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Resolution of β-blockers on a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid: Unusual temperature effect

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Abstract

A chiral stationary phase (CSP) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid has been successfully employed in the liquid chromatographic resolution of eleven β-blockers containing a secondary amino functional group. As the result of an effort to find out the optimal mobile phase condition, the mixture of trifluoroacetic acid–triethylamine–ethanol–acetonitrile with the ratio of 0.1/0.5/20/80 (v/v/v/v) was concluded to be the best mobile phase condition, the separation (α) and the resolution factors (R_S) for the resolution of 11 β-blockers being in the range of 1.13–1.85 and 1.36–5.79, respectively. Surprisingly, in contrast to the resolution of other racemic compounds containing a primary amino functional group, the separation factors (α) for the resolution of β-blockers were observed to improve as the column temperature increased and these unusual chromatographic behaviors were rationalized as the entropically controlled enantioselectivity.
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1. Introduction

(+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** (Fig. 1), which was first prepared by Lehn and coworkers [1], has been successfully utilized as an effective chiral selector of liquid chromatographic chiral stationary phases (CSPs). For example, Machida and coworkers developed a CSP by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** to 3-aminopropylsilica gel in the presence of coupling agent EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinolone) [2]. We also developed a structurally well defined CSP (CSP **2**) by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic dianhydride derived from (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** to 3-aminopropylsilica gel [3,4]. CSP **2** was very successful in the resolution of various racemic primary amino compounds including α- and β-amino acids [4–6], α-amino acid derivatives [4,7], racemic amines, racemic amino alcohols [8], racemic fluoroquinolone antibacterials [3,9,10] and aryl α-amino ketones [11].

Even though the chiral recognition mechanism is still controversial, it has been generally accepted that the protonation of the primary amino functional group of analytes in a mobile phase containing a certain amount of acidic modifier and the complexation of the resulting primary ammonium ion ($R–NH_3^+$) of analytes inside the cavity of chiral crown ether ring of the CSP are essential for the chiral recognition [12,13] except for the chiral resolutions in gas phase [14,15]. Consequently, liquid chromatographic chiral resolutions on crown ether-based CSPs have been generally performed with a mobile phase containing a certain amount of acidic modifier and applied only in the resolution of primary amino compounds. Actually, the resolution of racemic secondary amino compounds on crown ether-based CSPs has been thought to be impossible. However, recently, Steffeck and coworkers reported the first separation of the enantiomers of racemic

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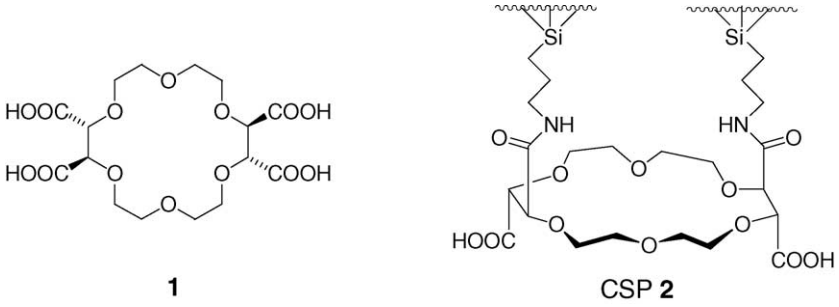

Fig. 1. Structures of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid **1** and CSP **2**.

secondary amino compounds on crown ether-based CSPs using CSP **2** [16]. In their short communication report, Steffeck and coworkers briefly demonstrated that five racemic secondary amino compounds including four β-blockers such as albuterol, atenolol, pindolol and propranolol show some resolution on CSP **2**. However, during our efforts to extend the use of CSP **2** to the resolution of other β-blockers, we found that the mobile phase condition reported by Steffeck and coworkers was not applicable to the resolution of several other β-blockers. Consequently, more widely applicable mobile phase condition should be explored for the resolution of various β-blockers on CSP **2**.

