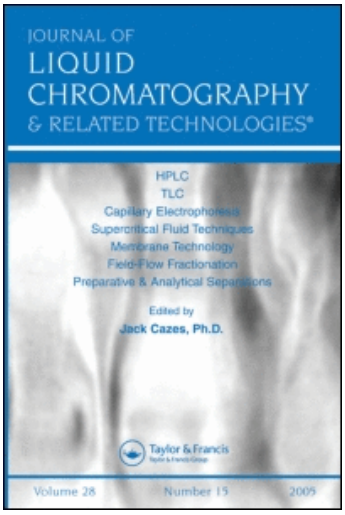

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Chromatographic Separation of Fluoxetine Hydrochloride Enantiomers by Cellulose Chiral Stationary Phase

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Abstract: Fluoxetine hydrochloride enantiomers were separated by chromatography on Tris-(3,5-dimethylphenyl carbamate) cellulose as a chiral stationary phase (CSP). The effects of mobile phase composition, flow rate, and column temperature on capacity factor, separation factor, and resolution were studied systematically. Best separation was obtained with a mobile phase composition of hexane/isopropyl alcohol/diethylamine of 98/2/0.2 (v/v/v) at 15°C. The optimal flow rate was 0.24 mL/min from the Van Deemter equation. However, baseline resolution ($R_S > 1.5$) was achieved under a flow rate of 0.8 mL/min. Thermodynamic parameters ΔH^0, $\Delta_{S,R}\Delta H^0$, and $\Delta_{S,R}\Delta S^0$ were calculated. In the temperature range examined, the enthalpic contribution to the enantiomer transfer energy was found to be more significant than the entropic one.

Keywords: Chiral separation, Chiral stationary phase, Fluoxetine hydrochloride, Derivatized cellulose, High performance liquid chromatography

INTRODUCTION

Fluoxetine (Figure 1) is a selective serotonin reuptake inhibitor used for the treatment of depression and obsessive compulsive disorders. So far the drug used is of racemate type, but the individual optical isomers do not have identical activity.[1,2] The S-isomer is effective for the treatment of migraine headaches while the R-isomer is for depression.[3,4] Thus, it is of significance to study the separation of the racemic fluoxetine hydrochloride.

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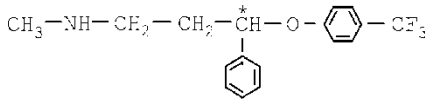

Figure 1. Structure of fluoxetine.

Several chromatographic methods have been published for the determination of fluoxetine enantiomers. Indirect methods include derivation with NBD-COCl and DBD-COCl,[5–7] S-(−)-N-trifluoroacetylprolyl chloride,[8,9] R-napthylethyl isocyanate,[10] or R-(−)-mandelic acid, followed by liquid chromatographic separation of the resulting diastereomers. Gas chromatographic separation of diastereomers after derivation with S-(−)-N-trifluoroacetyl-prolyl chloride has also been employed.[9]

Direct enantiomer separation methods include HPLC using derived cellulose,[11–13] protein based,[13] urea derivative,[14] antibiotic derivatives,[15,16] or cyclodextrin derivatives[17–20] as chiral stationary phases (CSPs) and GC using cyclodextrin as the chiral selector.[21] Investigations using capillary electrophoresis with cyclodextrin[22,23] or maltooligosaccharides[24] as chiral selectors have also been reported.

By using tris(3,5-dimethylphenyl carbamate) cellulose stationary phase and operating by the reverse mode, Gatti et al.[11] obtained a separation factor (α) of 1.10, Kaddoumi et al.[12] obtained a separation factor of 1.16 (with gradient elution). By the normal phase mode, Olsen et al.[13] achieved baseline separation of fluoxetine enantiomers. In addition, it was reported that column temperature affects the separation significantly.

Up to now, no systematical study of separation of fluoxetine enantiomers by using CSP has been reported. The present work intended to study the factors affecting the separation of fluoxetine enantiomers using Chiralcel OD-H column, and to optimize the operation conditions based on the kinetic and thermodynamic fundamentals.

