Short Communication

Enantiomeric Separation of Chiral Alcohols Using Novel Core-Shell Type Chiral Stationary Phase Coated with Helical Poly(diphenylacetylene) Derivative by High-Performance Liquid Chromatography

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Abstract

Enantiomeric separation ability on high-performance liquid chromatography (HPLC) of novel chiral stationary phase (CSP) was evaluated. A chiral selector of the CSP investigated in this study (SUMICHIRAL™ OA-SHELL P1) is one of helical poly(diphenylacetylene) derivatives which is coated on core-shell silica support. OA-SHELL P1 showed excellent chiral separation ability for a wide range of aromatic chiral alcohols and it was also effective for separation of some chiral carboxylic acids, ketones and lactones. It is considered to be a new option for enantiomeric separation of these chiral compounds. OA-SHELL P1 is used in normal phase mode with a mobile phase of simple composition and one of its features is easy method development. The analysis time is relatively short even with conventional HPLC system since a core-shell support with particle size of 2.6 µm is used.

Keywords: Enantiomeric separation; Chiral stationary phase; Helical poly(diphenylacetylene); Chiral alcohol; Core-shell

1. Introduction

In the field of fine chemicals, chiral compounds are becoming increasingly important. In research and development of chiral chemicals, it is an important issue to accurately measure the optical purity of the chiral compounds and HPLC methods have been frequently used for this purpose. Many types of CSPs for HPLC have been developed [1-6] but there are still several chiral compounds that cannot be separated using commercially available CSPs. Chiral compounds having an amino group or a carboxyl group can be separated relatively easily by conventional CSPs but chiral compounds only with hydroxyl group as a functional group sometimes have difficulty in separation. Therefore, chiral separation methods after derivatization are still used for chiral alcohols. Chiral alcohols are in demand as chiral precursors in the development of medicines, agricultural chemicals etc. and supplied as chiral building blocks for the synthesis of chiral compounds. It would be effective for these researches if a novel CSP with particularly superior performance for direct enantiomeric

separation of alcohol compounds will be developed.

Maeda et al. reported a novel type of CSP using helical poly(diphenylacetylene) derivatives as chiral selectors [7,8] and chiral recognition ability toward various racemates has been evaluated. It has been shown that the enantiomeric separation ability of these CSPs varies depending on substituents of pendant phenyl rings of the helical polymer [7]. SUMICHIRAL™ OA-SHELL P1 is a new type of CSP which is coated with one of these helical poly(diphenylacetylene) derivatives as chiral selector. We have examined the chiral separation ability of OA-SHELL P1 in detail toward various racemates and found that this chiral selector has superior performance for the separation of many aromatic chiral alcohols.

Recently, superficially-porous particles so called core-shell has attracted attention as an excellent support for HPLC columns [9,10]. The most important advantages achieved by the application of core-shell particles are the significant reduction in the time of analysis and consequently in the volume of mobile phase solvents. In the

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frequently used columns such as octadecyl silylated silica gel, the core-shell support is becoming widespread. The applications of core-shell support to CSP have also been reported [11,12] but there are currently few commercially available core-shell CSPs. In OA-SHELL P1, core-shell particles are used as a support, so the reduction of chiral separation time is expected. The aims of this article are to reveal the chiral separation characteristics of OA-SHELL P1 and to propose a new option of CSP for separation of various chiral compounds.

2. Experimental

2.1. CSP

SUMICHIRAL™ OA-SHELL P1 are manufactured by Sumika Chemical Analysis Service Ltd. (Osaka, Japan). The structure of its chiral selector is shown in Fig. 1, which is coated on core-shell silica support. The particle size of the support is 2.6 µm, the pore size is 25-28 nm and the specific surface area is 40-60 m²/g. The size of the column used in this study is 3.0 mm i.d. × 150 mm.

Fig. 1. Structure of helical poly(diphenylacetylene) derivative as the chiral selector of SUMICHIRAL™ OA-SHELL P1.