β-Blockers have been widely used to control pulmonary disease, hypertension, heart failure, migraine headaches and angina pectoris [17,18]. Especially, the (*S*)-enantiomers of β-blockers have been known usually more potent than the (*R*)-enantiomers [19]. Moreover, it has been reported that some (*R*)-enantiomers are toxic and present undesirable side effects [20]. In this instance, the separation of the enantiomers of β-blockers on CSP **2** is of great importance because this method can provide the very valuable means in the exact determination of the enantiomeric composition of β-blockers even though Pirkle-type CSPs [21,22], polysaccharide-based CSPs [23], macrocyclic antibiotic-based CSPs [24–26], and other CSPs [27] have been previously utilized in the liquid chromatographic resolution of β-blockers. However, the use of CSP **2** in the resolution of β-blockers was limited to only four β-blockers as mentioned above. In this study, we wish to find out an improved mobile phase condition for the resolution of β-blockers on CSP **2** and extend the use of CSP **2** to the resolution of a wide variety of β-blockers including β_1-, β_2- and nonspecific β-blockers. In addition, we wish to report the unusual temperature effect for the resolution of β-blockers on CSP **2**.

2. Experimental

Chromatography was performed with an HPLC system consisting of a Waters model 515 HPLC pump, a Rheodyne model 7725i injector with a 20 μl sample loop, a YoungLin M720 Absorbance detector (variable wavelength) and a YoungLin Autochro Data Module (Software: YoungLin Autochro-WIN 2.0 plus). The temperature of the chiral column was controlled by using a Julabo F30 Ultratemp 2000 cooling circulator. Chiral column (150 mm × 4.6 mm I.D.) packed with CSP **2** were available from previous study [11].

Among the β-blockers used in this study (Fig. 2), nonspecific β-blockers (**3**–**6**) and β_1-blockers (**7**, **8**) were provided by Dr. Welch when he was a research scientist at Regis Chemical Company (Morton Grove, Illinois, USA) and all β_2-blockers (**9**–**13**) were obtained from the Laboratory of Medicinal Chemistry, Shenyang Pharmaceutical University, China. Methanol, ethanol, isopropanol and acetonitrile were of HPLC grade from Fisher Scientific Korea Ltd. Acetic acid and triethylamine of super purity were purchased from Jusei Chemical Co., Ltd. (Japan). All solvents were filtered through a 0.45 μm filter and degassed before use.

Each of β-blockers shown in Fig. 2 was dissolved in methanol (usually 1.0 mg/ml) and then used for the resolution on CSP **2**. The usual injection volume was 2.0 μl.

3. Results and discussion

In this study, we tried to resolve eleven analytes including four nonspecific β-blockers (**3**–**6**), two β_1-blockers (**7, 8**) and five β_2-blockers (**9**–**13)** shown in Fig. 2 on CSP **2**. As a first attempt, we tried to resolve analytes **3**–**13** on CSP **2** with the use of usual aqueous mobile phases (for example, 50% methanol in water +10 mM sulfuric acid), which have been successfully utilized in the resolution of racemic primary amino compounds such as α-amino acids, amines and amino alcohols [3–8]. However, the secondary amino analytes shown in Fig. 2 were not resolved at all on CSP **2** with the use of usual aqueous mobile phase. Consequently, as a standard, we selected one mobile phase, the mixture of acetic acid–triethylamine–methanol–acetonitrile with the ratio of 0.1/0.1/50/50 (v/v/v/v), which was originally utilized by Steffeck and coworkers in the resolution of four β-blockers (albuterol, atenolol, pindolol and propranolol) [16]. And then, we tried to find out improved mobile phase condition from the trends of resolution behaviors observed with the variation of mobile phase composition from the standard.

