Chromatography **2016**, *37*, 65-71

Original Paper

Enantiomeric Separation of Chiral Amines and Amino Alcohols Using Acetylated β-Cyclodextrin Stationary Phase by High-Performance Liquid Chromatography

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Abstract

Enantiomeric separation ability on high-performance liquid chromatography (HPLC) was compared between native and acetylated β-cyclodextrin stationary phases. In the chiral stationary phases (CSP) used in this work, β-cyclodextrin is chemically bonded to aminopropyl silica gel *via* sugar chain spacer. The acetylated β-cyclodextrin phase (SUMICHIRAL™ OA-7700) exhibits excellent performance for the separation of chiral amines and amino alcohols in reversed phase mode. Conventional phosphate buffer can be used in mobile phase without special modifier and the analytical method is easy to develop. OA-7700 is very useful practically for the determination of optical purity of many important chiral amines. It can separate enantiomers of 1-phenyl-2-(*p*-tolyl)ethylamine (PTE) which is used for industrial scale resolution of chrysanthemic acids and norephedrin (phenylpropanolamine) which is regulated as stimulant raw material.

Keywords: Enantiomeric separation; Chiral stationary phase; Acetylated β-cyclodextrin; Chiral amine; Chiral amino alcohol

1. Introduction

Many cyclodextrin-based CSPs for HPLC have been reported [1-8], and they have shown abilities to separate enantiomers of many chiral compounds, especially for neutral chiral molecules with aromatic units, but hardly reported for chiral amines. We have reported chiral stationary phase bonded with β-cyclodextrin to the silica gel *via* new type of spacer which contains sugar chain (Fig. 1, SUMICHIRAL™ OA-7000) [9]. In separating enantiomers using OA-7000, sharp peaks are obtained compared to the conventional β-cyclodextrin stationary phases which have alkyl chain as spacer. We speculated that it was due to the effect of hydrophilic spacer moiety which prevented secondary interactions between the silica gel and the sample molecules. OA-7000 can separate many acidic and neutral chiral compounds in reversed phase mode. Cyclodextrin-based CSPs are also useful for determination of not only enantiomers but also geometrical isomers. Many chiral and achiral analytical methods using OA-7000 were reported, for analytes such as episesamin monocatechol epimer [10], isoflavan derivatives [11], epicatechin [12], glabridin [13] and fluorous-tag compounds [14], but OA-7000 does not have the enough performance for separation of chiral amines and amino alcohols.

Chiral amines are very important compounds industrially, for example, they are used as medicine chemical intermediates and chiral reagents for diastereomeric salt formation method. Accurate and simple methods by HPLC for determination of enantiomeric excess of chiral amines are therefore required. The performances of acetylated β-cyclodextrin stationary phases for several chiral compounds were investigated [7-8], but to our knowledge, systematic evaluation for chiral amines has not been reported. In this study, we payed attention to the acetylated β-cyclodextrin stationary phase (SUMICHIRAL™ OA-7700) which has the same skeletal formula as OA-7000 and the enantioselectivities of native and acetylated β-cyclodextrin stationary phases for many chiral amines were compared. We found that the acetylated β-cyclodextrin phase has superior performance for the separation of chiral amines.

The aims of this article are to describe chromatographic details of the separation for chiral amines by the acetylated

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Received: 29 October 2015
Accepted: 2 February 2016
J-STAGE Advance Published: 10 February 2016
DOI: 10.15583/jpchrom.2015.036

β-cyclodextrin phase and to propose a new option of CSP for chiral amines and amino alcohols.

2. Experimental

2.1. CSPs

Cyclodextrin-bonded phases, SUMICHIRAL™ OA-7000 and OA-7700 were used which are manufactured by Sumika Chemical Analysis Service Ltd. (Osaka, Japan). The chiral selector of OA-7000 are the native β-cyclodextrin bonded to aminopropyl silicagel *via* α-D-glucopyranosyl-(4→1)-*O*-α-D-glucopyranosiduronic acid (Fig. 1). SUMICHIRAL™ OA-7700 is prepared by acetylating the hydroxyl groups of OA-7000, its spacer is the same skeletal formula as OA-7000. The particle size of packing materials is 5 μm and the column dimension is 250 mm × 4.6 mm i.d.

