Chromatographic Enantiomer Separation of Diphenylalanine on Chiral Stationary Phases Derived from Chiral Crown Ethers

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The methods that allow a simple and accurate assessment of the enantiomeric purity of chiral compounds have been developed, because they are frequently required for those in the fields of pharmaceutical chemistry and biochemistry.[1] Among various techniques, liquid chromatographic separation of the enantiomers on chiral stationary phases (CSPs) has been known to be one of the most accurate and convenient means in determining the enantiomeric purity of chiral compounds. In an effort to discover potent and orally bioavailable thrombin inhibitors, the unique amino acid, diphenylalanine (DPA) has been used as an essential chiral precursor.[2-5] In the process of the preparation of intermediates of these compounds, analytical methodology for the enantiomeric purity of prepared DPA was required.

For the direct resolution of DPA, it was found that the use of chiral columns of other types, such as cyclodextrins and polysaccharides etc was not effective. Since Crownpak CR CSP derived from crown ether (Figure 1) has been widely used for the enantioresolution of amino acids, it was employed for separation of DPA enantiomers.[6,7] For the resolution of DPA analyte on Crownpak CR under any HPLC conditions, however, the base-line separation was not obtained. As the best example, Crownpak CR chiral column provided long retention times (about 60 min) and only partial separation (separation factor 1.09) using pH 2 $HClO_4$: MeOH = 85 : 15 (V/V) as a mobile phase (Figure 1). The retention times are decreased with an increase in methanol concentration on Crownpak CR chiral column. However, since the Crownpak CR chiral column is prepared by dynamic coating of chiral crown ether on reversed-phase packing, a mobile phase that contains more than 15% methanol should be avoided. The use of a mobile phase containing more than 15% methanol deteriorates the CSP performance due to the leaching of the chiral crown ether selector.[6,7] Moreover, the use of other organic solvents except methanol is not recommended for the safety of the column, because it may cause the column to be over-pressurized and lose capacity.

We recently reported the synthesis and evaluation of a new CSP prepared by covalently bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6-TA) to aminopropyl silica gel (Figure 2).[8-12] This CSP was successfully utilized

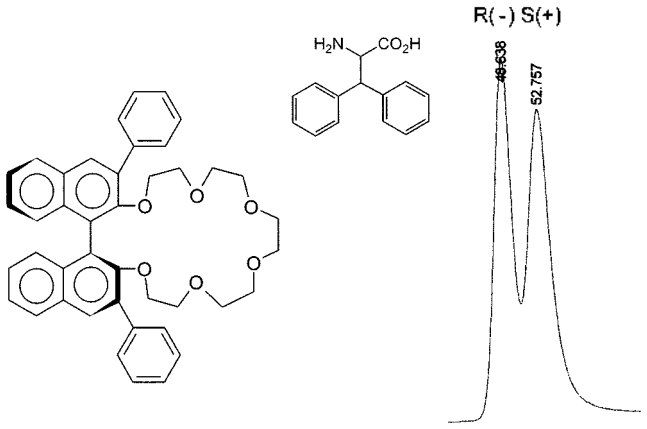

Figure 1. The structure of chiral crown ether utilized in the Crownpak CR(+) and the resolution of racemic DPA on Crownpak CR(+). Mobile phase = pH 2 $HClO_4$: MeOH = 85:15(V/V); Flow rate = 1 mL/min; UV 210 nm; Injection amount 1 μg.

in resolving not only various α-amino acids, but also their ester and amide derivatives. It was also found to be capable of separating the enantiomers of primary amines including amino alcohols and quinolone antibacterials. More recently, mechanistic studies for chiral recognition between CSP and amino acid enantiomers were investigated by NMR spectroscopy.[13] Therefore, it was expected that the enantiomers of DPA would be separated on the CSP derived from 18-C-6 TA. ChiroSil RCA(+) shown in Figure 2 is derived from (+)-18-C-6 TA, while ChiroSil SCA(−) is derived from (−)-18-C-6 TA.