First of all, in order to find out the effect of the ratio of methanol–acetonitrile in mobile phase, we resolved the analytes shown in Fig. 2 on CSP **2** with the variation of the ratio of methanol–acetonitrile in mobile phase at the constant

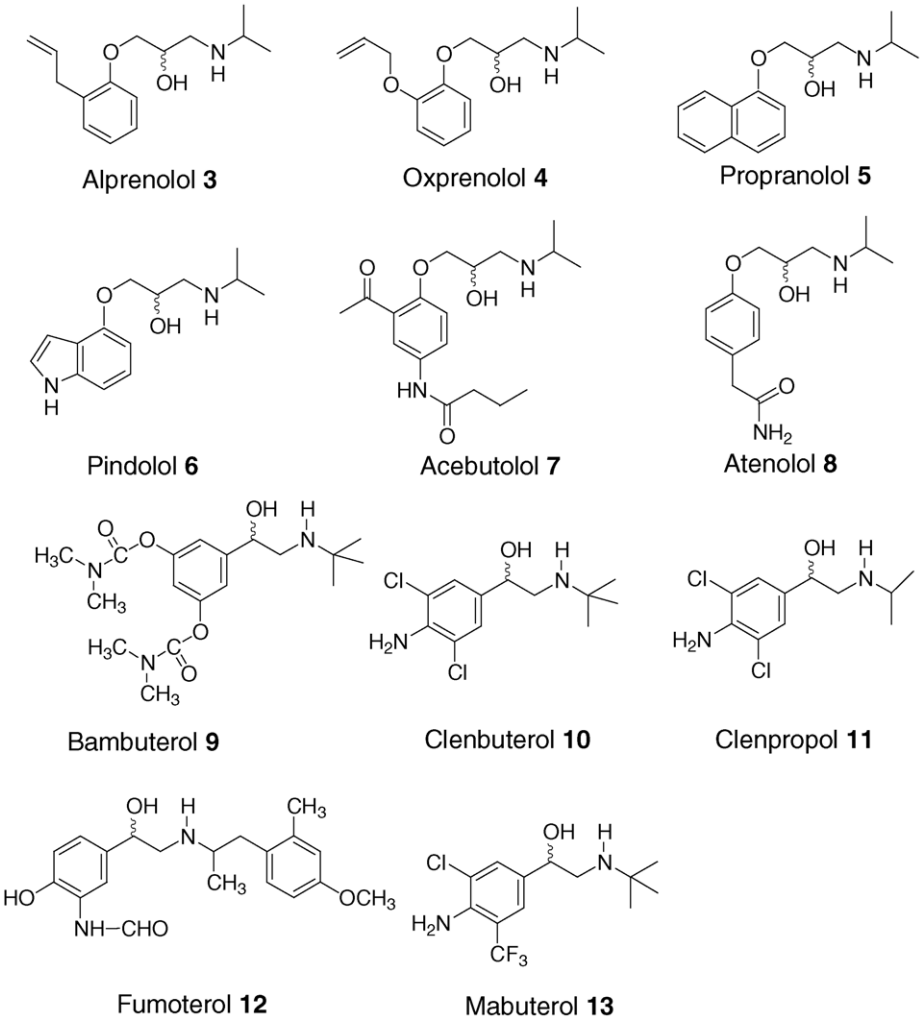

Fig. 2. Structures of β-blockers **3**–**13** used in this study.

ratio of acetic acid–triethylamine (0.1/0.1, v/v) and the chromatographic resolution results are summarized in Table 1. As the content of methanol in mobile phase decreases from 50 to 20%, the separation (α) and the resolution factor (R_S) slightly increase in general. However, the retention factor (k_1) increases significantly as shown in Table 1. When the methanol content was decreased further, the retention time was too long to be useful. As the content of protic polar

Table 1
Resolution of β-blockers **3**–**13** on CSP **2** with the variation of methanol content in acetonitrile as mobile phase at the constant ratio of acetic acid–triethylamine (acetic acid–triethylamine–methanol–acetonitrile, 0.1/0.1/x/100 − x, v/v/v/v)[a]

β-Blocker	x = 50			x = 35			x = 20		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
3	20.93	1.07	1.10	36.23	1.08	1.12	40.58	1.09	1.19
4	16.47	1.06	1.08	28.51	1.07	1.14	34.11	1.08	1.23
5	27.31	1.09	1.35	47.27	1.10	1.42	53.18	1.11	1.47
6	34.50	1.09	1.50	59.31	1.11	1.48	84.90	1.12	1.50
7	21.30	1.08	1.33	36.87	1.09	1.35	52.88	1.10	1.33
8	28.71	1.06	0.95	49.70	1.08	0.95	79.28	1.09	0.92
9	13.22	1.06	0.78	23.85	1.10	1.06	31.17	1.12	1.33
10	27.56	1.03	0.25	46.83	1.06	0.90	62.42	1.07	1.03
11	30.23	1.00		52.33	1.00		68.40	1.00	
12	51.42	1.00		89.01	1.02		169.22	1.02	
13	23.53	1.03	0.42	40.73	1.06	0.88	50.08	1.08	1.11