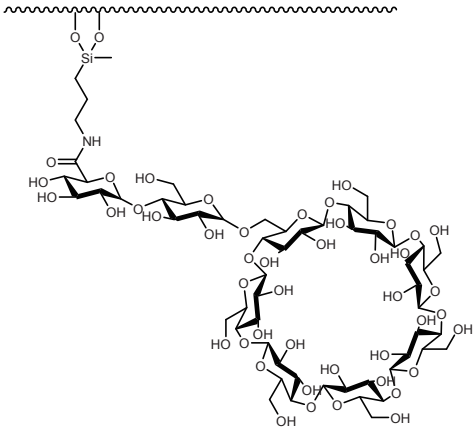


Fig. 1. Structure of stationary phase of SUMICHIRAL™ OA-7000 (native β-cyclodextrin) [9]. SUMICHIRAL™ OA-7700 has the same skeletal formula as OA-7000, but the hydroxyl groups are acetylated.

2.2. Reagents and chemicals

Methanol and acetonitrile were HPLC grade from Wako Pure Chemicals (Osaka, Japan). Among racemic compounds shown in Table 2 used as samples for the evaluation of CSPs, compounds 8, 12, 14, 16 and 18 were purchased from Wako Pure Chemicals, compound 3, 4, 7, 15, and 19 - 23 were from Tokyo Kasei (Tokyo, Japan) and all other compounds in Table 2 were from Sigma-Aldrich Japan (Tokyo, Japan). Ammonium acetate (NH_4Ac), potassium dihydrogenphosphate (KH_2PO_4), phosphoric acid and other reagents were all analytical reagent grade, also purchased from Wako Pure Chemicals. Compound 7, norephedrin (phenylpropanolamine) has been appointed to stimulant raw materials, it was treated under compliance according to the law of Japan.

Sample solutions for evaluation of CSPs were prepared by dissolving each racemic compounds in methanol or water at suitable concentration for each compound (about 1 - 10 mg/mL) and the injection volume to HPLC was 1 - 10 μL. 20 mmol/L phosphate buffer (pH 3.0) for mobile phase was prepared by adding phosphoric acid into 20 mmol/L KH_2PO_4 water solution in order to adjust the pH to 3.0.

2.3. Equipment

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. The mobile phases were degassed by ultra-sonication under vacuum for 10 min. The wavelength of an UV detector was set up to 254 nm or 210 nm. All analyses were carried out at room temperature (about 25ºC) and the flow rate of mobile phase was adjusted to 0.5 mL/min.

2.4. Evaluation of CSPs

The performance of each CSP was evaluated in reversed phase mode using mobile phase described in Table 2. 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. Comparison of two CSPs

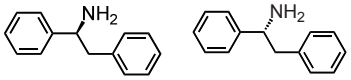
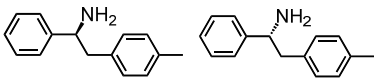
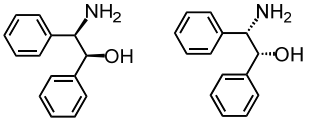
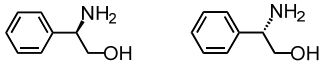
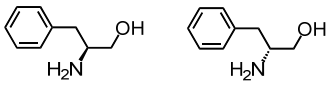
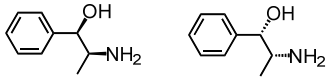
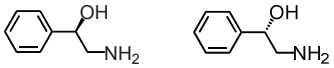
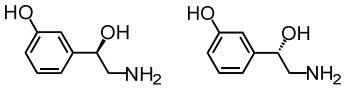
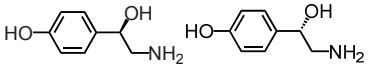
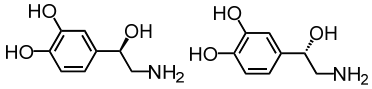
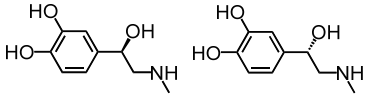
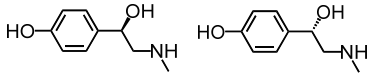
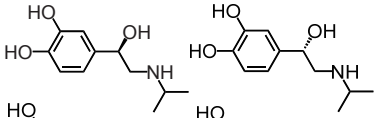
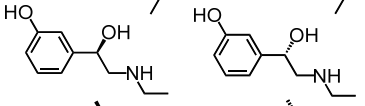
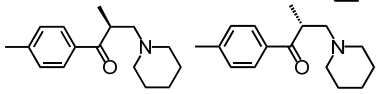
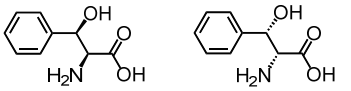
The performance of each CSP for chiral amines is summarized in Table 1, retention factor (k') and enantiomeric separation factor (α) of each chiral compound are shown in Table 2. Compounds 1-11 in Table 2 are primary amines and amino alcohols, all of these eleven compounds can be separated at $\alpha > 1.1$ by OA-7700, whereas OA-7000 can separate only three compounds. For secondary amines (compounds 12-15) and amino acids (compounds 17-19), OA-7700 also exhibited superior performance. On the other hand, OA-7000 which is native β-cyclodextrin phase is effective for separation of neutral chiral compounds, especially flavanone (compound 21) enantiomers were separated at large separation factor ($\alpha > 2.2$) by OA-7000, but OA-7700 could not separate them at all. OA-7000 has been adopted for a lot of determination methods for flavonoid derivatives [10-12].

