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ENANTIOSEPARATION OF FLUOXETINE ON A NEW β-CYCLODEXTRIN BONDED PHASE COLUMN BY HPLC

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ABSTRACT

A novel perphenyl carbamate of β-cyclodextrin immobilized onto aminized silica gel via multiple ureido chemical bonds was synthesized and its enantioseparation ability was evaluated as chiral stationary phase (CSP) through separation study of fluoxetine (FLU) on high performance liquid chromatography under reversed phase condition. The optimal separation condition of FLU was found and a good separation was obtained. The influences of mobile phase composition, ionic strength, pH, flow rates of mobile phase, and column temperature on retention behavior, resolution of (*R*)-FLU and (*S*)-FLU were studied. The results showed that the new CSP had good separation ability and built a strong basis for the large-scale separation of FLU.

Key Words: Enantioseparation; β-Cyclodextrin; Fluoxetine

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INTRODUCTION

Fluoxetine (FLU) [± *N*-methyl-3-[(α,α,-αtrifluoro-*p*-tolyl)oxy]-propylamine] is a new antidepressant drug. The structure is shown in Fig. 1. The activity is based on the selective inhibition of 5-hydroxytryptamine (5-HT) recapture in the presynaptic neurons of the central nervous system. Due to its selectivity, it has been investigated extensively as a possible antidepressant with a low incidence of unwanted effects. Clinical studies have shown FLU to be at least as effective as the tricyclic antidepressants in treating major depressive disorders. Since the enantiomers have the different pharmacodynamic and metabolization mechanics, their enantioseparation is very important (1–3).

Even though much progress has been achieved in the recent years in the separation of structural and positional isomers of a number of compounds, most of the effort has been directed at developing complex mobile phases that can provide the required selectivity on the otherwise nonselective or moderately selective columns. However, in the last few years, some attention has been directed towards the development of tailor-made column packing materials specially designed to provide multipoint or specific chemical interactions with the analysis, thus yielding more selective separations (4–8). Among such column packings are a number of stable cyclodextrin bonded phases that were first described in the literature in 1984 (9). These packings consist of chiral cyclodextrin molecules linked to silica gel via a 6–10 atom spacer. Both the linkage and the cyclodextrin are hydrolytically stable under standard LC conditions. The attachment is such that the cyclodextrin molecules remain physically intact. This allows cyclodextrin columns to effect numerous chemical separations by selectively including, in solution, a wide variety of organic and inorganic guest molecules into the cyclodextrin cavity.

Cyclodextrins are toroidally shaped oligosaccharides formed by the action of *Bacillus macerans* amylase on starch. These macrocyclic molecules

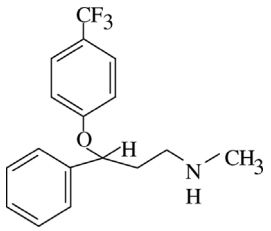

Figure 1. The structure of fluoxetine (FLU).

contain 6–12 glucose units bonded through α-(1,4)-glycosidic linkages. The physical shape of the molecule is that of a truncated cone, with an internal cavity whose dimensions are determined by the number of glucose units. Due to the orientation of the glucose units, there are no hydroxyls on the interior of the cavity, thus rendering it hydrophobic. When using aqueous–organic mobile phases, retention is predominantly due to inclusion complex formation. Separations are readily achieved since molecules of different chemistry size, shape, and special geometry form inclusion complexes may be caused by a variety of interaction, including hydrophobic interactions with the interior of the cyclodextrin cavity, hydrogen bonding with the hydroxyl groups at the periphery of the cavity, the release of high-energy water or modifier during complex formation, or a combination of the above factors (10).

Recently, a new β-cyclodextrin phenyl isocyanate bonded chiral stationary phase (CSP) was developed in the Chemistry Department of National University of Singapore. The new procedure afforded structurally well-defined CSPs and easily controlled batch–batch reproducibility. This CSP is quite stable and can be used in most of the high performance liquid chromatography (HPLC) solvents. Many drug enantiomers that do not have enantioseparation effect on native β-cyclodextrin column in reversed phase were separated very well on this new CSP. A US patent is pending.

In this paper, the β-cyclodextrin phenyl isocyanate bonded stationary phase will be used to investigate the retention time and resolution of FLU under the different LC conditions. The optimal operation conditions will be established. Since this new CSP can be produced in large scale, this work will build a basis for preparative enantiosepartion of FLU.

PREPARATION OF CHIRAL STATIONARY PHASES AND COLUMN

This CSP was prepared by a pre-derived procedure. Perfunctionalised cyclodextrins were first synthesized, purified, and characterized, and then chemically anchored on the surface of the aminized silica gel via the hydrolytically stable urethane linkage. General synthetic route is shown in Fig. 2.