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature, 20 °C; k_1, retention factor of the first eluted enantiomer; α, separation factor; R_S, resolution factor.

solvent in mobile phase decreases, mobile phase-analyte interaction is inferred to decrease because of the decreased hydrogen bonding interaction between the protic polar solvent and analytes. In this instance, the retention time should increase as the methanol content in mobile phase decreases.

The effect of the variation of the acetic acid–triethylamine ratio in mobile phase on the chromatographic behaviors for the resolution of β-blockers on CSP **2** was examined by increasing or decreasing the acetic acid or the triethylamine content from the standard ratio of acetic acid–triethylamine (0.1/0.1, v/v) in mobile phase. In addition, the effect of the use of other acids instead of acetic acid and the use of other amines instead of triethylamine in mobile phase on the chromatographic behaviors for the resolution of β-blockers on CSP **2** was also examined.

Increasing the content of acetic acid or triethylamine from the standard ratio of acetic acid–triethylamine (0.1/0.1, v/v) in mobile phase decreases the retention factors (k_1) quite much. In contrast, decreasing the content of acetic acid and/or triethylamine in mobile phase increases the retention factors (k_1) very much. A part of the chromatographic resolution behaviors observed with the variation of the content of acid-triethylamine in mobile phase are included in Table 2. At the higher content of acetic acid and/or triethylamine in mobile phase, the ionic strength of mobile phase should increase. In this instance, the interaction between the mobile phase and analytes is expected to increase and consequently, the retention of analytes should decrease at the higher content of acetic acid and/or triethylamine. The separation (α) and the resolution factors (R_S) were found to show the increasing trends except for a few cases when the content of triethylamine in mobile phase increases while no significant trends were observed when the content of acetic acid in mobile phase increases.

When diisopropylethylamine was used as an amine additive in mobile phase instead of triethylamine, the retention time of analytes was too long to be useful. However, when trifluoroacetic or formic acid was used instead of acetic acid as an acid additive in mobile phase, the separation (α) and the resolution factors (R_S) were improved quite much along with some degree of increase of retention factors (k_1). Between the two acids, trifluoroacetic acid was slightly better than formic acid in terms of separation (α) and the resolution factors (R_S). The examples for the resolution of β-blockers on CSP **2** with the use of a mixture of trifluoroacetic acid–triethylamine–methanol–acetonutrile as mobile phase are included in Table 2. As shown in Table 2, the use of trifluoroacetic acid instead of acetic acid improved the separation (α) and the resolution factors (R_S) quite much. For example, clenpropol **11** has not been resolved at all with the use of acetic acid as an acid additive in mobile phase. However, it was resolved with the use of trifluoroacetic acid as an acid additive in mobile phase. Considering the retention time of analytes, the best ratio of trifluoroacetic acid–triethylamine in mobile phase was concluded to be 0.1:0.5 (v/v).

By decreasing the content of methanol in mobile phase, we were able to slightly increase the separation (α) and the resolution factors (R_S) as shown in Table 1. However, decreasing the content of methanol further than 20% in mobile phase was useless because of the too long retention of analytes. As an alternative to decrease the protic polar property of the component of mobile phase, methanol, we tried to use ethanol or 2-propanol, expecting the improved resolution results. As shown in Table 3, when ethanol or 2-propanol was used as a polar protic component of mobile phase instead of methanol at the constant ratio of trifluoroacetic acid–triethylamine (0.1/0.5, v/v), the separation (α) and the resolution factors (R_S) were improved as expected. When 2-propanol was used, the separation (α) and the resolution factors (R_S) were improved more than when ethanol was used. However, the retention factors (k_1) were always increased too much when 2-propanol was used as a polar protic component of mobile phase. In