Table 1 shows the effect of acid additives for the resolution of DPA on ChiroSil SCA(−). These data show that chromatographic parameters such as enantioselectivities and retention times are greatly influenced by the nature of acid additive in the mobile phase. An increase in the concentration of water or acid additive in the mobile phase has a tendency of the increase of the retention times. Among several mobile phases used, the best separation is obtained using 80% methanol/water(V/V) containing sulfuric acid or perchloric acid as an acid additive. The typical chromatogram for resolution of the enantiomers of DPA is shown in Figure 3. The ChiroSil column affords good resolution and very short retention times within 5 min, while the Crownpak CR affords poor resolution and long retention times (Figure 1).

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Table 1. Effect of mobile phase for the separation of the enantiomers of DPA on ChiroSil SCA(−)

Acid Additive	Methanol: water (V V)	t_1^a	t_2	$k'_1{}^b$	k'_2	α^c	R_s^d
10 mM H_2SO_4	100:0	3.51	4.59	0.52	0.99	1.89	2.49
10 mM H_2SO_4	80:20	3.88	4.44	0.16	0.33	2.04	2.67
10 mM H_2SO_4	50:50	4.60	5.16	0.26	0.42	1.58	2.04
10 mM H_2SO_4	15:85	6.39	7.06	0.72	0.90	1.25	1.25
10 mM H_2SO_4	0:100	6.74	7.17	1.13	1.26	1.12	0.23
8.5 mM H_2SO_4	15:85	4.54	4.97	0.58	0.73	1.25	1.21
2 mM $HClO_4$	80:20	2.64	3.19	0.27	0.53	1.99	2.54
8.5 mM $HClO_4$	15:85	6.23	7.04	0.84	1.08	1.28	1.91
10 mM $HClO_4$	0:100	6.42	7.08	0.81	1.00	1.23	1.21
8.5 mM CF_3COOH	15:85	5.21	5.82	0.75	0.96	1.27	1.52
10 mM CF_3COOH	0:100	7.07	7.41	1.27	1.38	1.09	0.06

Mobile phase: Methanol:water (V V) containing acid additive; Flow rate = 1 mL/min; UV 210 nm; Temperature ambient (about 25 °C). [a]Retention time (min) for the first eluted enantiomer. [b]Capacity factor for the first eluted enantiomer. [c]Separation factor. [d]Resolution factor.

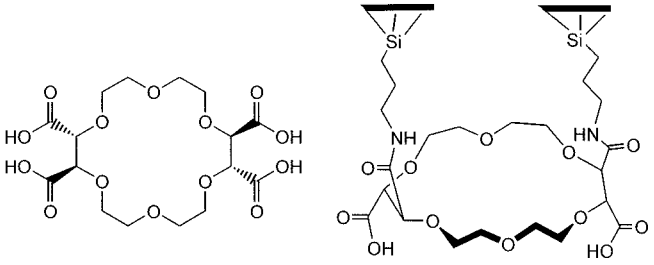

Figure 2. The structure of (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid (18-C-6 TA) and ChiroSil RCA(+) derived from (+)-18-C-6 TA.

Elution orders were determined for the configurationally known DPA, the (S)-enantiomer being strongly retained on ChiroSil SCA(−) CSP. These observations are consistent with the previously reported results,[11] the (R)-enantiomers of the investigated amino acids being more strongly retained than the (S)-enantiomers on the CSP derived from (+)-18-C-6 TA. On Crownpak CR(+) the elution of the (R)-isomer of DPA prior to the (S)-isomer was observed, as shown in Figure 1. Since the elution orders of the enantiomers of DPA and other amino acids on Crownpak CR(+) afford the opposite on the CSP derived from (+)-18-C-6-TA, it might be said that ChiroSil SCA(−) CSP is equivalent to Crownpak CR(+) in terms of elution order.[6,11] The signs of optical rotation of the two DPA enantiomers were determined by an in-line polarimetric detector set at 589nm (Figure 3).