All chemicals were purchased from Fluka, Fisher, and TCI and directly used without any further purification. Silica gel was supplied by Hypersil (UK) with a particle size of 15 μm. All solvents were purchased from Fluka of analytical grade and were distilled before use. Empty column (250 × 4.6 mm) assembly was purchased from Phenomenex (USA). The column was packed using an Alltech pneumatic HPLC pump (Alltech, USA).

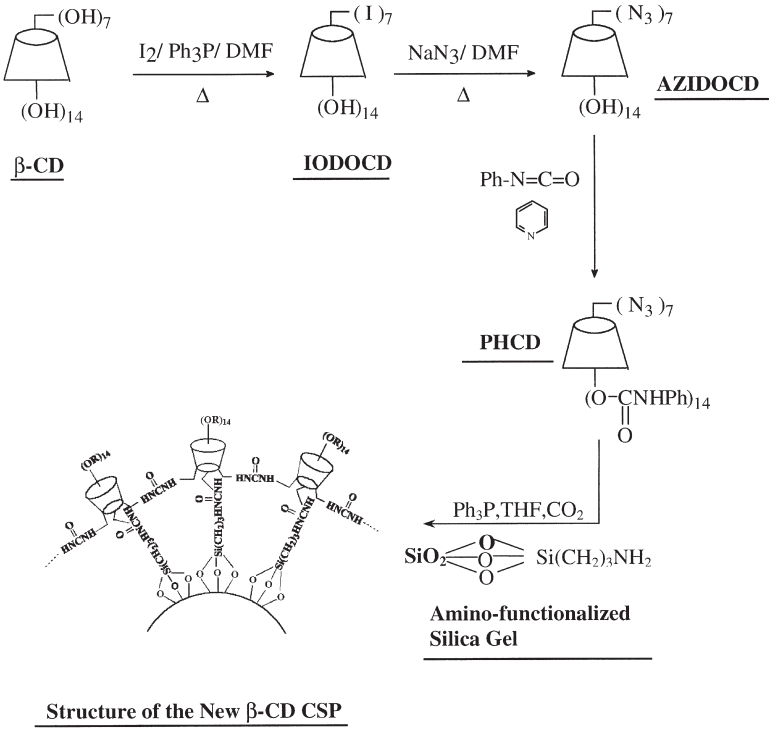

Figure 2. General synthetic route to the new CSP.

EXPERIMENTAL FOR SEPARATION OF FLUOXETINE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

The liquid system consisted of a series 200 LC Pump, series 200 Autosampler, 785A UV/Vis detector. The chromatograms were obtained by using a TurboLC1 chart recorder. The column temperature was controlled through a Polyscience water bath.

A β-cyclodextin phenyl isocyanate bonded, 250×4.6 mm column was packed in Chemistry Department of National University of Singapore. The pH readings were obtained using a ESL0227 PH meter (model HG-3). Detection was accomplished at a wavelength of 225 nm.

Mobile phases were prepared using methanol and buffer in different ratios by volume. Buffers were prepared using triethylamine solutions of different ionic strengths (e.g., 0.5–2.5%), which were adjusted by addition of glacial acetic acid to the desired pH (e.g., 3.5–6.5).

Methanol was distilled and filtered before using. Triethylamine and glacial acetic acid were of analytical grade. The concentration of the working standard solution of the enantiomer is 10 μg/mL. To evaluate the reproducibility of the retention time, each run was triplicated. The void volume of the column was determined by pure methanol (11).

EXACTING FLUOXETINE PROCEDURE FROM CAPSULES

Each green and white capsule, printed with Lilly 3105 and Prozac 20 mg, contains: FLU HCl equivalent to FLU 20 mg. Nonmedicinal ingredients: silicone and starch. The capsule shell contains FD and C Blue No. 1, iron oxide yellow, titaniumdioxide, gelatin, sodium lauryl sulfate, sodium propionate, benzyl alcohol, methylparaben, carboxymethylcellulose sodium, and edetate calcium disodium.

The content of 10 capsules, a quantity of the powder representing 20 mg of FLU hydrochloride, was mixed with 0.5 mL 0.1 *N* HCl and 20 mL of water, stirred for 3 min using a stirring rod. The mixture was filtered, the first 2 mL of the filtrate was rejected and then some was collected. Then the filtrate was added by 0.1 *N* Na_2CO_3 until the pH was equal to 10.

Add 10 mL of diethylether to the mixture to extract FLU, and shake for 3 min. The mixed solvent was then kept at rest for 5 min. The upper liquid (organic phase) was collected. The operation was triplicated. The collected diethylether was vacuum dried and yellow liquid was obtained. The yellow liquid is FLU (12).