Table 2
Resolution of β-blockers **3**–**13** on CSP **2** with the variation of the ratio of acetic or trifluoroacetic acid–triethylamine in mobile phase (acetic or trifluoroacetic acid–triethylamine–methanol–acetonitrile, *x*/*y*/20/80, v/v/v/v)[a]

β-Blocker	Acetic acid:triethylamine (*x*:*y*)									Trifluoroacetic acid:triethylamine (*x*:*y*)					
	x:*y* = 0.1:0.1			*x*:*y* = 0.1:0.25			*x*:*y* = 0.1:0.5			*x*:*y* = 0.1:0.25			*x*:*y* = 0.1:0.5		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
3	40.58	1.09	1.10	26.12	1.13	1.46	15.42	1.15	1.68	58.24	1.23	2.63	22.40	1.22	2.59
4	34.11	1.08	1.23	21.95	1.11	1.61	13.10	1.13	1.68	45.18	1.22	2.40	18.41	1.21	2.69
5	53.18	1.11	1.47	34.88	1.15	2.00	20.17	1.17	2.11	77.70	1.24	3.43	29.23	1.24	3.00
6	84.90	1.12	1.50	60.52	1.17	2.05	37.88	1.20	2.00	131.49	1.24	2.67	56.43	1.25	2.82
7	52.88	1.10	1.33	35.33	1.14	1.94	21.02	1.16	1.33	73.99	1.26	3.45	29.71	1.25	2.94
8	79.28	1.09	0.92	51.28	1.14	1.45	30.34	1.15	1.65	109.89	1.21	1.92	42.69	1.20	1.42
9	31.17	1.12	1.33	19.74	1.20	1.35	11.68	1.23	1.67	44.93	1.57	4.52	17.20	1.55	4.62
10	62.42	1.07	1.03	43.62	1.12	1.37	27.57	1.14	1.36	96.65	1.42	4.57	41.27	1.38	4.11
11	68.40	1.00		43.06	1.00		24.80	1.00		96.45	1.08	1.20	36.32	1.06	0.71
12	169.22	1.02		81.66	1.07	0.67	38.01	1.08	0.85	200.21	1.20	2.05	56.42	1.18	1.80
13	50.08	1.08	1.11	34.62	1.14	1.56	21.65	1.16	1.74	81.72	1.47	5.81	33.05	1.43	4.71

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature, 20 °C; k_1, retention factor of the first eluted enantiomer; α, separation factor; R_S, resolution factor.

Table 3
Resolution of β-blockers **3**–**13** on CSP **2** with the variation of the type of alcohol component in acetonitrile as mobile phase at the constant ratio of trifluoroacetic acid–triethylamine-alcohol component-acetonitrile (0.1/0.5/20/80, v/v/v/v)[a]

β-Blocker	Methanol			Ethanol			2-Propanol		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
3	22.40	1.22	2.59	29.35	1.26	2.12	41.82	1.31	2.48
4	18.41	1.21	2.69	24.61	1.22	2.29	34.76	1.29	2.49
5	29.23	1.24	3.00	38.59	1.28	3.05	54.00	1.35	3.28
6	56.43	1.25	2.82	83.38	1.31	2.67	126.45	1.40	2.86
7	29.71	1.25	2.94	45.60	1.29	2.90	74.61	1.35	2.80
8	42.69	1.20	1.42	67.30	1.27	1.87	112.21	1.31	2.64
9	17.20	1.55	4.62	22.52	1.85	4.21	32.77	2.25	4.93
10	41.27	1.38	4.11	53.61	1.59	4.37	73.52	1.87	5.46
11	36.32	1.06	0.71	48.61	1.13	1.58	68.28	1.19	2.17
12	56.42	1.18	1.80	98.08	1.23	1.36	198.44	1.30	1.68
13	33.05	1.43	4.71	43.07	1.64	5.79	60.87	1.91	6.62

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. Temperature, 20 °C; k_1, retention factor of the first eluted enantiomer; α, separation factor; R_S, resolution factor.

this instance, we concluded that the best mobile phase for the resolution of β-blockers on CSP **2** is the mixture of trifluoroacetic acid–triethylamine–ethanol–acetonitrile with the ratio of 0.1/0.5/20/80 (v/v/v/v).

