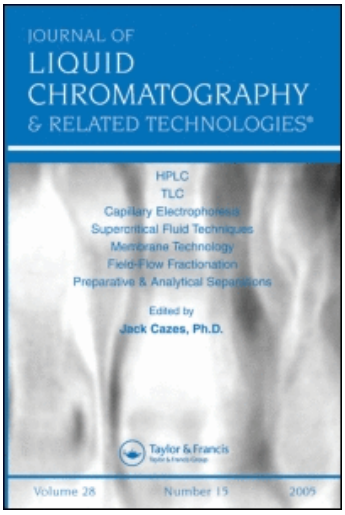

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A new diamide-type chiral stationary phase for chiral resolution by normal and reversed phase HPLC

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A New Diamide-Type Chiral Stationary Phase for Chiral Resolution by Normal and Reversed Phase HPLC

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ABSTRACT

In this work, a new diamide-type chiral stationary phase was developed by chemically bonding the *N*-stearoyl-*L*-leucine on the aminopropylsilica gel. It has been successfully used for the chiral resolution of the benzoyl-, dinitrobenzoyl-, and trifluoroacethyl amino acids in the modes of normal phase, as well as for the underivatized amino acids in the mode of reversed phase. It was found that the hydrogen bonding makes significant contribution to the chiral resolution. It has been demonstrated that the chiral resolution, by the use of diamide-type chiral stationary phase (CSP), can be achieved without the π–π interaction between the CSP and analytes.

Key Words: Chiral stationary phase; Diamide; High performance liquid chromatography; Amino acid; Amine.

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(A) Synthesis of Chiral Stationary Phase

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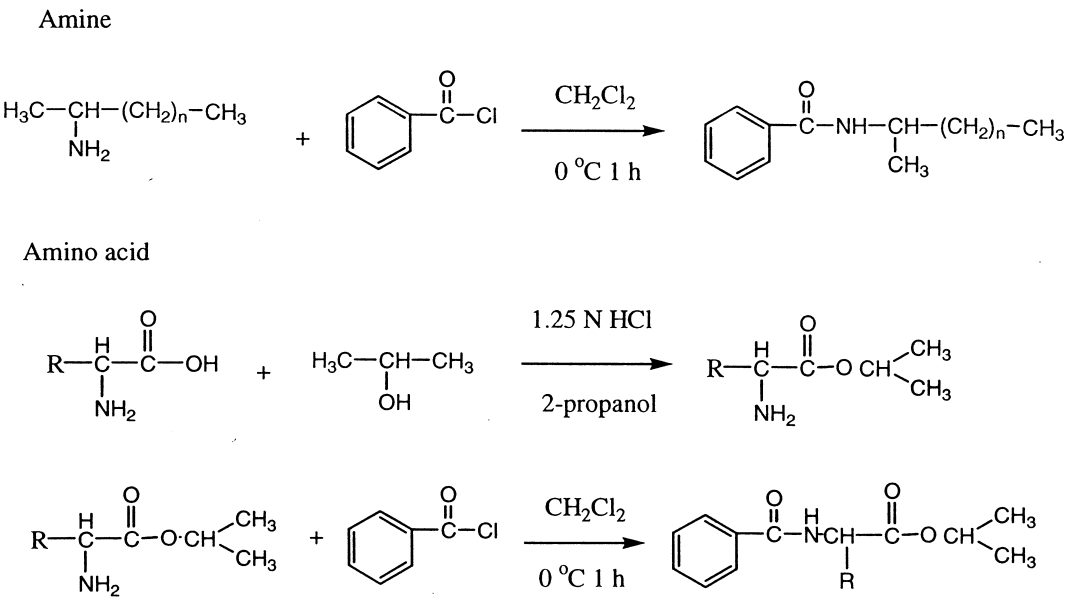

(B) Derivatization of amine and amino acids

Figure 1. Schemes of preparation of CSP (A) and sample derivatization (B).