2.2. Reagents and chemicals

HPLC grade *n*-hexane, 2-propanol and ethanol used as mobile phase solvents were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Among racemic compounds used as samples for the evaluation of CSP, compounds 1-3, 12-14, 16 and 19-20 in Tables were also purchased from FUJIFILM Wako Pure Chemical and compounds 4-11, 15, 17-18 and 21-26 were from Tokyo Chemical Industry (Tokyo, Japan). (S)-1-phenylethanol for the evaluation of elution order and trifluoroacetic acid were also purchased from FUJIFILM Wako Pure Chemical. Sample solutions for evaluation of CSP were prepared by dissolving these racemic compounds in 2-propanol at suitable concentration for each (about 1-10 mg/mL) and the injection volume to HPLC was 1-10 µL.

2.3. Equipment and conditions

Chromatographic separations were carried out using a HITACHI L-6000 HPLC system and L-4000 UV detector (Hitachi High-Tech Science, Tokyo, Japan) with data processing software were used. Normal phase mode was

adopted for separation and the composition of mobile phase is described in the caption of each Table and Figure. The mobile phases were degassed by ultra-sonication under vacuum for 10 min before analyses. The wavelength of an UV detector was set up to 254 nm. All analyses were carried out at room temperature (about 25 °C) and the flow rate of mobile phase was adjusted to 0.2 mL/min.

2.4. Evaluation of enantioselectivity

The performance of CSP was evaluated from retention factors and separation factors of various samples. The retention factor (*k'*) was calculated according to the equation $k' = (t_r - t_0) / t_0$, where *t*₀ is hold up time and *t*_r is each retention time. The enantiomeric separation factor (α) was calculated using $\alpha = k'_2 / k'_1$, where *k'*₁ and *k'*₂ are the retention factors of the first and second enantiomers respectively.

3. Results and discussion

3.1. Difference in enantioselectivity by functional groups of analytes

Enantioselectivity of OA-SHELL P1 toward racemates of several ethylbenzene derivatives is summarized in Table 1. These test compounds have the same structure except functional groups and the enantioselectivity greatly varied depending on the functional groups. It showed enantiomeric separation ability for alcohol and carboxylic acid, but no separation was observed for other compounds. The retention time of ester and hydrocarbon was very short even when the 2-propanol concentration in mobile phase was lowered to 1 %, while that of 1-phenylethylamine was too long. OA-SHELL-P1 was not regarded as a suitable CSP for these compounds. A very large separation factor was observed for 1-phenylethanol among these compounds, suggesting that OA-SHELL P1 can be a new effective option of CSP for the separation of chiral alcohols.

Table 1. Enantioselectivity of OA-SHELL P1 toward racemates of ethylbenzene derivatives with different functional group.

No	Compound name (Racemate)	Substituent (R)	<i>k'</i> ₁	α
1	1-phenylethanol	-OH	1.57	1.60
2	2-phenylpropionic acid	-COOH	5.21	1.24
3	α -methylbenzyl acetate	-OCOCH ₃	0.58	1.00
4	2-phenylpropionitrile	-CN	1.70	1.00
5	1-phenylethylamine	-NH ₂	11.48	1.00
6	sec-butylbenzene	-CH ₂ CH ₃	0.17	1.00

Mobile phases : *n*-hexane/2-propanol (95/5, v/v) for 1, *n*-hexane/2-propanol (99/1, v/v) for 3, 4, 6, *n*-hexane/2-propanol/trifluoroacetic acid (99/1/0.1, v/v) for 2, *n*-hexane/2-propanol/trifluoroacetic acid (90/10/0.1, v/v), for 5.

3.2. Enantiomeric separation of alcohol racemates

In order to clarify the enantiomeric separation of OA-SHELL P1 in detail, various alcohol racemates having different structure were used to evaluate the performances. These results are shown in Table 2.

Table 2. Retention factors of the first enantiomer (k'_1) and enantiomeric separation factors (α) for aromatic alcohol racemates on OA-SHELL P1.