The most interesting part for the resolution of β-blockers on CSP **2** is the effect of the column temperature on the chromatographic resolution behaviors. The effect of the column temperature on the resolution of β-blockers on CSP **2** was investigated with selected five β-blockers (**4**, **5**, **9**, **11** and **13**) and the chromatographic behaviors with the variation of the column temperature are summarized in Table 4. As shown in Table 4, the retention factors (k_1) decrease quite much as the column temperature increases. At higher temperature, the rate of equilibration for the formation of the two diastereomeric complex should increase and consequently the two chromatographic peaks become sharper. In this instance, the retention factors (k_1) should decrease and the resolution factors (R_S) increase as the column temperature increases as shown in Table 4. However, the trends of separation factors (α) with the variation of column temperature are quite surprising. In every case for the resolution of primary amino compounds on crown ether-based CSPs, separation factors (α) have been reported to decrease as the column temperature increase [5,6,8–11,28]. In contrast, for the resolution of β-blockers containing a secondary amino group on CSP **2**, the separation factors (α) increase as the column temperature increases as shown in Table 4.

The characteristics of the separation factors (α) with the variation of the column temperature can be rationalized based on the plots of the natural logarithm of the retention factors (k) of the two enantiomers or the separation factor (α) against the reciprocal of absolute temperature (known as van't Hoff plot) [8,29,30]. The relation associated with the separation factors (α), the retention factors (k) of the two enantiomers and the thermodynamic parameters for the separation of two enantiomers on a CSP is expressed by the following Eqs. (1)–(4), where the subscripts 1 and 2 correspond to the first and the second eluted enantiomer respectively [30–34].

$$\Delta\Delta G = \Delta G_2 - \Delta G_1 = -RT\ln\alpha = -RT\ln\frac{k_2}{k_1} \quad (1)$$

$$\Delta\Delta G = \Delta G_2 - \Delta G_1 = (\Delta H_2 - T\Delta S_2) - (\Delta H_1 - T\Delta S_1) = \Delta\Delta H - T\Delta\Delta S \quad (2)$$

$$\ln k_2 = \frac{-\Delta H_2}{RT} + \frac{\Delta S_2}{R} + \ln\phi$$
$$\ln k_1 = \frac{-\Delta H_1}{RT} + \frac{\Delta S_1}{R} + \ln\phi \quad (3)$$

$$\ln\alpha = \frac{-\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R} \quad (4)$$

However, it should be noted that the retention (k) and separation factors (α) include the contributions coming from

Table 4
Resolution of selected β-blockers on CSP **2** with the variation of column temperature at the constant ratio of trifluoroacetic acid–triethylamine–ethanol–acetonitrile (0.1/0.5/20/80, v/v/v/v)[a]

β-Blocker	10 °C			20 °C			30 °C			40 °C		
	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S	k_1	α	R_S
4	29.16	1.21	2.26	24.61	1.22	2.29	19.69	1.25	2.62	15.94	1.26	3.35
5	46.58	1.27	2.23	38.59	1.28	3.05	30.10	1.29	3.12	24.23	1.30	3.43
9	28.91	1.72	4.00	22.52	1.85	4.21	18.77	1.87	4.25	15.12	1.90	5.46
11	60.51	1.10	0.78	48.61	1.13	1.58	37.04	1.15	1.65	29.40	1.16	2.12
13	53.75	1.62	4.76	43.07	1.64	5.79	32.88	1.69	6.24	25.87	1.70	7.41

[a] Flow rate: 1.0 ml/min. Detection: 254 nm UV. k_1, retention factor of the first eluted enantiomer; α, separation factor; R_S, resolution factor.

both non-enantioselective and enantioselective interactions between the CSP and analytes [34–37]. In this event, the apparent separation factors are always less than the intrinsic separation factors. Consequently, the direct calculation of the thermodynamic parameters according to the above equations from experimental values of the retention (k) and separation factors (α) at different temperatures might lead to erroneous results. However, the non-eanatioselective and enantioselective contributions are not easy to separate [35] and consequently, the relation associated with the separation factors (α) and the thermodynamic parameters for the separation of two enantiomers on a CSP is usually described by the above equations [34].