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INTRODUCTION

The liquid chromatographic resolution of enantiomers on chiral stationary phases (CSPs) has been known as one of the most accurate and convenient means of determining the enantiomeric composition of chiral compounds.[1,2] A number of CSPs based on proteins,[3] cellulose derivatives,[4] cyclodextrins,[5] macrocyclic antibiotics,[6] low molecular mass chiral molecules,[7,8] and crown ethers[9,10] have been developed for successful enantioseparations.

Diamide-type CSPs are one type of low molecular CSP used in chiral gas chromatography (GC) and liquid chromatography (LC). Gil-Av and Hobo reported enantioseparation with *N*-lauroyl-*L*-valine-tert-butylamide by GC.[11,12] Dobashi et al. reported on extended scopes of ennatiomer resolution with chiral diamide phases in LC.[13] Previous works in our laboratory studied the immobilization method of diamide-type CSPs and examined their enatioselectivies.[14,15] It has been known that these CSPs possess both π–π interaction and hydrogen bonding sites for chiral recognition. However, it is not quite clear which interaction of π–π or hydrogen bonding dominates the chiral recognition. In order to answer this question and know the mechanism of chiral resolution of diamide-type CSPs, in the present work, our aim was focused on the preparation of diamide-type CSPs, which have no π–π interaction site. In other words, we like to explore the possibility using CSP, which only offers the interaction of hydrogen bonding for chiral LC. A new diamide-type CSP, which chemically bonded the *N*-stearoyl-*L*-leucine on aminopropylsilica gel was developed in this work, as shown in Fig. 1(A). The applications for chiral resolutions of amine and amino acids in normal and reversed phases HPLC have been investigated.

EXPERIMENTAL

Instrumentation and Chromatography

The chromatographic equipment consisted of a Shimadzu (Kyoto, Japan) Model LC-6A pump, a Rheodyne (Cotati, CA) Model 7125 sampling valve with a 25 μL loop, a Shimadz Model SPD-6AV UV–Vis spectrophotomeric detector, and Shimatz C-R5A integrator. The column (250 × 4.0 mm I.D.) was packed with modified silica gel using the slurry packing technique. The detection wavelength used was 254 nm. Flow rate of mobile phases were 1 mL min^{-1}. The mobile phases for normal and reversed phases were hexane/2-propanol (97.5 : 2.5) and water/methanol (60 : 40), respectively. All the experiments were carried out at room temperature (about 25°C).

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Sample Preparation

Amino acids and amines were derivatized to benzoyl (BEN), 3,5-dinitrobenzoyl (DNB), and trifluoroacethyl (TFA) derivatives as test samples. The scheme of reactions are shown in Fig. 1(B). The procedures were carried out according to the previous works in our laboratory with modifications.[14] They are briefly described as follows.

Derivatization Procedures for Amines

One hundred milligram of amine racemates was dissolved with 5 mL of dichloromethane in a 100-mL flask with a drying tube. A few drops of benzoyl chloride were added and stirred by an electromagnetic method for 2 h in an ice bath. After removing the solvent by the rotary evaporator, samples were dissolved in a ethyl acetate and transferred to a separating funnel for extraction, and washed successively with 5% $NaHCO_3$, water, and 0.1 M HCl. The extracted samples were purified by column chromatography using silica gel columns and *n*-hexane/ethyl acetate (10 : 1) as the mobile phase. After removing the solvent, it was dissolved in *n*-hexane and kept for use.

Derivatization Procedures for Amino Acids

One hundred milligrams of amino acid racemates were esterified with 1.25 M HCl–isopropanol solution at 120°C for 2 h under reflux. After removing the solvent by rotary evaporator, samples were dissolved in ethyl acetate and transferred to a separatory funnel for extraction. The esterified amino acids were derivatized, extracted, and purified by the same procedure as for amines.