No	Compound name (Racemate)	Structure	k'_1	α
1	1-phenylethanol		1.57	1.60
7	1-phenyl-1-propanol		1.51	1.62
8	1-phenyl-1-butanol		1.65	1.17
9	1-phenyl-1-pentanol		1.40	1.25
10	1-phenyl-2-propanol		1.24	1.44
11	1-(p-tolyl)ethanol		1.14	1.21
12	1-(4-chlorophenyl)ethanol		1.70	1.25
13	1-(2-chlorophenyl)ethanol		1.35	1.43
14	1-(3-chlorophenyl)ethanol		1.87	1.87
15	trans-2-phenyl-1-cyclohexanol		1.42	3.55
16	1-(1-naphthyl)ethanol		2.03	1.30
17	1-phenyl-3-butene-1-ol		1.89	1.55
18	2-phenyl-4-pentene-2-ol		0.81	1.26
19	2-phenyl-2-butanol		0.80	1.42
20	2-phenyl-1-propanol		2.08	1.21
21	2-phenoxypropanol		2.30	1.14

Mobile phase: *n*-hexane/2-propanol (95/5, v/v) for all samples.

All racemates in Table 2 were separated with relatively high separation factor. The separation was performed in normal phase mode and the mobile phases were mixtures of *n*-hexane and 2-propanol for all samples. It can be said that OA-SHELL P1 has excellent separation ability for a wide range of aromatic chiral alcohols with a simple mobile phase composition. As a result of examining the elution order of compound 1, (S)-enantiomer was eluted first. Typical chromatograms are shown in Fig. 2. For neutral compounds such as alcohols, it was not necessary to add other modifiers in mobile phase and good peak shape was obtained. The support of OA-SHELL P1 was core-shell silica with a particle size of 2.6 μm and analysis was done by conventional HPLC without using ultra high-performance liquid chromatography, but the analysis time was within about 10-15 minutes.

Fig. 2. Typical chromatograms of enantiomeric separation for chiral secondary alcohols using OA-SHELL P1. (a) 1-(1-naphthyl)ethanol, (b) 1-phenyl-2-propanol. Mobile phase is *n*-hexane/2-propanol (95/5, v/v) for both samples. Retention times of each enantiomer are indicated on the peak tops.

Compounds 1 and 7-17 are chiral secondary alcohols which hydroxyl groups bond to asymmetric carbon directly. High separation factors were obtained for most of these racemates. In these compounds, as the substituent of the asymmetric carbon becomes larger, the separation factor tends to decrease and this phenomenon was different from many conventional chiral stationary phases. Compounds 18 and 19 were tertiary alcohols and they also showed good separation although the separation factors were smaller than those of secondary alcohols having a similar structure. Compounds 20 and 21 are primary alcohols which hydroxyl group is not bonded to the asymmetric carbon and the β -carbon of hydroxyl group is asymmetric, OA-SHELL P1

also showed separation ability for these chiral primary alcohols. OA-SHELL P1 is generally considered to have excellent enantiomeric separation ability for various aromatic alcohols regardless of their structures.

3.3. Enantiomeric separation of racemates other than aromatic alcohol

Performance evaluation results for chiral compounds other than aromatic alcohol are shown in the Table 3. As shown in the Table 1, OA-SHELL P1 also has enantiomeric separation ability for carboxylic acid in addition to alcohols. It showed good separation toward *N*-protected amino acids where the carboxyl group is directly bonded to the asymmetric carbon, such as *N*-benzoyl and *N*-acetyl derivatives. A small amount of trifluoroacetic acid was added to the mobile phase for the separation of compounds having a carboxyl group. OA-SHELL P1 was also effective for separation of some chiral carbonyl compounds and lactones.

Table 3. Enantioselectivity of OA-SHELL P1 toward several chiral ethylbenzene derivatives.