Model van't Hoff plots of the retention factors (k) of the two enantiomers against the reciprocal of absolute temperature for the separation of enantiomers on a CSP are shown in Fig. 3. In the plots of Fig. 3, the slope of each line is related to the enthalpy ($-\Delta H/R$) of adsorption and the intercept is related to the entropy ($\Delta S/R$) associated with adsorption of the enantiomer according to the Eq. (3) [30,31]. If the two lines in Fig. 3 are parallel with each other (the slopes of the two lines are equal), the free energy difference ($\Delta\Delta G$) should be constant with the variation of the column temperature for the adsorption of the two enantiomers and consequently the separation factor (α) should be constant. However, in general, the two lines intersect at a certain temperature (isoelution temperature), where the free energy difference ($\Delta\Delta G$) is zero (at this temperature the $\Delta\Delta H$ term exactly negates the $T\Delta\Delta S$ term) and the separation of enantiomers is not observed. Below the isoelution temperature (right part from the intersect point of the two lines in Fig. 3), enantiomer 2 is retained longer than enantiomer 1 and both the enthalpy difference ($\Delta\Delta H$, $\Delta H_{\text{enantiomer 2}} - \Delta H_{\text{enantiomer 1}}$) and the entropy difference ($\Delta\Delta S$, $\Delta S_{\text{enantiomer 2}} - \Delta S_{\text{enantiome 1}}$) are negative. Overall, $\Delta\Delta G$ value is negative below the isoelution temperature and the negative $\Delta\Delta G$ value originated from the negative enthalpy difference ($\Delta\Delta H$) increases as the column temperature decreases. This means that the separation factor (α) increases as the column temperature decreases. In general, the isoelution temperatures are typically well above the temperature region normally used for liquid chromatography [30] and consequently the separation factors (α) for the resolution of most racemic analytes on CSPs increases as the column temperature decreases.

In contrast, above the isoelution temperature (left part from the intersect point of the two lines of Fig. 3), enantiomer 1 is retained longer than enantiomer 2 (namely, the elution order is changed) and both the enthalpy difference ($\Delta\Delta H$, $\Delta H_{\text{enantiomer 1}} - \Delta H_{\text{enantiomer 2}}$) and the entropy difference ($\Delta\Delta S$, $\Delta S_{\text{enantiomer 1}} - \Delta S_{\text{enantiomer 2}}$) are positive. In this instance, the negative $\Delta\Delta G$ value originated from the positive entropy difference ($\Delta\Delta S$) should increase as the column temperature increases. At this point it should be noted that the change of sign of $\Delta\Delta H$ and $\Delta\Delta S$ below and above the isoelution temperature is based on the definition of the separation factor (α), namely α = (large retention factor)/(small retention factor). However, under the circumstance that the separation factor (α) is defined as (retention factor of enantiomer 2)/(retention factor of enantiomer 1) both below and above the isolution temperature, the sign of $\Delta\Delta H$ and $\Delta\Delta S$ should be not changed and the sign of $\Delta\Delta G$ changes from negative to positive as the temperature changes from below to above the isoelution temperature.

Especially for the resolution of β-blockers on CSP **2**, the isoelution temperature seems to be casually very low and below the usual liquid chromatographic temperature region. Consequently the separation factor (α) related to the negative $\Delta\Delta G$ value should increase as the column temperature increases.