Synthesis of Chiral Stationary Phase

Preparation of Stearoyl Chloride

Stearic acid (300 g about 1 mol) dissolved in 350-mL of thionyl chloride was refluxed for 3 h and excess thionyl chloride was removed by distillation for 3 h at 76°C. The residue was distilled under reduced pressure at 182°C for 2 h to afford stearoyl chloride.

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Preparation of *N*-Stearoyl-*L*-Leucine and its Purification

One hundred and twenty gram of (1 mol) *L*-Leucine was dissolved with 2 M NaOH solution and stirred in a flask in an ice bath, and then 2 L of the stearoyl chloride (303 g) was dissolved in diethyl ether and 680 mL of 2 M NaOH. After removing the ice bath, the mixture was stirred for 18 h. The product was purified by column chromatography using a silica gel (200 meshes) column (47 mm × 150 cm) and mobile phase of *n*-hexane/ethyl acetate. The structure of the purified product was identified by NMR spectrum.

Determination of Optical Purity of the *N*-Stearoyl-*L*-Leucine

As a new prepared CSP, the optical purity should be determined. However, it is difficult to determine the optical purity of CSP directly; we determined the optical purity of *N*-stearoyl-*L*-leucine before immobilizing on the aminopropylsilica. As the immobilization reaction to silica was carried out at room temperature, it was reasonable to assume that the optical purity of the CSP was very close to the purity of *N*-stearoyl-*L*-leucine.

For the determination of the optical purity, a GC method was employed. In brief, *N*-stearoyl-*L*-leucine (10 g) was placed in a glass tube, identical amounts of 6 M HCI and 1.25 M HCl–isopropanal solutions were added. The mixture was reacted under reduced pressure. After the completion of esterification, the solvent was evaporated and the residue was dissolved in dichloromethane and frozen with liquid N_2. Then the trifluoroacetic anhydride was added and stored at room temperature for 1 h. After evaporation, the derivative (*N*-TFA-leucine-iso-propylester) was analysed by GC, the average optical purity was determined to be 93.5% ($n = 3$). A glass capillary column coated with *N*-stearoyl-*L*-valine-tert-butyramide stationary phase and a flame ionization detector were used.

Preparation of Chiral Stationary Phase

5.02 g of aminopropylsilica (Develosil-NH_2, 5 μm, Nomura Chemical), was reacted with *N*-stearoyl-*L*-leucine (10.16 g, ca. 0.03 mol) in the dry tetrahydrofuran in the presence of 1-ethoxycarbonyl-2-ethoxy- 1,2-dihydroquinone (EEDQ: 15.52 g, ca. 0.06 mol) for 18 h under N_2 atmosphere. The CSP beads were filtered with a Milipore 3-μm filter, and then washed successively with tetrahydrofuran, acetone, heptane, and diethyl ether.

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RESULTS AND DISCUSSION

Chiral Recognition of Diamide-Type Chiral Stationary Phase

As mentioned earlier, several interaction models have been proposed between solute molecules and the chiral diamide molecules.[2,16–18] As shown in Fig. 2(A), two aspects should be pointed out: (1) the solute–solvent interaction consists of three points, namely π–π and two hydrogen bonds; and the interaction of all three points occurs between one solute molecule and one diamide molecule. This work was focused on synthesizing the CSP without the phenyl group for studying the necessity for a phenyl group as