EXPERIMENTAL

Reagents

HPLC grade hexane and isopropyl alcohol were from B&J Brand (Muskegon, MI). Ethanol, 1-propanol, 1-butanol, and diethylamine (DEA) were purified with 0.45 μm solvent filter and ultrasonically degassed. Sample of racemic fluoxetine hydrochloride was from Lijing Ltd. (Taizhou, China).

Apparatus

The chromatograph system consisted of a Knauer model K-501 pump (Berlin, Germany), a fixed loop injection valve and a model K-2501 UV detector

(Berlin, Germany). Chromatograms were recorded at 1 Hz, and peak areas were determined by using an Eurochrom 2000 data acquisition system. Chiracel OD-H column was from Chiral Technologies (Exton, PA, USA), 25 cm × 4.6 mm i.d., packed with 5 μm tris(3,5-dimethylphenyl carbamate) cellulose CSP. Dead volume of the column was determined with 1,3,5-tri-*tert*-butylbenzene.

Sample Preparation

5 mg of fluoxetine HCl was dissolved in 2 mL alcohol and diluted to 25 mL with hexane and 0.2% diethylamine. The injection size was 20 μL.

UV Absorbance

In the literatures, UV wavelengths at 226, 254, 260, and 276 nm have been used for detection of fluoxetine.[11–20] In the present work the absorbance scanning of fluoxetine was examined. It was found that the absorbance of s-fluoxetine decreases rapidly as the wavelength increases from 226 to 254 nm (Figure 2). Therefore, 226 nm was applied in this work for fluoxetine detection.

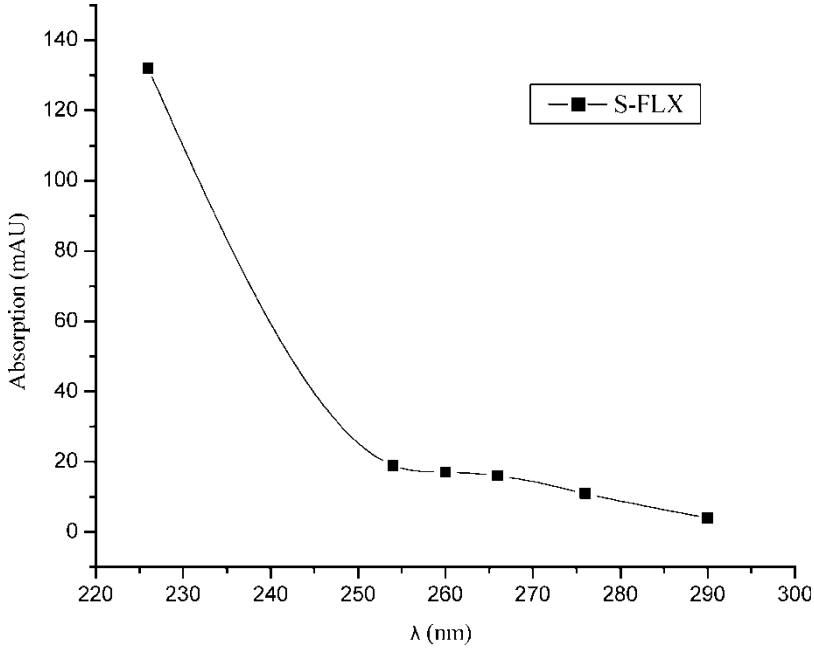

Figure 2. UV absorption spectrum of fluoxetine. Mobile phase: 98/2/0.2, hexane/isopropyl alcohol/diethylamine (v/v/v); Flow rate: 1.0 mL/min; Column temperature: 17°C.

RESULTS AND DISCUSSION

Composition of Mobile Phase

For the Chiralcel OD-H column, the manufacturer recommended hexane with alcohols as mobile phase. Wang et al.[25] proposed that alcohol can not only form a hydrogen bond with the carbamate of CSP, but also can change the environment of the chiral cavity and, consequently, affect the separation of enantiomers.

