Talanta 83 (2010) 286–290

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Preparation and application of rifamycin-capped (3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles as chiral stationary phase for high-performance liquid chromatography

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ARTICLE INFO

Article history:
Received 11 June 2010
Received in revised form 20 August 2010
Accepted 21 August 2010
Available online 27 August 2010

Keywords:
Chiral stationary phase
β-Cyclodextrin
Rifamycin
Chiral separation
High-performance liquid chromatography

ABSTRACT

Rifamycin-capped (3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles (RCD-HPS), a new type of substituted β-cyclodextrin-bonded chiral stationary phase (CSP) for high-performance liquid chromatography (HPLC), have been synthesized by the treatment of bromoacetate-substituted-(3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles (BACD-HPS) with rifamycin SV in anhydrous acetonitrile. The stationary phase is characterized by means of elemental analysis and Fourier-transform infrared spectroscopy. This new CSP has a chiral selector with two recognition sites: rifamycin and β-cyclodextrin (β-CD). The chromatographic behavior of RCD-HPS was studied with several disubstituted benzenes and some chiral drug compounds under reversed-phase HPLC mobile phase conditions. The results show that RCD-HPS has excellent selectivity for the separation of aromatic positional isomers and enantiomers of chiral compounds due to the cooperative functioning of rifamycin and β-CD.

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1. Introduction

Separation of chiral compounds is important in many fields [1,2] including natural products research, stereospecific organic synthesis, pharmaceutical industry and environmental studies. The development and application of new chiral stationary phases (CSPs) with high enantioselectivities to separate chiral molecules is one of the most active areas of high-performance liquid chromatography (HPLC) [2]. Many chiral separations have been successfully accomplished using β-cyclodextrin-based [3–5] silica particles as CSPs in HPLC. However, one drawback in utilizing cyclodextrins has been the low binding constants for most guest molecules [4]. In contrast, it was reported that crown ether-capped β-CDs [6] exhibited high binding constants for several guest molecules. We previously prepared a series of crown ether/cyclam-capped β-CD-bonded silica particles [7] and used as CSPs in capillary electrochromatography [1] and HPLC [8]. This type of CSPs has a chiral selector with several recognition sites: cyclodextrin, crown ether/cyclam and the latter's flexible sidearm. These CSPs showed excellent enantioselectivity for a wide range of chiral drug compounds due to the cooperative functioning of crown ethers/cyclams and β-CDs. Since rifamycin has similar cavity structure [9] and metal ion binding property [10] as crown ethers and cyclams, it is of interest to prepare rifamycin-capped β-CD-bonded silica particles to study their chiral separation selectivities when used as stationary phases in HPLC.

Rifamycin is an antibiotic macrocycle that has been used as a chiral selector in HPLC [9]. Like other antibiotic macrocycles, rifamycin contains several functional groups, e.g., phenol groups and stereogenic centres, which allow for multiple interactions with chiral solutes. Interestingly, it was reported that use of some antibiotic macrocyles as chiral selector additives for β-CD-based CSPs in HPLC exhibited better chiral separation than β-CD-based CSPs alone [11] due to the cooperative functioning of these macrocyles and β-CDs. However, many antibiotic macrocyles and their derivatives with high UV/vis absorption characteristics or poor solubility in water are not suitable to be used as mobile phase additives for direct detection [2]. Alternatively, they can be bonded onto silica support to be used as CSPs in HPLC to separate chiral compounds [2,8]. Recently, it has been reported that use of a new type of erythromycin-appended β-CD as chiral selector in capillary electrophoresis provided better enantioseparation than erythromycin or β-CD selector alone [12]. Rifamycin also showed better binding properties for many guest molecules than crown

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doi:10.1016/j.talanta.2010.08.031