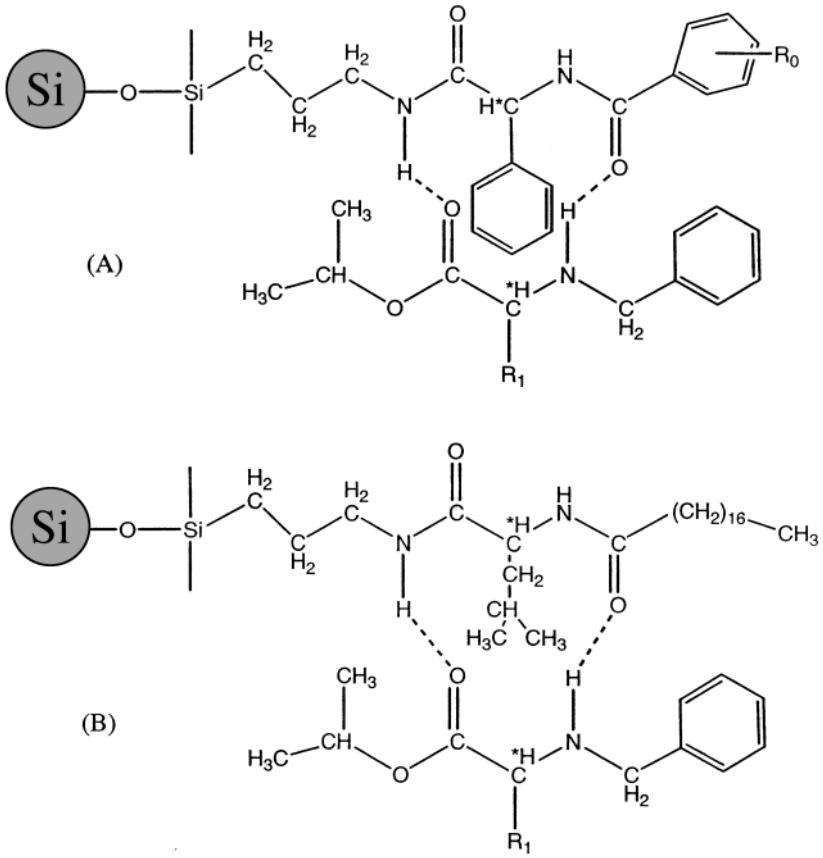

Figure 2. Chiral recognition models of diamide-type CSPs.

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the acyl moiety and phenylglycine as the chiral moiety in Fig. 2(A). As shown in Fig. 2(B), the chiral recognition is mainly based on the two hydrogen bondings between the CSP and the solute and the steric stereoselectivies of substituent groups of the chiral centers in both CSP and solute.

Chiral Resolution of Amino Acid Derivatives by Normal Phase HPLC

The BEN derivatization is shown in Fig. 1(B). Benzoyl derivatives of amine and amino acid were resolved by normal phase HPLC, the results are listed in Table 1, and representative chromatograms were shown in Fig. 3. It was demonstrated that the CSP show excellent enantioselectivies for amino acid derivatives, although they did not show enantiselectivities for amine derivatives. Previous work in our laboratory reported diamide-type CSPs containing chiral center of phenylglycine.[14] It was considered that the π–π interaction between CSP and analytes plays an important role, in addition to the hydrogen bonding. However, this work demonstrated that chiral resolution could be achieved in the absence of phenyl groups. On the other hand, it suggests that hydrogen bonding should be considered the main interaction for chiral resolutions.

To examine the effect of the phenyl group in the analytes on the chiral resolution, we also investigated the chiral resolutions of TFA and DNB derivatives by using this CSP. The chromatograms are shown in Figs. 4 and 5.

Table 1. Chiral resolution of benzoyl derivatives of amine and amino acid on CSP by normal-phase HPLC.

Samples	k_1'	k_2'	α
2-Octylamine	3.86	3.86	1.0
α-Phenylethylamine	12.48	12.48	1.0
DL-valine	1.29	1.67	1.29
DL-tyrosine	4.08	5.47	1.36
DL-norvaline	2.11	3.00	1.42
DL-alanine	3.78	4.81	1.27
DL-phenylalanine	2.37	3.36	1.42
DL-leucine	1.95	3.06	1.57
DL-phenylglycine	2.29	2.59	1.13
DL-norleucine	1.68	2.50	1.49
DL-proline	4.94	4.94	1.0
DL-β-amino acid	4.50	4.50	1.0