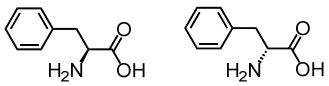
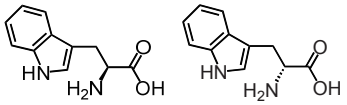
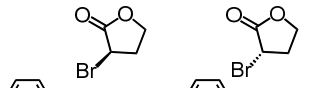
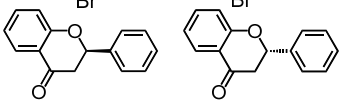
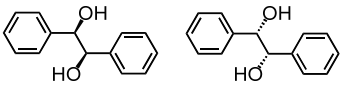
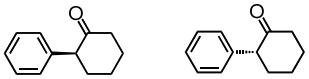
Table 1. Summary of the number of enantiomeric separated compounds at $\alpha > 1.1$ on each CSP.[a]

Chiral compounds	CSP (SUMICHIRAL™)	
	OA-7000	OA-7700
primary amines [b]	3/11	11/11
secondary amines [b]	0/4	4/4
amino acids	0/3	1/3

[a] Number of separated compounds / investigated compounds.
[b] Including amino alcohols.

Table 2. Retention factor (k') and enantiomeric separation factor (α) of chiral compounds on SUMICHIRAL™ OA-7000 and OA-7700 in reversed phase mode [a]

No	Compound name	Structure (racemates)	CSP SUMICHIRAL™	k'$_1$ 1st peak [b]	α	Mobile phase [c] (v/v)
1	1-phenylethylamine		OA-7000 OA-7700	0.07 1.31	1.00 1.21	Phos.Buf./methanol (98/2)
2	1,2-diphenylethylamine		OA-7000 OA-7700	0.44 2.98	1.18 1.64	Phos.Buf./methanol (60/40)
3	1-phenyl-2-(*p*-tolyl)ethylamine (PTE)		OA-7000 OA-7700	0.77 3.50	1.10 1.34	Phos.Buf./methanol (60/40)
4	(*RS/SR*)-2-amino-1,2-diphenylethanol		OA-7000 OA-7700	0.59 3.68	1.20 1.81	Phos.Buf./methanol (95/5)
5	phenylglycinol		OA-7000 OA-7700	0.03 0.67	1.00 1.29	Phos.Buf.
6	phenylalaninol		OA-7000 OA-7700	0.68 5.18	1.00 1.11	Phos.Buf.
7	(*RS/SR*)-norephedrine		OA-7000 OA-7700	0.58 3.52	1.00 1.24	Phos.Buf./methanol (98/2)
8	2-amino-1-phenylethanol		OA-7000 OA-7700	0.27 2.16	1.00 1.23	Phos.Buf.
9	norphenylephrine		OA-7000 OA-7700	0.57 2.71	1.00 1.43	20 mmol/L KH_2PO_4
10	octopamine		OA-7000 OA-7700	0.78 1.53	1.00 1.21	20 mmol/L KH_2PO_4
11	noradrenaline		OA-7000 OA-7700	0.35 0.69	1.00 1.38	20 mmol/L KH_2PO_4
12	adrenaline		OA-7000 OA-7700	0.48 0.98	1.00 1.32	20 mmol/L KH_2PO_4
13	synephrine		OA-7000 OA-7700	0.94 2.05	1.00 1.21	20 mmol/L KH_2PO_4
14	isoproterenol		OA-7000 OA-7700	0.62 1.23	1.00 1.27	20 mmol/L KH_2PO_4
15	etilefrine		OA-7000 OA-7700	2.03 4.27	1.05 1.49	20 mmol/L KH_2PO_4
16	tolperison		OA-7000 OA-7700	2.00 3.83	1.26 1.00	20 mmol/L NH_4Ac /methanol (40/60)
17	(*RS/SR*)-3-phenylserine		OA-7000 OA-7700	0.44 0.85	1.00 1.00	20 mmol/L KH_2PO_4

