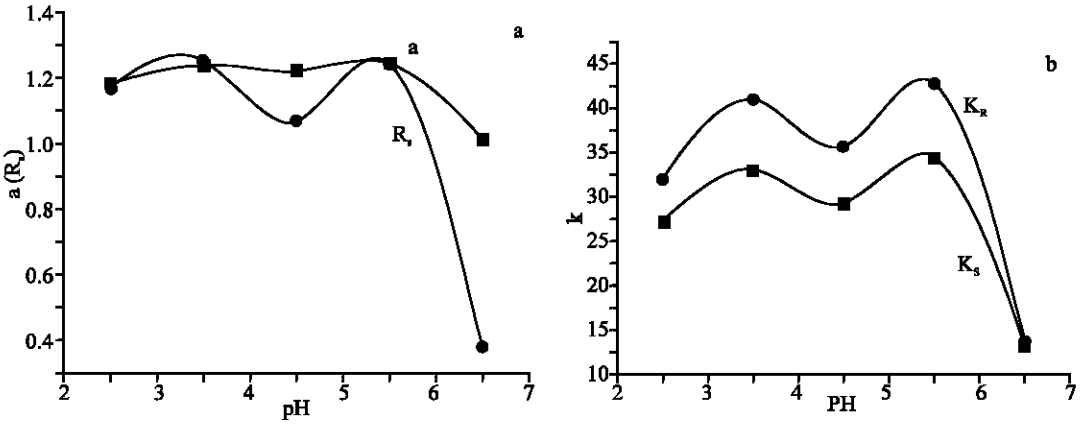

Fig. 3: Effect of pH on (a) capacity factor and (b) selectivity and resolution. Chromatographic conditions: 85:15 (v/v) aqueous, 1% TEA/ ethanol containing 20 mmol L^{-1} HP-β-CD

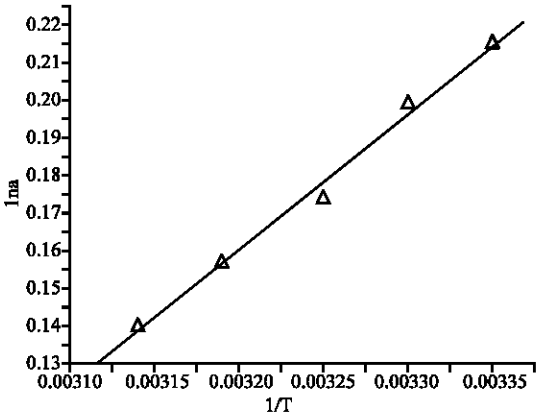

Fig. 4: The plot of lnα vs. 1/T for enantioseparation of naproxen

where, $\Delta_{R,S}$ ΔG, $\Delta_{R,S}$ ΔH and $\Delta_{R,S}$ ΔS represent the differences of enthalpy and entropy for a given pair of enantiomers, respectively. *R* is the gas constant and *T* the absolute temperature.

In order to study the influence of temperature on retention and enantioselectivity, the same experiments were carried out at the range of 25-45°C using 25 mmol L^{-1}HP-β-CD with 0.5% TEA at pH 4.0 containing 15% ethanol. According to lnα versus 1/T (Fig. 4), 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 remained unchanged and corresponding thermodynamic parameters are temperature-independent, the correlative thermodynamic parameters can be obtained from the slope or intercept of the straight lines. The Gibbs–Helmholtz parameters, $\Delta_{R,S}$ΔH and $\Delta_{R,S}$ΔS can be calculated from the plots, they are apparent, the negative

Table 2: Thermodynamics parameters of the enantioseparation of naproxen enantiomers

Regression Eq.	r^2	$-\Delta_{R,S}\Delta H$ (J/mol)	$-\Delta_{R,S}\Delta S$ (J/mol*K)	$\Delta_{R,S}\Delta G$ (298K)/ (J/mol)	R_S (298K)	α (298K)
Y = -0.0.9970 +361.7x	0.996	3007	8.289	-535.6	1.15	1.244

values indicate that the separation is enthalpy driven and the inclusion process is exothermic. The negative entropy of chiral separation is unfavourable 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 racemic naproxen was achieved on an achiral C_{18} column with HP-β-CD as CMPA. A relatively loose analytical conditions offers moderate and simple method for the confection of mobile phase and the mobile phase without using any buffer salt is in favor of the maintenance of good state of HPLC column system.

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 α versus 1/T. It was found that the enantioseparation is enthalpy driven and the inclusion process is exothermic.

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