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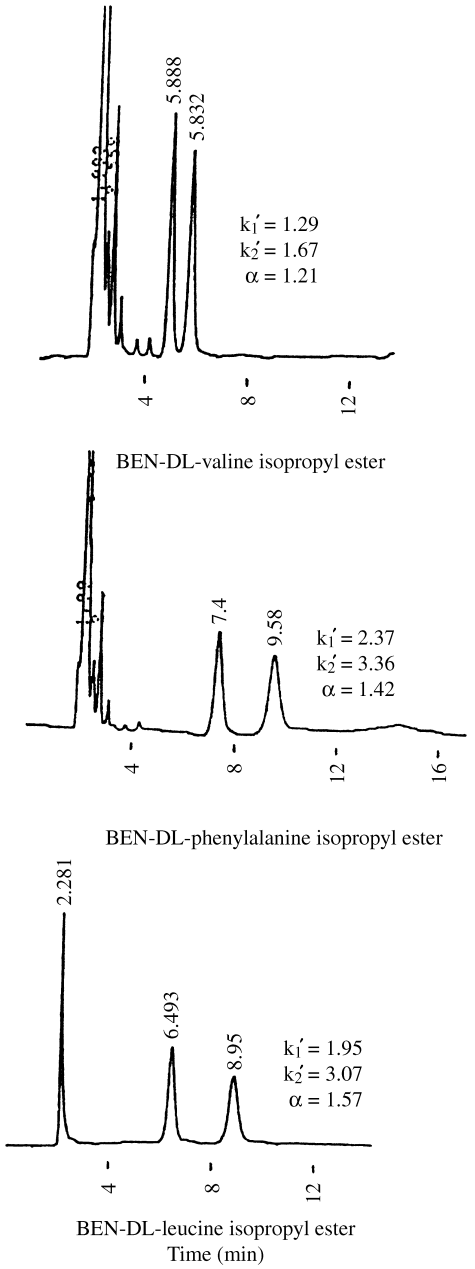

Figure 3. Chiral resolutions of benzoyl derivatives of amino acids on CSP by normal phase HPLC.

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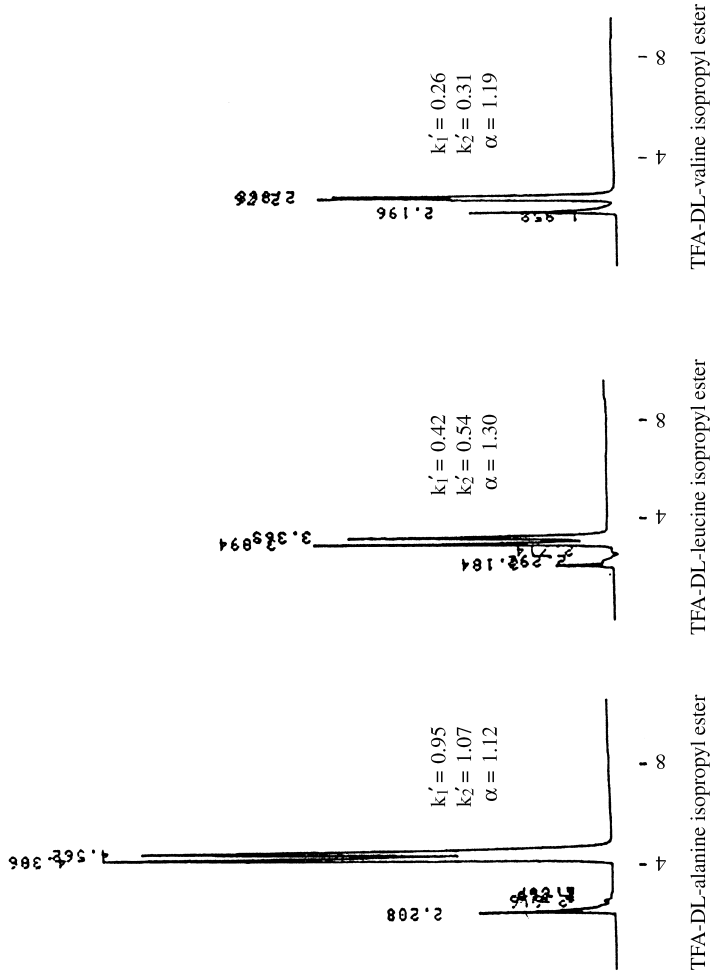

Figure 4. Chiral resolutions of TFA derivatives of amino acids on CSP by normal phase HPLC.