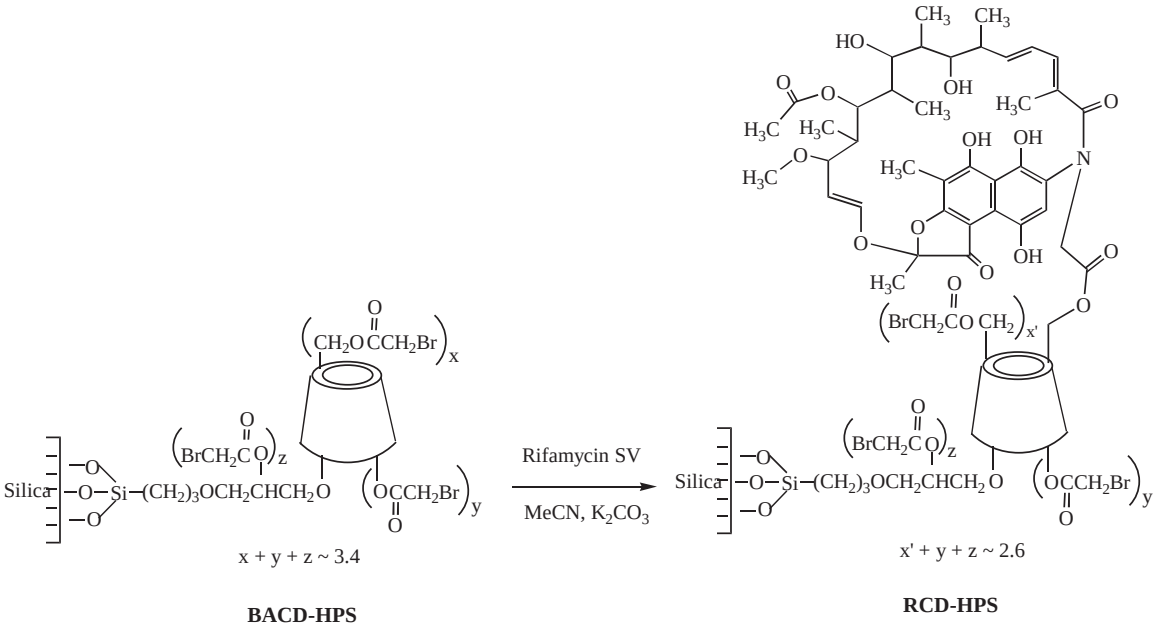

Fig. 1. Synthetic scheme for preparation of RCD-HPS.

ethers [10]. These results suggest that the development of a new type of rifamycin-capped β-CD-bonded CSPs will exhibit better chiral separation performance than the previously reported β-CD-bonded CSPs [1,11] and crown ether/cyclam-capped β-CD-bonded CSPs [2,7,8] for a wide range of chiral compounds.

In this report, we describe a convenient synthetic method to prepare rifamycin-capped (3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles (RCD-HPS) via reaction of bromoacetate-substituted-(3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles (BACD-HPS) with rifamycin SV in anhydrous acetonitrile. This is the first report of the method involving convenient successive multiple-step liquid–solid phase reactions on the silica surface to synthesize rifamycin-capped β-CD-bonded silica particles. The chromatographic performance of this new CSP is evaluated with several disubstituted benzenes and some chiral drug compounds under reversed-phase HPLC conditions.

2. Experimental

2.1. Instrumentation

HPLC was carried out on an Agilent Technologies (Waldbronn, Germany) Model 1100 system with a binary pump, an autosampler and a multiple wavelength UV detector. Chromatographic data were recorded and processed using Chemstation software (Rev. A.09.03). Elemental analysis was performed on a Perkin Elmer (Norwalk, CT, USA) 2400 elemental analyzer. A Bio-Rad (Hercules, CA, USA) Model FTS165 instrument was used for Fourier-transform infrared spectroscopic analysis.

2.2. Chemicals

Rifamycin SV was purchased from Sigma–Aldrich (St. Louis, MO, USA) and was dried in 0.1-mm Hg vacuum (1 mm Hg = 133.322 Pa) at 80 °C for 12 h. β-CD was purchased from Merck (Hohenbrum, Germany) and was dried in 0.1-mm Hg vacuum at 120 °C for 12 h. Premium Rf spherical silica gel (3 μm, 100 Å) was obtained from Sorbent Technologies (Atlanta, GA, USA). Analytical-grade sodium hydride and disubstituted benzene derivatives were all purchased from Fluka (Buchs, Switzerland). Analytical-grade acetic acid, triethylamine (TEA), and chiral drug compounds including 2-amino-1,2-diphenylethanol, benzyl mandelate, dichloroprop, indapamide, indoprofen, ketoprofen, labetalol, metoprolol, α-methylbenzylamine, 1-(1-naphthyl)ethylamine, 1-phenyl-1-propanol, pindolol, propanolol, proglumide, tyrosine, and warfarin were obtained from Sigma–Aldrich. 3-Glycidoxypropyltrimethoxysilane (97%) and HPLC-grade solvents methanol, acetonitrile, and acetone were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Milli-Q (Bedford, MA, USA) water purification system.