In the present work hexane was used as the major constituent of the mobile phase. Three normal alcohols (ethanol, 1-propanol, and 1-butanol) and isopropanol was added to hexane to study the effect of polarity of mobile phase on the chromatographic behavior of fluoxetine enantiomers. Meanwhile 0.2% diethylamine was added to enhance the resolution. It can be seen from the results in Table 1, addition of normal alcohols to the mobile phase results in a decrease of capacity factors k' of the enantiomers. Moreover, the greater the polarity of the alcohol (polarity of ethanol, 1-propanol, 1-butanol, and isopropanol are 4.3, 4.0, 3.91, and 3.9, respectively), the weaker is the interaction between enantiomers and CSP, and the capacity factor is less. Resolutions is generally poor with addition of normal alcohols. Much better resolution was obtained by addition of isopropanol to the mobile phase.

Further study was carried out on the effect of isopropanol concentration. It can be seen (Figure 3) that the capacity factor decreases exponentially with the isopropanol content in the mobile phase, i.e., the capacity factors decrease rapidly as the isopropanol content increases up to 2%, and then gradually level off. As regards to the resolution (Figure 4), the enantiomers are baseline resolved when the isopropanol concentration is less than 2%. Further increasing of isopropanol concentration results in rapid decrease of resolution. To compromise between resolution and elution time (and solvent consumption as well), the optimal composition of hexane/isopropanol would be 98/2(v/v).

Diethylamine Concentrations

The effect of the addition of diethylamine (DEA) is listed in Table 2. It can be seen that the effect of DEA on the capacity factor is negligible. Meanwhile, the resolution of fluoxetine enantiomers is enhanced by adding DEA up to 0.2% in the mobile phase, but by further increasing the DEA concentration the resolution again becomes less. This is consistent with the conclusion that for a basic analyte, addition of proper quantities of DEA can restrain the nonstereoselective adsorption of Si-OH group remaining on the CSP supporter and enhance the chiral recognition of CSPs.[26] On the other hand, too much

Table 1. Effect of mobile phase composition on the resolution of fluoxetine

Mobile phase	Composition (v/v)	Capacity factor		Separation factor (α)	Resolution (R_S)	Number of theoretical plates	
		k'_S	k'_R			N_S	N_R
Hexane/ethanol/DEA							
Hexane/1-propanol/DEA	95/5/0.2	1.30	1.36	1.05	−	+	+
	98/2/0.2	2.49	2.55	1.03	−	+	+
Hexane/1-butanol/DEA	95/5/0.2	1.51	1.56	1.03	−	+	+
	95/5/0.2	1.69	1.72	1.02	−	+	+
Hexane/IPA/DEA	80/20/0.2	0.84		+	−	6605	
	90/10/0.2	1.46	1.56	1.07	0.87	7768	598
	95/5/0.2	2.34	2.53	1.08	1.16	7773	7494
	97/3/0.2	3.35	3.63	1.09	1.25	6483	5522
	98/2/0.2	4.25	4.79	1.13	2.11	8290	7481
	98.5/1.5/0.2	5.26	5.88	1.12	1.88	7074	6345
	99/1/0.2	6.67	7.42	1.11	1.52	4891	4827

Flow rate: 0.5 mL/min. Column temperature: 13.6□.
" − ": not resolved, " + ": severe overlapped peaks