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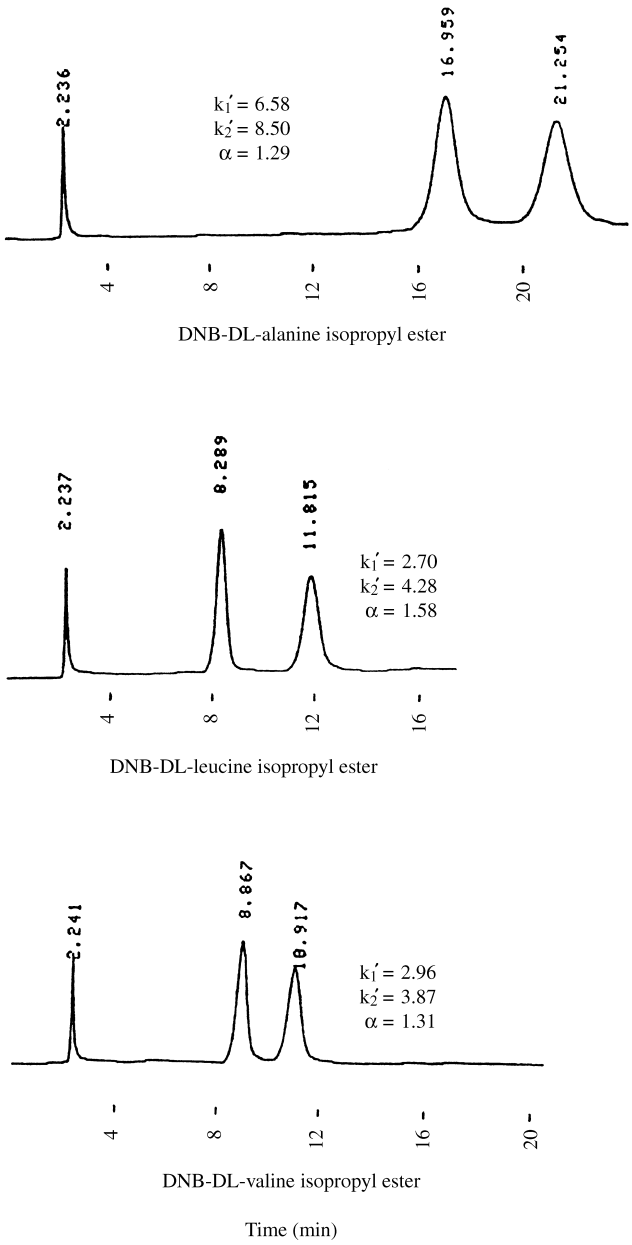

Figure 5. Chiral resolutions of DNB derivatives of amino acids on CSP by normal phase HPLC.

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Comparing the separation factors of BEN and DNB derivatives, we can notice that similar separation factors were obtained. However, the separation factors of TFA derivatives were smaller than those of both BEN and DNB derivatives. These results suggest that phenyl groups of analytes benefit the chiral resolution. However, the interaction between the phenyl groups is not dominative.