No	Compound name	Structure (racemates)	CSP SUMICHIRAL™	k'1 1st peak b	α	Mobile phase (v/v)
18	phenylalanine		OA-7000 OA-7700	0.92 2.94	1.00 1.07	20 mmol/L KH_2PO_4
19	tryptophan		OA-7000 OA-7700	1.29 5.03	1.00 1.13	20 mmol/L KH_2PO_4
20	α-bromo-γ-butyrolactone		OA-7000 OA-7700	0.58 4.37	1.00 1.06	water/methanol (50/50)
21	flavanone		OA-7000 OA-7700	0.64 1.39	2.22 1.00	water/acetonitrile (60/40)
22	(*RR/SS*)-hydrobenzoin		OA-7000 OA-7700	4.58 6.29	1.28 1.40	water/methanol (70/30)
23	1-phenylethanol		OA-7000 OA-7700	1.76 2.57	1.00 1.00	water/acetonitrile (80/20)
24	2-phenylcyclohexanone		OA-7000 OA-7700	2.59 2.75	1.00 1.00	water/methanol (50/50)

[a] All analyses were monitored by UV detection at 254 nm.
[b] k'1 is the retention factor of the first elution peak of enantiomers.
[c] "Phos.Buf." is 20 mmol/L phosphate buffer (pH3.0).

The difference of chemical structure between OA-7000 and OA-7700 is only with and without acetylation of their hydroxyl groups, but the enantioselectivity of each CSP is remarkably different. These results indicate that the derivatization of hydroxyl groups of cyclodextrin plays an important role in the chiral recognition. Host-guest interaction of acetylated β-cyclodextrin for amino compounds is supposed to be stronger than that of native β-cyclodextrin, because the retention time of amino compounds obtained with OA-7700 tends to be longer than OA-7000. It is inferred that the hydrogen bond between acetyl groups of stationary phase and amino groups of eluents potentiate the host-guest interaction of cyclodextrin, but further examination will be needed for the explanation of diastereomeric recognition mechanisms by cyclodextrin phases.

3.2. Enantiomeric separation of primary amines

Compounds 1-3 in Table 2 are hydrophobic primary amines, OA-7700 can separate the enantiomers of these three compounds at α > 1.2. Acidic phosphate buffer and methanol mixture was used as mobile phase and typical chromatograms are shown in Fig. 2.

Enantiomers which have only one primary amine and no other functional group are often difficult to separate directly. CSP including crown ether derivatives as chiral selector is an important choice for separation of these primary amines [15-17]. Crown ether-type CSPs can be used both in reversed phase and normal phase, but in reversed phase mode it is often necessary to contain perchloric acid or sulfuric acid in mobile phase, while cyclodextrin-type CSP

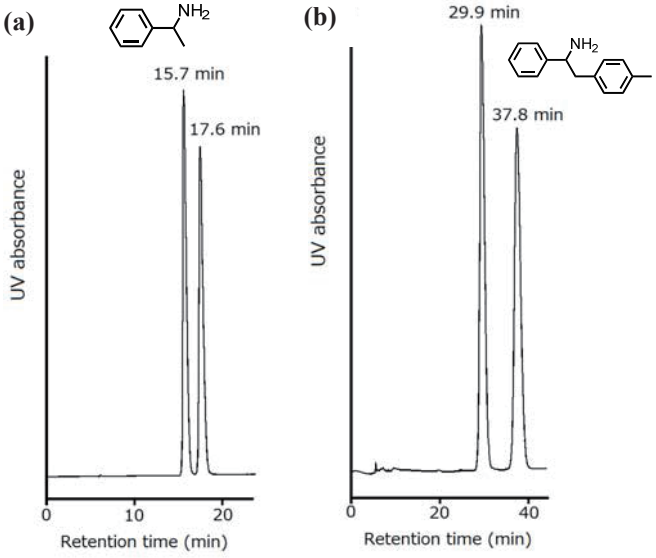


Fig. 2. Typical chromatograms of enantiomeric separation for chiral primary amines using OA-7700. (a) 1-Phenylethylamine, mobile phase: 20 mmol/L phosphate buffer (pH3.0) / methanol (98/2), (b)1-phenyl-2-(*p*-tolyl)ethylamine (PTE), mobile phase: 20 mmol/L phosphate buffer (pH3.0) / methanol (60/40). Both samples are dissolved in methanol at 2 mg/mL and the wavelength of UV detector was 210 nm. Retention times of each enantiomer are indicated on the peak tops.

can be used at reversed mobile phase with phosphate or acetate buffer. These conventional conditions of mobile phase resemble those of octadecylsilanized silica column which are familiar to many chromatographer and it is relatively easy to develop the determination methods using OA-7700.