2.3. Preparation of bonded silica particles RCD-HPS

The starting material BACD-HPS was prepared according to our previous reports [1,7]. The amount of anchored β-CD and substituted bromoacetate moieties in the BACD-HPS were 142 μmol g^{-1} and 482 μmol g^{-1}, respectively, as determined by elemental analysis. The degree of substitution of bromoacetate was calculated to be 3.4. The RCD-HPS particles were synthesized by treatment of BACD-HPS with excess rifamycin SV in anhydrous acetonitrile (MeCN) in the presence of potassium carbonate. The synthetic routine is shown in Fig. 1. Typically, 1.36 mmol (0.98 g) of rifamycin SV, which has been dried in vacuum at 80 °C for 12 h, was resolved in 50 mL anhydrous acetonitrile containing 10 mg of potassium carbonate. Then, 2 g of BACD-HPS, which has been dried in vacuum at 60 °C for 12 h, was added to the reaction mixture and the mixture was refluxed for 24 h under dry nitrogen gas. The RCD-HPS was filtered, washed successively with acetonitrile, acetone, water, and methanol, purified by Soxhlet extraction with acetone overnight and dried for 6 h under vacuum at 60 °C. An elemental analysis of C, 12.42%; H, 2.73%; gave the amount of rifamycin moieties in the bonded silica as 107 μmol g^{-1} in RCD-HPS. Accordingly, the degree of substitution was calculated as 0.8. The Fourier-transform infrared spectroscopic (FTIR) spectrum of the RCD-HPS particles showed a weak broad band at 3400–3600 cm^{-1} that is characteristic of the silanol stretching frequency for silica. It exhibited bands at 2850 cm^{-1} and 2925 cm^{-1} for the C–H aliphatic CH_2 stretching fre-

Table 1
Retention factors (k') for disubstitued benzenes on the RCD-HPS-packed column.

Solutes	Methanol:water (v/v)[a]				
	100:0	80:20	60:40	40:60	20:80
o-Nitroaniline	0.26	0.30	0.33	0.81	2.09
m-Nitroaniline	0.25	0.31	0.33	0.69	1.58
p-Nitroaniline	0.34	0.45	0.62	1.77	4.80
o-Nitrophenol	0.20	0.28	0.31	0.74	1.84
m-Nitrophenol	0.21	0.29	0.33	0.92	2.50
p-Nitrophenol	0.25	0.40	0.48	1.39	3.58

[a] Conditions: 150 mm × 2.0 mm I.D. stainless steel column packed with 3 μm RCD-HPS particles; 0.12 mL/min mobile phase flow rate; UV detection at 254 nm.

quencies. It also showed a band at 1725 cm^{-1} for the C=O stretching frequency and bands at 1505–1545 cm^{-1} for the aromatic C=C stretching frequencies for rifamycin moiety. This confirmed that the rifamycin was successfully introduced to the RCD-HPS. Since rifamycin only has one secondary acyl-amine (–CO–NH–) group which is more reactive than other functional groups, the rifamycin therefore is attached to the β-CD in RCD-HPS via the reaction of the acyl-amine group with the bromoacetate of the BACD-HPS, similar to the previously reported crown ether/cyclam-capped β-CD-bonded silica particles [7]. The typical structure of RCD-HPS is shown in Fig. 1.

2.4. Chromatographic procedures

The bonded silica RCD-HPS particles were packed into a 150 mm × 2.0 mm I.D. stainless steel column (Phenomenex, Torrance, CA, USA) by a modified balance-density slurry technique [1,8]. The mobile phases used were mixtures of methanol/water, acetonitrile/water, or acetonitrile/triethylamine–acetate (TEAA) buffer solutions, by volume ratios. The triethylamine–acetate buffer was prepared as reported [13,14] by dissolving the desired amount of pure triethylamine in water to achieve a 1% concentration and then adding glacial acetic acid to achieve the required pH. The samples were prepared by dissolving in the mobile phases and the concentrations were approximately 0.5–5 mmol/L. The volume injected was 5–10 μL. Chromatography was carried out at room temperature. The baseline perturbation resulting from injection of the mobile phase was used as void volume marker (t_0). Supporting evidence for chiral separation was supplied by the separation with detection at different UV wavelengths (e.g., 210 nm, 230 nm, 254 nm, 280 nm, etc.). All the reported retention data were based on at least duplicate determinations.

3. Results and discussion

3.1. Evaluation of the RCD-HPS-packed column using disubstituted benzenes

The chromatographic performance of RCD-HPS was investigated using mixtures of methanol/water as mobile phases and nitrophenols and nitroanilines as solutes. The influence of methanol content in the mobile phase on the retention behavior of solutes is shown in Table 1. It can be seen that the retention (k') of all solutes on the RCD-HPS-packed column generally increased when methanol content decreased, demonstrating that the RCD-HPS had some hydrophobic interaction with solutes. As shown in Table 1, *p*-nitroaniline always elutes last in the RCD-HPS-packed column. This suggests that the formation of an inclusion complex between the solute and the β-CD is important in the separation mechanism [13]. This is because of the larger binding constant of *p*-nitroaniline that results from its linear geometry, which allows optimal penetration into the β-CD cavity to form a more stable inclusion complex as previously reported in literatures [1,8,15].