The thermodynamic parameters for the resolution of selected five β-blockers (**4**, **5**, **9**, **11** and **13**) on CSP **2** were calculated based on the plots of the natural logarithm of separation factor (α) against the reciprocal of absolute temperature. All of the five β-blockers shown in Table 4 were found to show similar trends in their van't Hoff plots and one example of van't Hoff plot for the resolution of propranolol **5** on CSP **2** is presented in Fig. 4. Thermodynamic parameters calculated from the van't Hoff plots for the resolution of selected five β-blockers on CSP **2** are summarized in Table 5. As shown in Table 5, both the $\Delta\Delta H$ and $\Delta\Delta S$ values are positive and consequently the negative $\Delta\Delta G$ values are

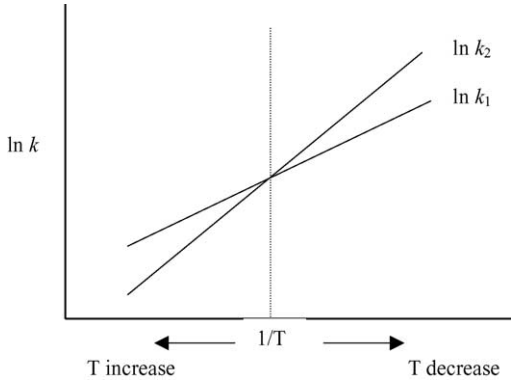

Fig. 3. A model van't Hoff plot between ln k and $1/T$ for the separation of two enantiomers (enantiomer 1 and enantiomer 2) on a CSP.

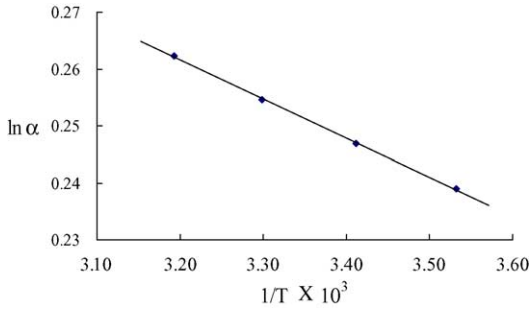

Fig. 4. A representative van't Hoff plot between ln α and $1/T \times 10^3$ for the resolution of propranolol **5** on CSP **2** with the chromatographic condition given in Table 4.

Table 5
Thermodynamic parameters calculated from the van't Hoff plots for the resolution of selected β-blockers (**4**, **5**, **9**, **11** and **13**) on CSP **2** summarized in Table 4

β-Blocker	$\Delta\Delta H^\circ$ (kJ/mol)	$\Delta\Delta S^\circ$ (J/mol K)	$\Delta\Delta G^\circ$ (kJ/mol) (20 °C)
4	1.07	5.36	−0.50
5	0.57	4.02	−0.61
9	2.31	12.80	−1.44
11	1.31	5.44	−0.28
13	1.29	8.58	−1.23

originated from the positive $\Delta\Delta S$ values. From these results, it is concluded that the enantioselectivity for the resolution of β-blockers on CSP **2** is entropically controlled.

In conclusion, CSP **2** was very successful in the resolution of various types of β-blockers shown in Fig. 2. As an effort to find out the optimal mobile phase condition, the mixture of trifluoroacetic acid–triethylamine–ethanol–acetonitrile with the ratio of 0.1/0.5/20/80 (v/v/v/v) was concluded to be the best mobile phase condition. Very interestingly and unusually, the separation factors (α) for the resolution of β-blockers on CSP **2** were found to increase as the column temperature increased while the separation factors (α) for the resolution of all other primary amino racemic compounds had been reported to decrease. From the thermodynamic consideration of the chromatographic resolution behaviors, the enantioselectivity for the resolution of β-blockers on CSP **2** was concluded to be entropically controlled. From these results, it should be noted that the chiral recognition mechanism for the resolution of β-blockers on CSP **2** is different from that for the resolution of racemic primary amino compounds, in which the enantioselective diasteromeric complex formation of the two enantiomers of the primary ammonium ions of analytes inside the chiral cavity of the crown ether ring of the CSP is essential for chiral recognition. In the resolution of β-blockers on CSP **2**, somewhat different type enantioselctive diastereomeric complex formation of the two enantiomers of analytes with the chiral crown ether moiety of the CSP utilizing hydrogen bonding interactions between the analyte and the chiral selector of the CSP seems to be responsible for the chiral recognition. However, the exact chiral recognition mechanism for the resolution of β-blockers on CSP **2** is not clear yet and the further study is needed to elucidate the chiral recognition mechanism.

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