RESULTS AND DISCUSSIONS

Our final objective in undertaking this work was to achieve an enantioseparation of FLU on a large scale. It is helpful for the target to investigate the relationships between composition of mobile phase, pH, ionic strength, temperature, and the two most important parameters: retention time and resolution, which are used to describe retention and separation behavior.

On the basis of the findings of this study, chromatographic conditions were chosen for the isocratic separation of the enantiomers under study. This study provides a basis to achieve the separation of the FLU in large scale.

Experiment 1: Effects of Mobile Phase Composition

The effect of mobile phase composition on the retention time and the resolution of the enantiomers were investigated by changing the methanol–buffer ratio in the mobile phase from 40:60 to 60:40 at room temperature. The

concentration of triethylamine in the buffer was 0.5% and the pH 4.0.The mobile phase flow rate was 0.5 mL/min.

Retention time of changing the volume composition was not linear as was the case with most reversed phase columns. From an operational point of view, this implied that at low methanol content there was a greater change in retention time on a β-cyclodextrin column.

A number of researchers had reported a linear relationship between the log of the retention factor, k, and the volume fraction of methanol in Me–buffer mixtures used as mobile phases in reversed-phase chromatography (13,14).

According to Snyder et al. (15), the dependence of the logarithm of the retention factor, k, on the volume fraction of organic component, ϕ_0, in the mobile phase was given by

$$\log k = \log k_w - S\phi_0 \quad (1)$$

where k_w is the retention factor obtained in water and S is the solvent strength as defined by Snyder et al.

The shapes of plots such as these were assumed to be due to structural variations in the solvent systems as the concentration of the organic modifier was changed. As the concentration of the organic component in the water was increased, a point was reached where at least some of the symmetrical aspects of the bulk water started to disappear, and one or more intermediate structures occurred (16). The concentration (or range of concentrations) at which this took place varies with the nature of the modifier. Finally, at very high organic component concentrations, the liquid structures of the pure organic phase began to predominate. The fact that methanol–water mixtures follow Eq. (1) over almost the whole composition range was tied to the fact that methanol could act as both a proton donor and a proton acceptor. Thus, methanol appeared to simply provide a dilution medium for water (17).

By plotting $\log k$ vs. methanol content in the mobile phase for the (*R*)-FLU and (*S*)-FLU, we found that the plots obtained were mostly linear, suggesting a reversed-phase mechanism for the interaction between solute and the stationary phase (Fig. 3).

From the results of the effect of the mobile phase composition on the resolution of (*R*)-FLU and (*S*)-FLU, we found that the resolution increased with the decrease in the concentration of the methanol in the mobile phase (Fig. 3).

When the concentration of the methanol in the mobile phase was decreased, the retention time increased. The enantiomers had more time to enter the cavity in the cyclodextrin and interacted with it, so the resolution of (*R*)-FLU and (*S*)-FLU was increased. But too long a retention time is not good, therefore we need to choose a proper concentration of methanol in the mobile phase.

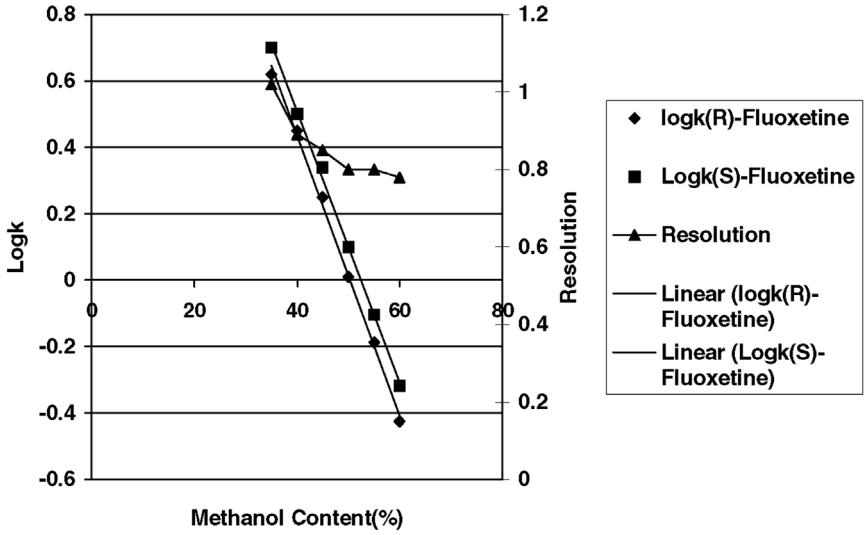

Figure 3. Effect of mobile phase composition on the logarithm of the retention factor and resolution for FLU.

According to the analysis of the results of the effect of the mobile phase composition on the (*R*)-FLU and (*S*)-FLU, the proper methanol vs. buffer ratio should be 40:60.