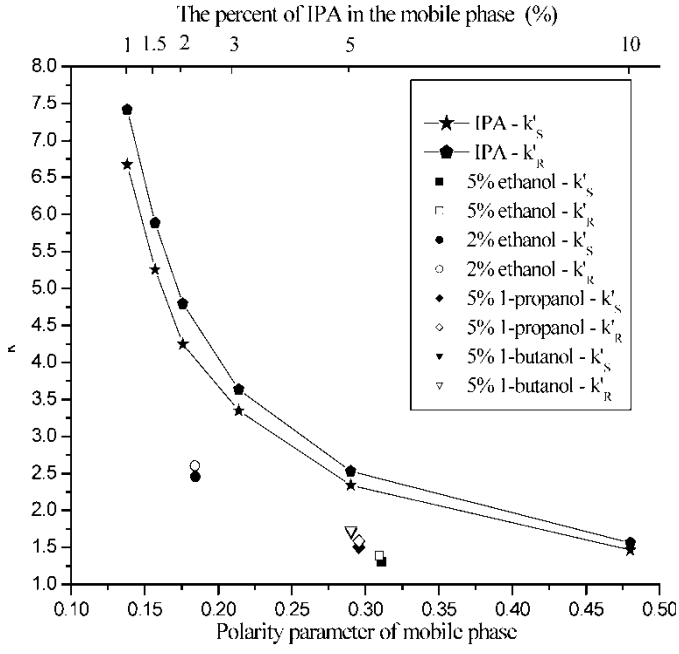

Figure 3. Effect of polarity parameter of mobile phase on k′. Flow rate: 0.5 mL/min; Column temperature: 15°C.

DEA may affect the chiral selectivity of CSP and the enantiomers are not resolved.

Flow Rate

The effect of the flow rate of mobile phase on the separation of fluoxetine enantiomers is listed in Table 3. As the flow rate increases, the capacity factors of the enantiomers and the separation factor almost do not change, this is consistent with the thermodynamic fundamentals that the equilibrium between two phases are not affected by the flow rate. Meanwhile, the resolution increases significantly as the flow rate decreases. This is due to the effect of the flow rate to the peak width of the chromatogram. The HETP versus u data were plotted in Figure 5 and fitted to the Van Deemter equation:

$$H = A + \frac{B}{u} + Cu \tag{1}$$

where H is the height of theoretical plates (mm), u is the linear velocity (cm/s). The obtained constants are listed in Table 4.

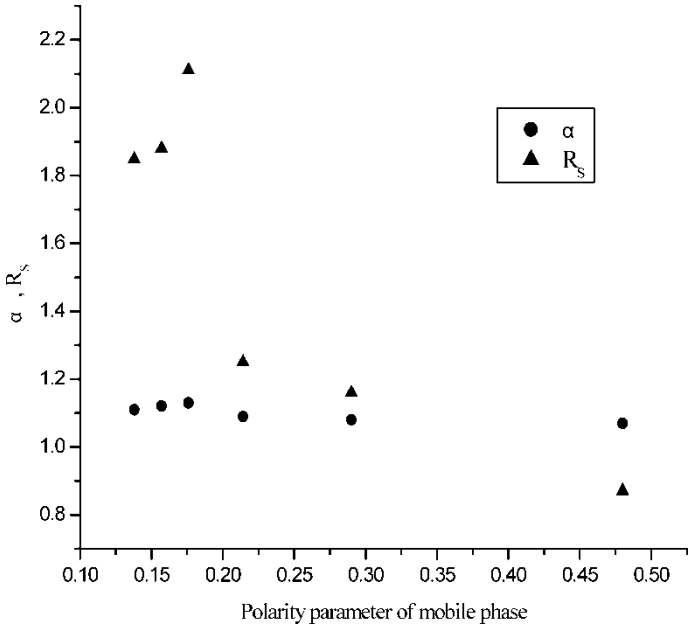

Figure 4. Effect of polarity parameter of mobile phase on α, R_S. Flow rate: 0.5 mL/min; Column temperature: 15°C.

The linear velocity corresponding to the minimum HETP (H_{min}), u_{opt} is 0.024 cm/s. In the range of linear velocity greater than u_{opt}, HETP increases with the linear velocity almost linearly, this results in that the resolution R_S decreases almost linearly with the increase of linear velocity.