No	Compound name (Racemate)	Structure	k'_1	α
22	<i>N</i> -benzoylleucine		1.27	1.49
23	<i>N</i> -acetylleucine		4.29	1.22
24	<i>trans</i> -stylobenoxide		4.24	1.35
25	pantolactone		4.22	1.34
26	2-phenylcyclohexanone		2.31	1.53

Mobile phases ; *n*-hexane/ethanol/trifluoroacetic acid (90/10/0.1, v/v) for 22, *n*-hexane/ethanol/trifluoroacetic acid (95/5/0.02, v/v) for 23, *n*-hexane/2-propanol (100/0.02, v/v) for 24, *n*-hexane/2-propanol (95/5, v/v) for 25, *n*-hexane/2-propanol (99/1, v/v) for 26.

3.4. Development of chiral separation method using OA-SHELL P1

OA-SHELL P1 is used in normal phase mode with a mobile phase of simple composition and one of its feature is easy method development. The general method development procedure is as follows. First, the standard mobile phase for neutral compounds such as aromatic alcohols is *n*-hexane/2-propanol (95/5, v/v) as shown in the Table 2. If retention is too slow or too fast, adjust the retention time by changing the concentration of 2-propanol. It is not usually necessary to add other modifiers for neutral

compounds. Using a 2-propanol-added mobile phase, high separation factor was obtained for many chiral aromatic alcohols, but when the separation factor is very large, peak tailing of later eluted enantiomer may increase such as compound 15 in Fig. 3 (a). In such a case, when 2-propanol was changed to ethanol, the peak shape was improved shown in Fig. 3 (b) although the separation factor was decreased.

Fig. 3. Chromatograms of enantiomeric separation of *trans*-2-phenyl-1-cyclohexanol using OA-SHELL P1. Mobile phases were (a) *n*-hexane/2-propanol (95/5, v/v), (b) *n*-hexane/ethanol (95/5, v/v). Retention times of each enantiomer are indicated on the peak tops.

When analyzing acidic compounds such as carboxylic acids, a small amount of trifluoroacetic acid was added. Since normal phase mode is used, it is expected to be applied to methods using supercritical fluid chromatography. When using a column with an inner diameter of 3 mm, the flow rate was suitably about 0.2 mL/min and the column pressure in that case was about 3 MPa.

3.5. Column durability of OA-SHELL P1

The durability of OA-SHELL P1 was evaluated using *trans*-2-phenyl-1-cyclohexanol (compound 15) as test sample at the concentration of 10 mg/mL in ethanol. The mobile phase, *n*-hexane/ethanol (95/5, v/v), was fed to the column continuously at the flow rate of 0.2 mL/min and the sample solution was injected after suitable time period. The results are shown in Fig. 3 by plotting retention factor of first enantiomer and separation factor. The retention parameter and separation ability did not change and the performance of OA-SHELL P1 was maintained until the washing time reaches 220 hours. The column pressure also did not change during the test period. Chromatograms before and after washing with mobile phase are shown in Fig. 4 and degradation of peak shape was not observed. OA-SHELL P1 has practically enough stability despite the

use of helical polymers as chiral selectors.

Fig. 4. Retention factor and Separation factor obtained from column durability test of OA-SHELL P1. Test sample was *trans*-2-phenyl-1-cyclohexanol and mobile phase was *n*-hexane/ethanol (95/5, v/v).

4. Conclusions

SUMICHIRAL™ OA-SHELL P1 showed excellent enantiomeric separation ability for a wide range of aromatic chiral alcohols. It was also effective for some chiral carboxylic acids, ketones and lactones. OA-SHELL P1 can also be a new option of CSP for these compounds. Its practical advantages are the simple analytical method development and the short analysis time of about 10 to 15 minutes.

From the viewpoint of CSP selection, it can be said that aromatic chiral compounds having hydroxyl groups directly bonded to asymmetric carbon can be separated with high probability by using OA-SHELL P1. It is well known that ligand exchange type CSP is effective for amino acids and hydroxyl acids [6,13]. We previously reported a particularly effective CSP for the separation of aromatic amines [14]. In this way, it can be said that selecting CSPs focusing on the functional group in the vicinity of asymmetric carbon is an effective way of thinking.

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