Experiment 2: Effects of Ionic Strength

The effect of ionic strength on the enantioseparation of (*R*)-FLU and (*S*)-FLU was investigated in the concentration range 0.5–2.5%(w/v) triethylammonium acetate (TEAA) at pH 4.0 and the room temperature. The mobile phase flow rate is 0.5 mL/min. The methanol vs. buffer ratio is 40:60.

As shown in Fig. 4, the retention time decreased with an increase in the concentration of triethylamine. It implied that the ionic strength could likely affect the enantiomers to enter into the cavity of the β-cyclodextrin. The behavior could be rationalized in terms of triethylamine acetate molecules being included in the CD cavity, thus competing with the solutes. This mechanism might be possible through the formation of a triethylamine acetate ion pair within the β-cyclodextrin cavity. The triethylamine acetate molecules interacted with the substituted functional groups at the opening of the β-cyclodextrin cavity, thereby blocking the entry of the enantiomers, thus the retention time was decreased.

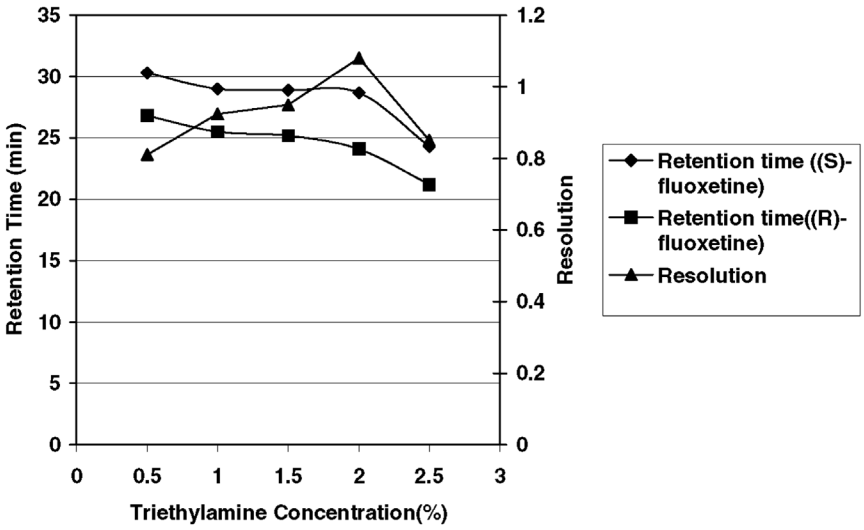

Figure 4. Effect of ionic strength on retention time and resolution.

The results of the effect of ionic strength showed that the resolution was increased with an increase in the ionic strength before the concentration of triethlamine was higher than 2%, and then the resolution was decreased with an increase in the ionic strength (Fig. 5). It showed that ionic strength could affect resolution. The nonlinear relationship between resolution and ionic strength implies that ionic strength could affect the interaction of the enantiomer molecules and β-cyclodextrin. There could be a difference in the effect to block the entry of the two enantiomers to β-cyclodextrin cavity such that the two enantiomers have a larger difference in the retention time. Thus, a better resolution might be obtained because of the ionic strength. But higher ionic strength could cause a decrease in the retention time, it would make the enantiomer molecules have no sufficient time to interact with cyclodextrin and cause a decrease in the resolution. When the concentration of triethylamine was 2%, the resolution was maximum and therefore a quantity of 2% triethylamine was chosen.

Experiment 3: Effects of Flow Rate

The effect of flow rate on the retention time, resolution of the enantiomeric separation of (*R*)-FLU and (*S*)-FLU was investigated by decreasing the flow rate from 1.0 to 0.2 mL/min at the room temperature. The concentration of triethylamine was 0.5% and pH 4.0.

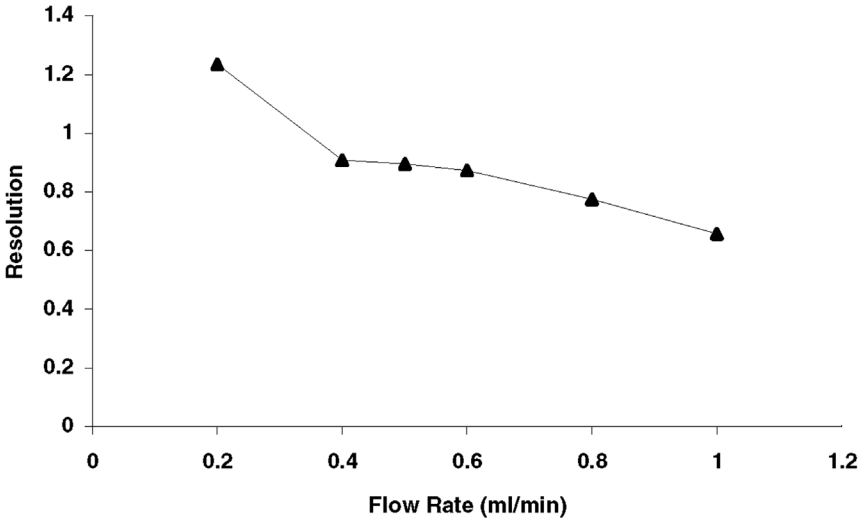

Figure 5. Effect of flow rate on resolution.