Table 2. Effect of diethylamine concentrations on the resolution of fluoxetine

DEA concentration (%)	Capacity factor		Separation factor (α)	Resolution (R_S)	Number of theoretical plates	
	k'_S	k'_R			N_S	N_R
0	4.18	4.34	1.04	0.31	1692	1370
0.1	4.26	4.61	1.08	1.02	4832	4205
0.2	4.14	4.59	1.11	1.40	4965	4537
0.3	4.41	4.73	1.07	0.90	4589	4221
0.4	/	/	/	–	/	/

Mobile phase: hexane/isopropanol (98/2, v/v). Flow rate: 1.0 mL/min. Column temperature: 15°C.

* "/": no peaks, "–": not resolved

Table 3. Effect of flow rate of mobile phase on the resolution of fluoxetine

Flow rate (mL/min)	Capacity factor		Separation factor (α)	Resolution (R_s)	Width of the peak (min)		Height of theoretical plates (mm)	
	k'_S	k'_R			W_S	W_R	$HETP_S$	$HETP_R$
0.2	4.24	4.84	1.14	2.18	3.39	3.66	0.0343	0.0324
0.4	4.17	4.71	1.13	2.00	1.75	1.87	0.0357	0.0346
0.6	4.13	4.67	1.13	1.85	1.23	1.32	0.0410	0.0412
0.8	4.10	4.63	1.13	1.74	0.94	1.07	0.0444	0.0478
1.0	4.14	4.59	1.11	1.40	0.78	0.92	0.0504	0.0551

Mobile phase: hexane/isopropanol/DEA (98/2/0.2, v/v/v). Column temperature: 15□.

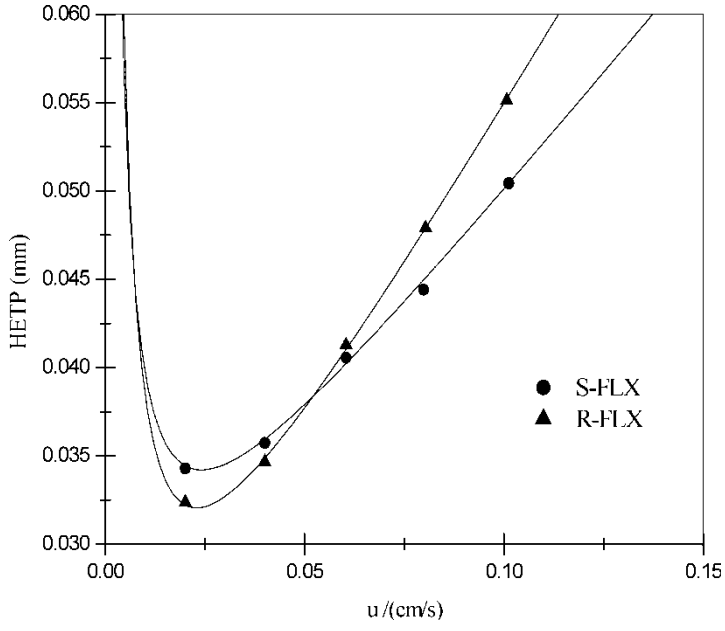

Figure 5. The Van Deemter plots of fluoxetine enantiomers.

For analytical purposes, the flow rate of 0.8 mL/min is appropriate because the baseline separation of the enantiomers is obtained and the elution time is the least. For the preparation of enantiomers, the flow rate corresponding to the minimum HETP would be the optimal condition to obtain larger production rates.

Column Temperature

As shown in Table 5, the capacity factor, the separation factor, and the resolution all increase with the decrease of the column temperature. The highest temperature allowable for baseline separation of fluoxetine enantiomers is 15°C.

Table 4. Constant A, B and C values and the $H_{\min}$, u_{opt} for fluoxetine enantiomers

Enantiomer	A	B	C	Correlation coefficient	H_{min} (mm)	u_{opt} (cm/s)
S-fluoxetine	0.0209	0.000160	0.276	0.99952	0.034	0.024
R-fluoxetine	0.0144	0.000201	0.385	0.99997	0.032	0.024

Table 5. Effect of column temperature on the resolution of fluoxetine

Column temperature (°C)	Capacity factor		Separation factor (α)	Resolution (R_s)	Number of theoretical plate	
	k'_S	k'_R			N_S	N_R
7	4.80	5.83	1.21	2.60	4911	4894
15	4.10	4.63	1.13	1.74	5627	5226
23	3.27	3.56	1.09	1.33	6875	6841
30	2.92	3.07	1.05	0.79	7859	7516

Mobile phase: hexane/isopropanol/DEA (98/2/0.2, v/v/v). Flow rate: 0.8 mL/min.