1-Phenyl-2-(*p*-tolyl)-ethylamine (PTE) is one of the chiral reagents for diastereomeric salt formation method which is used for industrial scale resolution of chrysanthemic acid [18] and it is necessary to determine the optical purity as chiral reagent. In chromatogram obtained using OA-7700 for separation of PTE shown in Fig. 2, baseline resolution is observed and OA-7700 is very useful for this purpose.

3.3. Enantiomeric separation of amino alcohols

Compounds 4-15 in Table 2 are primary and secondary amines with hydroxyl group, these amino alcohols are physiologically and pharmacologically important substances such as catecholamines. OA-7700 can separate all enantiomers of these twelve compounds, whereas OA-7000 can separate only two compounds at $\alpha > 1.05$. An example chromatogram for separation of norephedrin (compound 7) is shown in Fig. 3 and baseline separation was achieved. We have also investigated the effect of pH of phosphate buffer in mobile phase and Fig. 4 indicated the data at pH 3 to 7. Along with an increase of pH value, the retention time became longer. The pH value in mobile phase has a great effect on retention time but no appreciable difference in separation factor was observed. The suitable retention time and shape peaks were obtained under the acidic mobile phase at pH 3.0.

Another potential choice except β-cyclodextrin phase for enantiomeric separation of amino alcohols is chiral ligand exchange phase [19], which can separate many chiral 2-aminoethanol derivatives directly. However it is necessary to add copper sulfate in mobile phase and difficult to connect directly to liquid chromatography / mass spectrometry (LC/MS).

Norephedrin is a classic precursor of stimulant materials. Determination of enantiomeric excess of chiral stimulants and nonregulated drugs is necessary for the scientific criminal investigation. Several methods for chiral separation of norephedrin were reported, such as gas chromatography [20], capillary electrophoresis [21] and normal phase HPLC [22-23]. One of the advantages of acetylated β-cyclodextrin phase is to treat under reversed phase mode, popular phosphate buffer can be used and it provides speedy and practically useful analytical methods. Enantiomers of norephedrin also separated using ammonium acetate / methanol mobile phase with nearly the same performance as phosphate buffer and it may be applicable for LC/MS methods.

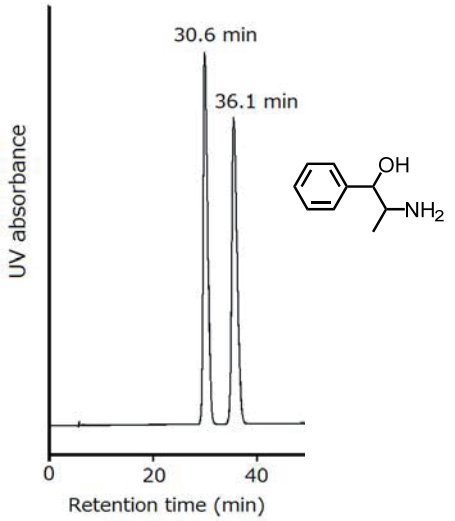


Fig. 3. Typical chromatogram of enantiomeric separation for norephedrin using OA-7700. Mobile phase: 20 mmol/L phosphate buffer (pH3.0) /methanol (98/2). Norephedrin is dissolved in methanol at 2 mg/mL and the wavelength of UV detector was 210 nm. Retention times of each enantiomer are indicated on the peak tops.

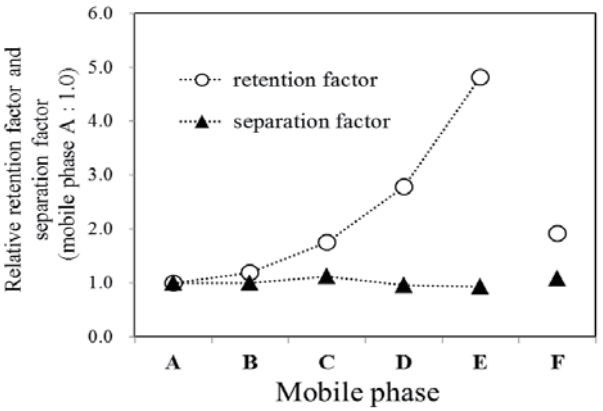


Fig. 4. Effect of pH in mobile phase on the separation of norephedrin enantiomers using OA-7700. Relative retention factors of second enantiomers and separation factors are plotted when the data from mobile phase A are 1.0. Mobile phases A to F are as follows;