It was found that the flow rate could affect the retention time strongly. It was clear that the retention time and the resolution decreased with an increase in the flow rate (Fig. 5). As the decrease in the flow rate caused the increase in the retention time, the enantiomer molecules had enough time to get intact with β-cyclodextrin, which was profitable to separation, the resolution was increased.

When the flow rate was 0.2 mL/min, the resolution was the maximum. But the retention time was too long, so it was ruled out. When the flow rate is 0.5 mL/min, the resolution was almost the same as when flow rate was 0.2 mL/min, but the retention time was shorter than the previous case. Therefore 0.5 mL/min was chosen.

Experiment 4: Effects of pH

The effect of pH on the retention time, resolution of the enantiomeric separation of (*R*)-FLU and (*S*)-FLU was investigated by increasing the pH from 3.5 to 6.5 at the room temperature. The concentration of triethylamine was 2% and the flow rate was 0.5 mL/min.

From the results of the effect of pH on the retention time, it was found that the retention time was increased with an increase in the pH (Fig. 6).

Hydrophoblic interactions between β-cyclodextrin cavity and the enantiomers were affected slightly by the pH. Retention depended on the ion-pair formation between the charged nitrogen group in the molecules and the

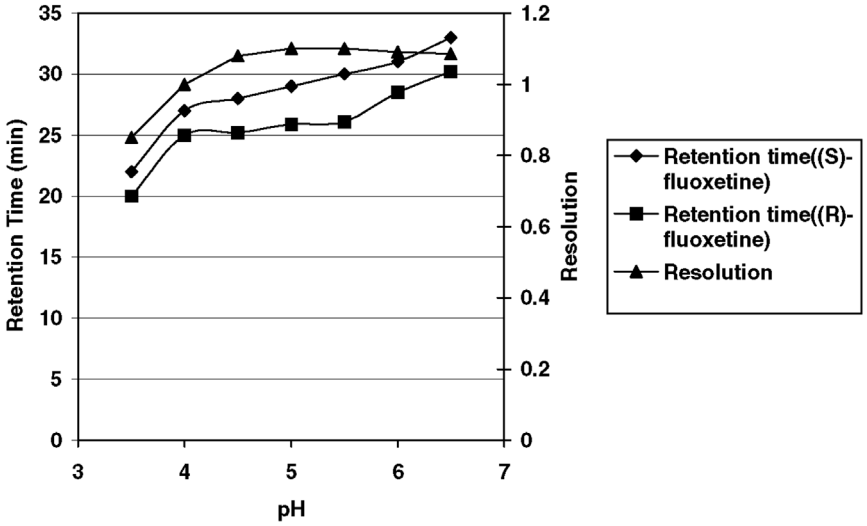

Figure 6. Effect of pH on retention time and resolution.

acetate anion, the degree of which increased with increasing pH, consequently, an increase in the retention time was observed.

By increasing the pH, the enantiomeric resolution increases until the pH was 5.0, reaching a maximum, which depended on the pK_α of the solutes. Even if the pH was increased above 5.0, the degree of ion-pair formation could not be changed strongly. So the resolution cannot be changed too much. Therefore the optimum pH should be 5.0.

Experiment 5: Effects of Temperature

The effect of temperature on the retention time, resolution of the enantiomeric separation of (*R*)-FLU and (*S*)-FLU was investigated by increasing the temperature from 20 to 60°C. The concentration of triethylamine was 2%, pH 5.0 and the flow rate 0.5 mL/min.

According to the results of the effects of temperature on the retention time, we found that the retention time was decreased as the temperature was increased.

This possibly followed the decrease in the binding constant to the β-cyclodextrin with increasing temperature. The effect of temperature on the retention was determined largely by the enthalpy was evaluated from the slope