The expression of capacity factor based on thermodynamics is as follows:[27,28]

$$\ln k' = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} + \ln \varphi^9 \tag{2}$$

where ΔH^0 and ΔS^0 are the change of enthalpy and entropy for the transfer of enantiomers from the mobile phase to the stationary phase, φ is the volume ratio of mobile phase and stationary phase.

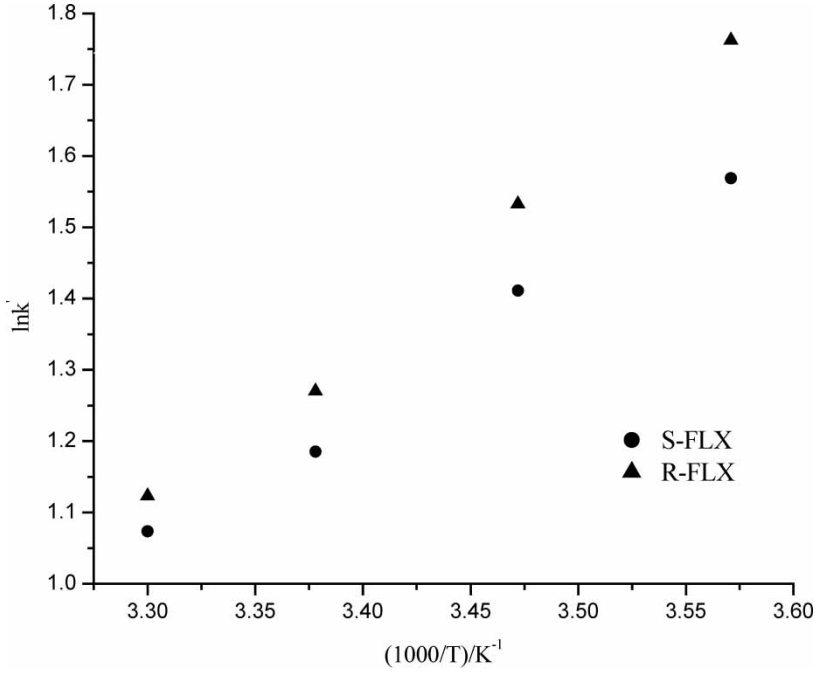

Figure 6. Plot of $\ln k' \sim 1/T$ for fluoxetine enantiomers.

Table 6. ΔH^0 and $\Delta S^0 + R \cdot \ln\varphi$; values for fluoxetine enantiomers

Enantiomers	$\Delta H^0/(\mathrm{KJ/mol})$	$\Delta S^0 + R \cdot \ln \phi/$ $(\mathrm{J/(mol \cdot K)})$	Correlation coefficient
S-fluoxetine	−15.7	−5.18	0.996
R-fluoxetine	−20.1	−6.85	0.998

The Van't Hoff plots of fluoxetine enantiomers (Figure 6) were found to have good linearity, with the correlation coefficients of 0.996. This indicates that no changes of retention mechanics occurred with the change of column temperature in our studies.

The data in Table 6 show the values of ΔH^0 and $\Delta S^0 + R \cdot \ln \varphi$ calculated from the slope coefficient and intercept of Van't Hoff plots in Figure 6.

The thermodynamic parameter, ΔH^0 indicates the thermal effect of the transfer of enantiomers from the mobile phase to the stationary phase. The negative value of ΔH^0 showed the adsorption of solute on CSP is a exothermal process.