Chiral Resolution of Amino Acids by Reversed Phase HPLC

The CSP was also employed for the chiral resolutions of underivatized amines and amino acids by a reversed phase HPLC. The samples examined are listed in Table 2; the representative chromatograms are shown in Fig. 6. It was shown that only the CSP had enantioselectivies for four amino acids, but the resolutions were not too good. Even when the concentrations of methanol in the mobile phase were changed to 30, 50, and 60%, the resolutions were not achieved. As shown in Tables 1 and 2, the retention times in the reversed phase mode were longer than those in the mode of normal phase. It indicates that the hydrophobic interaction between the CSP and analytes plays a significant role in the reversed phase mode. On the other hand, the fact that separation selectivities are not so good in the mode reversed phase suggests that the hydrogen bonding between the CSP and analytes in the mode of reversed phase become more difficult than that in the mode of normal phase.

CONCLUSIONS

The CSP developed was successfully used for the chiral resolutions by both modes of normal and reversed phases. It has been demonstrated that

Table 2. Chiral resolution of amine and amino acid on CSP by reversed-phase HPLC.

Samples	k_1'	k_2'	α
α-Phenylethylamine	8.29	8.29	1.0
DL-valine	12.37	12.37	1.0
DL-norvaline	12.40	12.85	1.04
DL-alanine	4.50	4.50	1.0
DL-phenylalanine	28.12	29.46	1.05
DL-leucine	22.90	24.19	1.06
DL-norleucine	27.52	28.82	1.05

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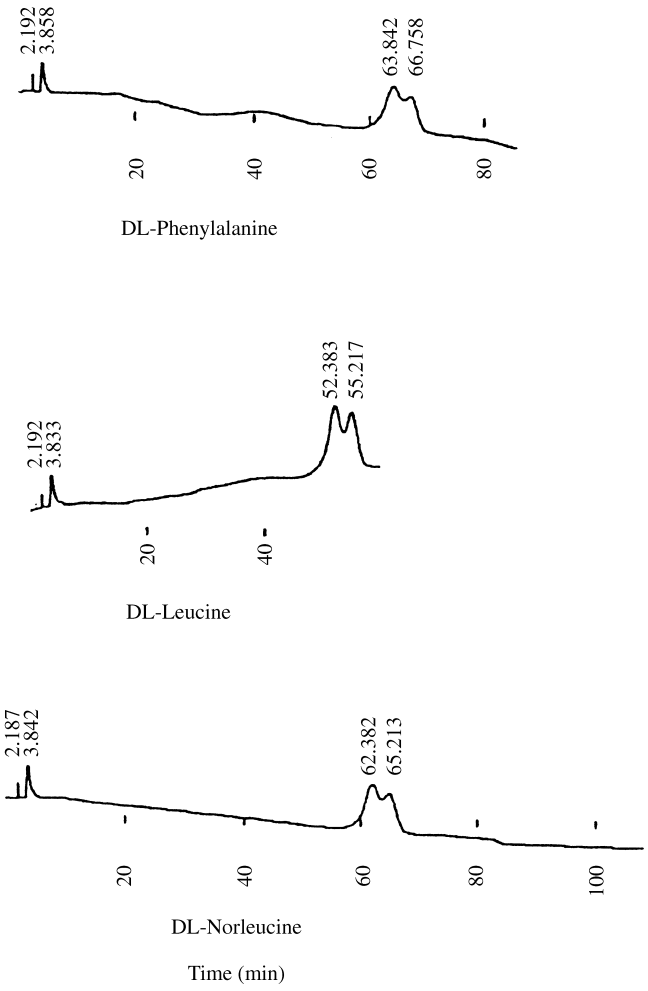

Figure 6. Chiral resolutions of underivatized amino acids on CSP by reversed phase HPLC.

CSP showed excellent enantioselectivities in the normal phase mode. The fact that present CSP does not possess the phenyl group suggests that the hydrogen bonding between the CSP and analytes dominates interaction for the chiral resolution. Further, it has been demonstrated CSP can be used for chiral resolution of underivatized amino acids in the reversed phase mode.