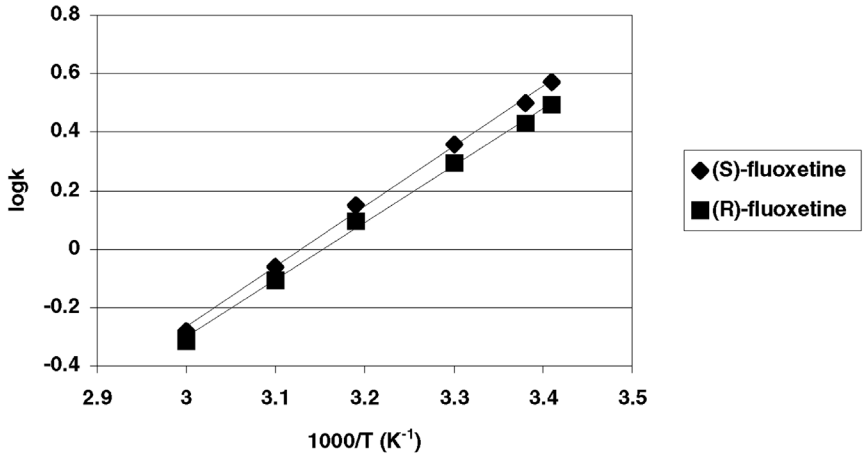

Figure 7. Van't Hoff plot for the retention of FLU in 20–60°C temperature range.

of van't Hoff plots, that was, plots of $\log k$ vs. the reciprocal of the absolute temperature (18). According to Snyder (19), the dependence of the retention factor k on temperature in reversed-phase liquid chromatography was given by:

$$\frac{\mathrm{d}(\log k)}{\mathrm{d}(1/T)} = \frac{\Delta H^0}{4.57} \tag{2}$$

where k is the retention factor, T the absolute temperature (K), and ΔH^0 the enthalpy in calories.

Figure 7 shows a van't Hoff plot for FLU. Since the plots were quite linear, it was possible to determine the enthalpy of binding from the slope of the van't Hoff plot. The enthalpies of binding for (*R*)-FLU and (*S*)-Fluxetine are 9.361 and 8.845 kJ/mol, respectively.

With the increase in the temperature, the resolution was decreased. The relationship between them was linear (Fig. 8). Since the binding constant decreased as the temperature was increased, the retention time decreased, the enantiomeric molecules could not interact with stationary phase, and the resolution decreased.

EVALUATION OF ELUTION ORDER

There are several requirements which are necessary for chiral recognition to occur in CD bonded phase. First, an inclusion complex should be formed

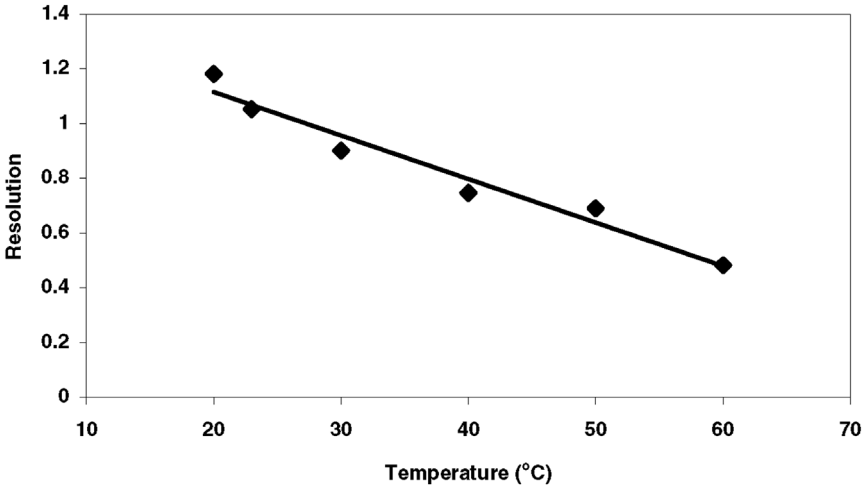

Figure 8. Effect of temperature on resolution.

between the solute and the CD, which can provide a relatively "tight fit" between the hydrophobic part of the solute and the CD cavity. In addition, the chiral center or a substituent attached to the chiral center must be near and interact with the "mouth" of the CD cavity (11).

(*R*)-enantiomer can produce a strong $\pi-\pi$ interaction with *R* chiral center in CSP, but has weak hydrogen bond strength with *S* chiral center in CSP. It can make (*R*)-enantiomer interact strongly (hydrogen bond) with methanol in mobile phase and elute faster than (*S*)-enantiomer. On the contrary, (*S*)-enantiomer can produce strong hydrogen bond strength with *S* chiral center and has weak interaction with the methanol in the mobile phase. So it can be thought that (*S*)-enantiomer is eluted out later (20).