The relationship of separation factor ($\alpha \llcorner k'_R/k'_S$) with temperature can be obtained from Equation (2) as follows:

$$\ln \alpha = -\frac{\Delta_{R,S}\Delta H^0}{RT} + \frac{\Delta_{R,S}\Delta S^0}{R} \tag{3}$$

where $\Delta_{R,S}\Delta H^0$ and $\Delta_{R,S}\Delta \mathrm{S}^0$ are the difference of enthalpy and entropy change of enantiomers from the mobile phase to the stationary phase.

The separation factor decreases with the increase of temperature, as shown in Figure 7. The data listed in Table 7 are the values of $\Delta_{R,S}\Delta H^0$ and $\Delta_{R,S}\Delta \mathrm{S}^0$ calculated from the slope coefficient and intercept of plot in Figure 7. Within our experimental range (280 K $<$ T $<$ 303 K), both two enantiomers of fluoxetine all satisfied $|\Delta_{R,S}\Delta H^0| > |\mathrm{T}\Delta_{R,S}\Delta S^0|$, and the chiral separation is a enthalpic control process.

CONCLUSIONS

Tris (3,5-dimethylphenyl carbamate) cellulose stationary phase was used in the normal phase to separate the enantiomers of fluoxetine. The effects of

Table 7. Thermodynamic parameters of chiral resolution of fluoxetine enantiomers

Analyte	$\Delta_{R,S}\Delta H^0/(\mathrm{KJ/mol})$	$\Delta_{R,S}\Delta S^0/$ $(\mathrm{J/(mol \cdot K)})$	Correlation coefficient
Fluoxetine enantiomers	−4.25	−13.6	0.992

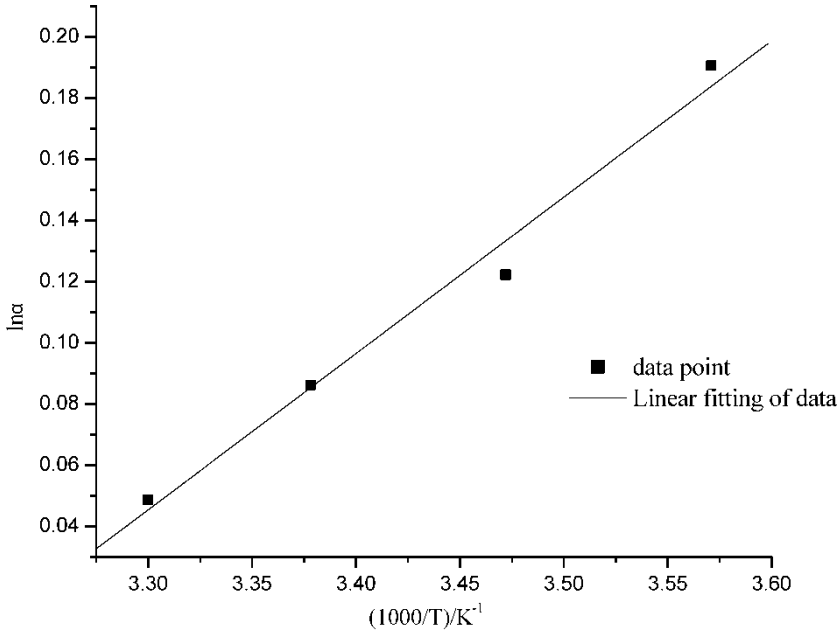

Figure 7. Plot of $\ln \alpha \sim 1/T$ for the chiral resolution of fluoxetine.

composition of mobile phase, flow rate, and column temperature on resolution have been systematically studied.

The optimal composition of mobile phase is hexane/isopropanol/DEA (98/2/0.2, v/v/v).

The optimal linear velocity from Van Deemter equation is 0.024 cm/s, but the enantiomers are baseline separated ($R_S > 1.5$) at the linear velocity of 0.08 cm/s. Column temperature should be lower than 15°C to obtain baseline separation.

The thermodynamic parameters were calculated from the Van't Hoff plots. In the temperature range examined, the enthalpic contribution to the enantiomer transfer energy was found to be more significant than the entropic one.

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