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REFERENCES

1. Allenmark, S.G. *Chromatographic Enantioseparation: Methods and Applications*; Ellis Horwood: Chichester, 1988.
2. Ahuja, S. Ed. *Chiral Separations by Liquid Chromatography*; American Chemical Society: Washington, D.C., 1991.
3. Allenmark, S.; Bomgren, B.; Boren, H. Direct resolution of enantiomers by liquid affinity-chromatography on albumin-agarose under isocratic conditions. J. Chromatogr. **1982**, *237*, 473–477.
4. Okamoto, Y.; Kawashima, M.; Hatada, K. Useful chiral packing materials for high-performance liquid chromatographic resolution of enantiomers: phenylcarbamates of polysaccharides coated on silica gel. J. Am. Chem. Soc. **1984**, *106*, 5357–5359.
5. Ward, T.J.; Armstrong, D.W. Improved cyclodextrin chiral phases: a comparison and review. J. Liq. Chromatogr. **1986**, *9*, 407–423.
6. Armstrong, D.W.; Tang, Y.; Chen, S.; Zhou, Y.; Bagwill, C.; Chen, J.R. Macrocyclic antibiotics as a new class of chiral selectors for liquid chromatography. Anal. Chem. **1994**, *66*, 1473–1484.
7. Pirkle, W.H.; Welch, C.J.; Lamm, B.; Design, synthesis and evaluation of an improved enantioselective naproxen selector. J. Org. Chem. **1992**, *57*, 3854–3860.
8. Oi, N. Enantiomer separation by HPLC with chiral stationary phases derived from low molecular selectors. Chromatography **1993**, *14*, 61R–70R.
9. Sogah, G.D.Y.; Cram, D.J. Host–guest complexation. 14. Host covalently bound to polystyrene resin for chromatographic resolution of enantiomers of amino acid and ester salts. J. Am. Chem. Soc. **1979**, *101*, 3035–3042.
10. Shinbo, T.; Yamaguchi, T.; Nishimura, K.; Sugiura, M. Chromatographic separation of racemic amino acids by use of chiral crown ether-coated reversed-phase packings. J. Chromatogr. **1987**, *405*, 145–153.
11. Watabe, K.; Gil-Av, E.; Hobo, T.; Suzuki, S. Effect of aplar diluents on the behavior of chiral stationary phases in gas chromatography. Binary mixtures of *N*-lauroyl-*L*-valine-tert-butylamide with squalane and *n*-tetracosane. Anal. Chem. **1989**, *61*, 126–132.
12. Hobo, T.; Suzuki, S.; Watabe, K.; Gil-Av, E. Effect of dilution by achiral solvents on enantiomer separation with *N*-lauroyl-*L*-valine tert-butylamide. Anal. Chem. **1985**, *57*, 362–364.
13. Dobashi, A.; Dobashi, Y.; Kinoshita, K.; Hara, S. Extended scope of enantiomer resolution with chiral diamide phase in liquid chromatography. Anal. Chem. **1988**, *60*, 1985–1987.
14. Sato, K.; Nakano, H.; Hobo, T. Effect of sample derivatization in liquid chromatographic separations of amine and amino acid enantiomers using diamide-type stationary phase. J. Chromatogr. A **1994**, *666*, 463–470.

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15. Hanno, Y.; Sugimoto, Y.; Ihara, T.; Nakagama, T.; Hobo, T. Study on immobilization method of diamide type chiral selector for HPLC enantiomer separation. Chromatography, **1994**, *15* (4), 190–191.
16. Finn, J.M. In *Chromatographic Chiral Separations*; Zief, M., Crane, L.J., Ed.; Marcel Dekker, Inc.: New York, 1988, 53.
17. Gil-Av, E.; Nurok, D. Resolution of optical isomers by gas chromatography of diastereomers. Adv. Chromaotgr. **1974**, *10*, 99–172.
18. Weinstein, S.; Leiseerowits, L.; Gil-Av, E. Chiral secondary amides. 2. Molecular packing and chiral recognition. J. Am. Chem. Soc. **1980**, *102*, 2768.

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