A : 20 mmol/L phosphate buffer (pH3.0) / methanol (98/2)
B : 20 mmol/L phosphate buffer (pH4.0) / methanol (98/2)
C : 20 mmol/L phosphate buffer (pH5.0) / methanol (98/2)
D : 20 mmol/L phosphate buffer (pH6.0) / methanol (98/2)
E : 20 mmol/L phosphate buffer (pH7.0) / methanol (98/2)
F : 20 mmol/L ammonium acetate in water / methanol (98/2)

3.4. Column durability of acetylated β-cyclodextrin phase

The durability of OA-7700 was evaluated using etilefrine (compound 15) as model sample, which is anti-hypotensive drug. The column was washed with pH 3.0 of phosphate buffer continuously at the flow rate of 0.5 mL/min and etilefrine was determined after suitable time period. The results are shown in Fig. 5 by plotting retention factor of second enantiomer and separation factor. The retention

parameter and enantioselectivity did not change and the performance of OA-7700 were maintained until the washing time reaches at least 312 hours. Chromatograms before and after 312 hours washing with acidic mobile phase are shown in Fig. 6, degradation of peak shape is not observed. As far as column durability is concerned, OA-7700 is stable under acidic condition and it is confirmed that OA-7700 is useful practically.

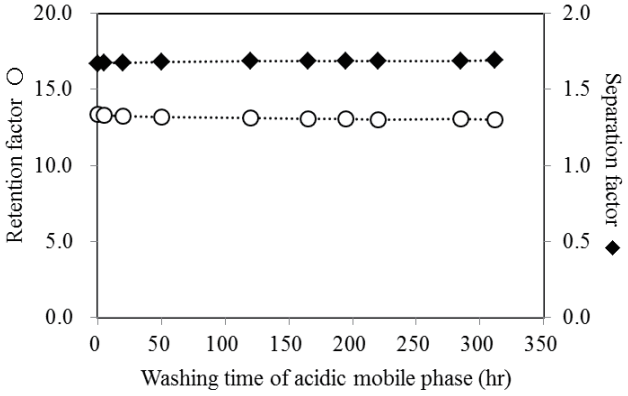


Fig. 5. Column durability of OA-7700 under acidic condition. Sample:etilefrine, Mobile phases: 20 mmol/L phosphate buffer (pH3.0) /methanol (80/20).

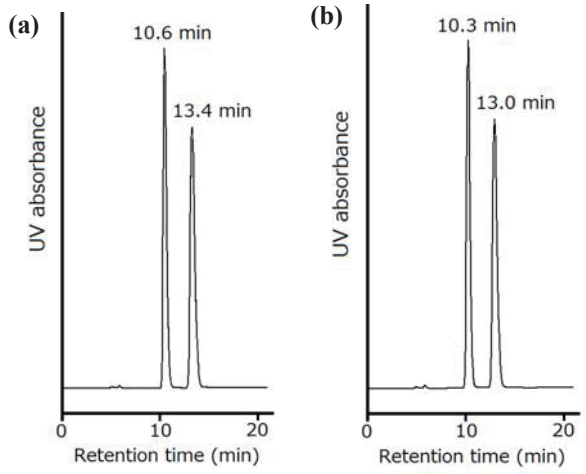


Fig. 6. Chromatograms of etilefrine enantiomers obtained during column durability test of OA-7700. (a) Initial, (b) after 312 hours washing with acidic mobile phase. Mobile phase: 20 mmol/L phosphate buffer (pH3.0) / methanol (80/20). Retention times of each enantiomer are indicated on the peak tops.

4. Conclusions

The acetylated β-cyclodextrin stationary phase, SUMICHIRAL^{TM} OA-7700 is very effective for the separation of many chiral amines and amino alcohols including physiologically and industrially important compounds compared with native β-cyclodextrin phase. It can be used in conventional reversed phase mode and the analytical methods are relatively easy to develop. OA-7700 is expected to be a new potential option of CSP for chiral amines and amino alcohols.

Acknowledgment

We gratefully acknowledge the contribution of Mr. Y. Matsumoto (Sumika Chemical Analysis Service, Ltd.) for this study.

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