CONCLUSION

In the paper, we had studied the effect of mobile phase composition, ionic strength in the mobile phase, flow rate, pH, and temperature on retention time, resolution of (*R*)-FLU and (*S*)-FLU, using the new β-cyclodextrin bonded column. The experimental results showed that all these factors could affect the retention time and resolution. The optional operation conditions were found. When methanol–buffer ratio is 40:60, flow rate 0.5 mL/min, the concentration of triethylamine in the mobile phase is 2%, pH is 5.0, and the temperature is 23°C,

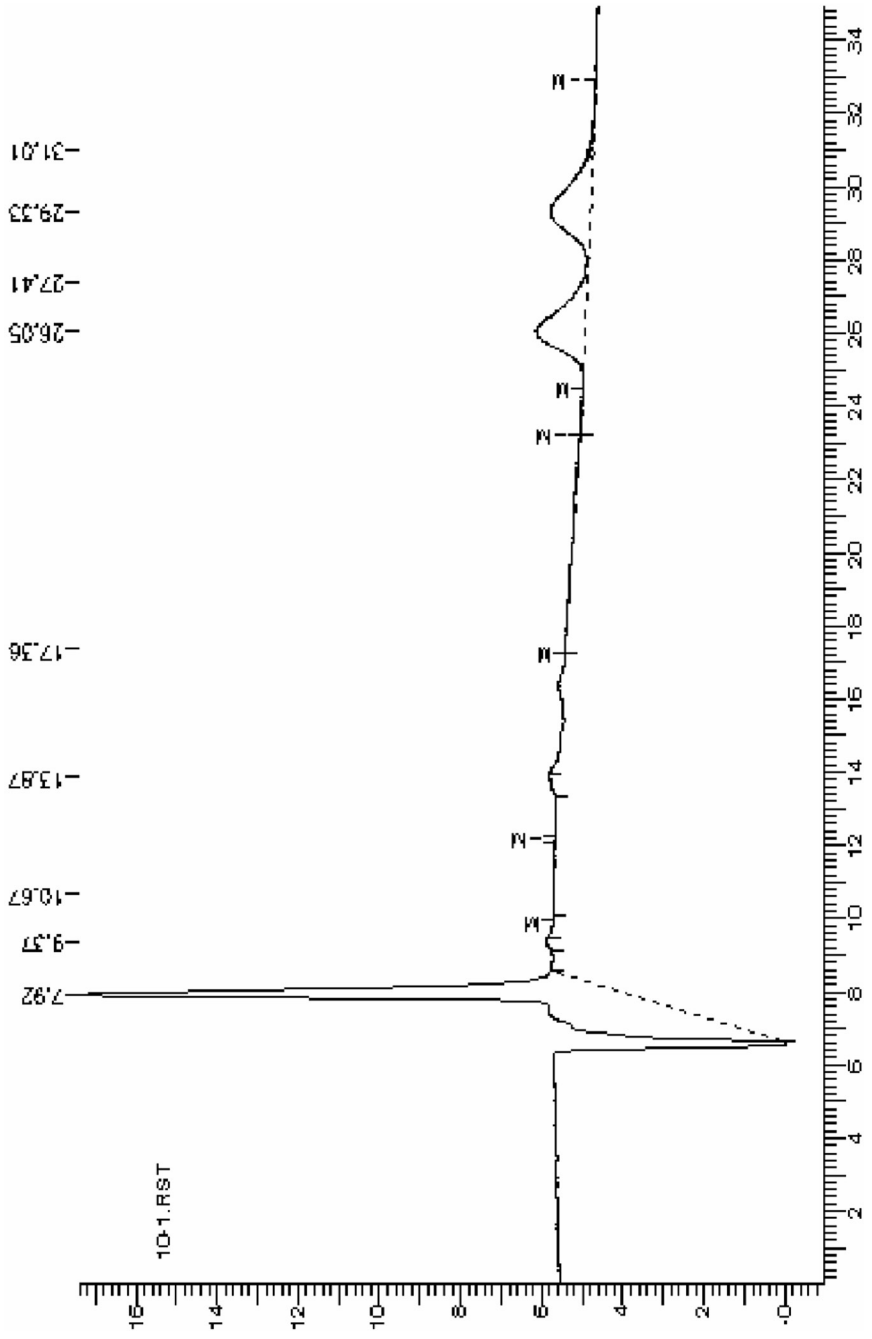

Figure 9. Chromatogram of (*R*)-FLU and (*S*)-FLU.

the retention time 29.02 and 25.72 min, respectively, the resolution was 1.130. The chromatogram of (*R*)-FLU and (*S*)-FLU was shown in Fig. 9. This work gave us a basis for the separation of FLU on a large scale, which will be our future work.