Figure 4 shows chromatograms of determination of the enantiomeric purity of an enantiomerically enriched DPA (S:R = 100:1) on ChiroSil RCA(+) and SCA(−), respectively. The (−)-(R)-DPA is strongly retained on ChiroSil RCA(+) CSP, while the (+)-(S)-enantiomer is strongly retained on ChiroSil SCA(−) CSP. Since ChiroSil column is commercially available in either enantiomeric form, it has the advantage of the reversal of elution order (Figure 4).[14,15] It is often a desirable feature for enantiomeric purity determinations and for preparative separations.

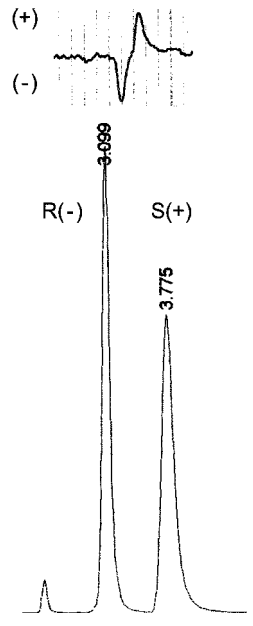

Figure 3. Resolution of racemic DPA on ChiroSil SCA(−); Mobile phase = 10 mM H_2SO_4 in water: MeOH = 20:80(V/V); Flow rate = 1 mL/min; UV 210 nm; Injection amount 1 μg. Signs of optical rotation were determined using polarimetric detector.

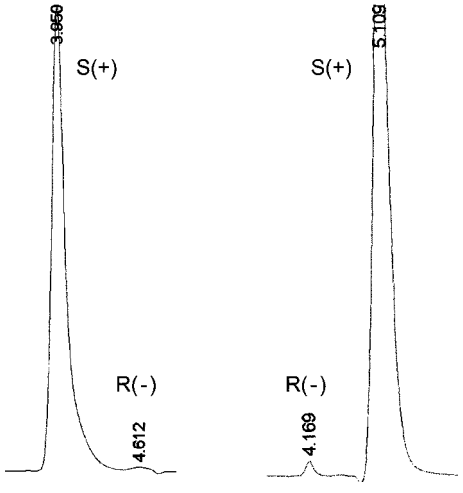

Figure 4. Chromatograms of the resolution of optically enriched DPA [S(+): R(−) =100:1] on ChiroSil RCA(+) (the left) and ChiroSil SCA(−) (the right); Mobile phase = water: MeOH = 20:80(V/V) containing 10 mM H_2SO_4; Flow rate = 1 mL/min; UV 210 nm; Injection amount 1-2 μg.

In summary, liquid chromatographic direct resolution of DPA was achieved on the Crownpak CR column and the ChiroSil column, which are classfied as crown ether type CSP. The ChiroSil column is much more effective than the Crownpak CR column in terms of column stability as well as the degree of enantioseparation. Owing to the advantages of the ChiroSil column shown in this study over the Crownpak CR column, the chromarographic method using the ChiroSil column is expected to be useful for resolution of other amino acids or related compounds in analytical and preparative separations.

Experimental Section

Chromatography was performed at room temperature using an HPLC Breeze system consisting of a Waters model 1525 binary pump, a Rheodyne model 7125 injector with a 20 μL loop, a dual absorbance detector (Waters 2487 detector). Signs of optical rotation were determined using a Shodex OR-2 (Showa Denko, Japan) as an inline chromatography detector. Crownpak CR(+) was purchased from Daicel Chemical Company (Tokyo, Japan). ChiroSil RCA(+) column derived from (+)-18-C-6 TA and ChiroSil SCA(−) column derived from (−)-18-C-6 TA were purchased from RS Tech corporation (Daejon, Korea). The racemic diphenylalanine was prepared according to the reported procedure[2] and D- and L-diphenylalanines were purchased from Synthetech Inc. (Albany, OR, USA) and Sh-Icon Inc. (Shanghai, China), respectively. The optically enriched DPA (S : R = 100 : 1) analyte using these obtained samples was prepared.

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