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REFERENCES

1. Piperaki, S.; Penn, S.G.; Goodall, D.M. Systematic Approach to Treatment of Enantiomeric Separations in Capillary Electrophoresis and Liquid Chromatography II. A Study of the Enantiomeric Separation of Fluoxetine and Norfluoxetine. J. Chromatogr. A **1995**, *700*, 59–67.
2. Olsen, B.A.; Wirth, D.D.; Larew, J.S. Determination of Fluoxetine Hydrochloride Enantiomeric Excess Using High-Performance Liquid Chromatography with Chiral Stationary Phases. J. Pharm. Biomed. Anal. **1998**, *17*, 623–630.
3. Piperaki, S.; Pariss-Poulou, M. Evaluation of the Chromatographic Behavior of Fluoxetine and Norfluoxetine Using Different Cyclodextrins as Mobile Phase Additives and Fluorimetric Detection. J. Liq. Chromatogr. Rel. Technol. **1996**, *19* (9), 1405–1421.
4. Hilton, M.L.; Chang, S.C.; Gasper, M.P.; Pawlowska, M.; Armstrong, D.W.; Stalcup, A.M. Comparison of the Enantioselectivity of Phenethyl- and Naphthylethyl-Carbamate Substituted Cyclodextrin Bonded Phases. J. Liq. Chromatogr. **1993**, *16* (1), 127–147.
5. Armstrong, D.W.; Stalcup, A.M.; Hilton, M.L.; Duncan, J.D.; Faulkner, J.R., Jr; Chang, S.C. Derivatized Cyclodextrins for Normal-Phase Liquid Chromatographic Separation of Enantiomers. Anal. Chem. **1990**, *62*, 1610–1615.
6. Hargitai, T.; Okamoto, Y. Evaluation of 3,5-Dimethylphenyl Carbamoylated α-, β, and γ-Cyclodextrins as Chiral stationary Phases For HPLC. J. Liq. Chromatogr. **1993**, *16* (4), 843–858.
7. Okamoto, Y.; Yashima, E. Polysaccharide Derivatives for Chromatographic Separation of Enanitomers. Angew. Chem. Int. Ed. **1998**, *37*, 1020–1043.
8. Chang, S.C.; Reid, G.L., III; Chen, S.; Chang, C.D.; Armstrong, D.W. Evaluation of a New Polar-Organic High-Performance Liquid Chromato-

graphic Mobile Phase for Cyclodextrin-Bonded Chiral Stationary Phases. Anal. Chem. **1993**, *12* (4), 144–153.
9. Paleologou, M.; Li, S.; Purdy, W.C. Liquid Chromatographic Retention Behavior and Separation of Chlorophenols on a β-Cyclodextrin Bonded-Phase Column. Part I. Monoaromatic Chlorophenols: Retention Behavior. J. Chromatogr. Sci. **1990**, *28*, 311–318.
10. Armstrong, D.W. Chiral Stationary Phases for High Performance Liquid Chromatographic Separation of Enantiomers: A Mini-Review. J. Liq. Chromatogr. **1984**, *7* (s-2), 353–376.
11. Piperaki, S.; Parissi-Poulou, M. Use of Cyclodextrins as Chiral Selectors for Direct Resolution of the Enatiomers of Fluoxetine and Its Metabolite Norfluoxetine by HPLC. Chirality **1993**, *5*, 258–266.
12. Pramar, Y. Quantitation of Fluoxetine Hydrochloride in Capsules Using High-Performance Liquid Chromatography. Drug Dev. Ind. Pharm. **1992**, *18* (2), 257–264.
13. Karch, K.; Sebastian, I.; Halasz, I.; Engelhardt, H.J. Optimization of Reversed-Phase Columns. J. Chromatogr. **1976**, *122*, 171–184.
14. Karger, B.L.; Gant, J.R.; Harkopf, A.; Weiner, P.H. Hydrophoblic Effects in Reversed-Phase Liquid Chromatography. J. Chromatogr. **1976**, *128*, 65–78.
15. Snyder, L.R.; Dolan, J.W.; Gant, J.R. Gradient Elution in High Performance Liquid Chromatography I. Theoretical Basis for Reversed-Phase System. J. Chromatogr. **1979**, *165*, 3–30.
16. Franks, F. *Water. A Comprehensive Treatise*; Plenum Press: New York, 1975; Vol. 4, Chap. 1.
17. Paleologou, M.; Li, S.; Purdy, W.C. Liquid Chromatographic Retention Behavior and Separation of Chlorophenols on a β-Cyclodextrin Bonded-Phase Column. Part II. Monoaromatic Chlorophenols: Separation. J. Chromatogr. Sci. **1990**, *28*, 319–323.
18. Melander, W.R.; Horvath, C. *High Performance Liquid Chromatography—Advances and Perspectives*; Academic Press: New York, 1980; Vol. 2, 195.
19. Snyder, L.R. Temperature-Introduced Selectivity in Separation by Reversed-Phase Liquid Chromatography. J. Chromatogr. **1979**, *179*, 167–172.
20. Wang, Z.X.; Yun, Z.H. Separation of Fluoxetine Enantiomers by High Performance Liquid Chromatography with Urea Derivative as Chiral Stationary Phase. Chin. J. Anal. Chem. **1997**, *25* (4), 464–467.

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