Baseline separations of all the positional isomers of the nitrophenols and nitroanilines were achieved on the RCD-HPS-packed column. Typical chromatogram depicting the separation of the isomers of *o*-, *m*-, *p*-nitrophenol is shown in Fig. 2. As shown in Fig. 2, the elution order of nitrophenol isomers on RCD-HPS-packed column was $o < m < p$ when using mixture of methanol/water (20:80, v/v) as mobile phase. The elution order was different from that when using an octadecyl-packed column [16], which was $p < m < o$. Under the same mobile phase conditions, the elution order of nitrophenol isomers on RCD-HPS ($o < m < p$) was also different from that on BACD-HPS ($m < o < p$) which was the starting material to prepare RCD-HPS [1,13]. This indicates that the rifamycin in the new CSP has

Table 2
Typical enantioseparation data for chiral compounds on RCD-HPS column.

Solutes	Mobile phase[a]	Separation data[b]		
		k_1'	α	R_S
1-(1-Naphthyl)ethylamine	Methanol/water (5:95, v/v)	1.26	1.36	1.27
1-Phenyl-1-propanol	Methanol/water (5:95, v/v)	2.03	1.87	2.83
Indapamide	Methanol/water (10:90, v/v)	3.05	1.18	1.09
Warfarin	Methanol/water (20:80, v/v)	5.45	1.22	1.21
Labetalol	Methanol/water (40:60, v/v)	1.48	1.10	1.03
Propanolol	Methanol/water (40:60, v/v)	1.16	1.18	0.87
Tyrosine	Methanol/water (40:60, v/v)	0.24	2.47	1.25
Metoprolol	Acetonitrile/water (20:80, v/v)	0.75	2.03	1.21
2-Amino-1,2-diphenylethanol	Acetonitrile/TEAA, pH 4 (10:90 v/v)	0.30	2.46	2.50
Benzyl mandelate	Acetonitrile/TEAA, pH 4 (10:90, v/v)	2.13	1.16	0.69
Dichloroprop	Acetonitrile/TEAA, pH 7 (5:95, v/v)	0.75	1.61	1.52
α-Methylbenzylamine	Acetonitrile/TEAA, pH 7 (5:95, v/v)	0.37	3.48	3.36
Proglumide	Acetonitrile/TEAA, pH 7 (5:95,v/v)	0.65	1.39	1.03
Indoprofen	Acetonitrile/TEAA, pH 7 (10:90, v/v)	1.32	1.18	1.06
Ketoprofen	Acetonitrile/TEAA, pH 7 (10:90, v/v)	1.40	1.08	0.75
Pindolol	Acetonitrile/TEAA, pH 7 (10:90, v/v)	0.34	1.64	1.75

[a] TEAA = 1% triethylamine–acetate buffer.
[b] k_1' is the retention factor for the enantiomer eluted out first; α is the selectivity; R_S is the resolution.

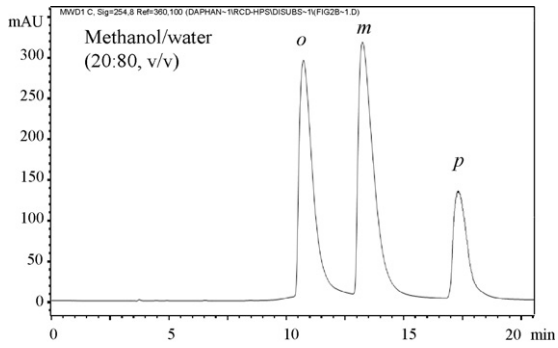

Fig. 2. Typical chromatogram for separation of positional isomers of *o*-, *m*- and *p*-nitrophenol.

an important contribution to the separation. It also suggests that some extra interactions, e.g., hydrogen bonding and π–π interactions [9,10], are imparted after rifamycin was introduced into the RCD-HPS.

3.2. Separation of the chiral drug compounds

The chromatographic performance of the RCD-HPS-packed column for separation of some chiral drug compounds was also studied under reversed-phase HPLC conditions. Fig. 3 shows the progressive separation of the enantiomers of 1-phenyl-1-propanol as the mobile phase composition comprising of methanol/water was varied. Increasing the proportion of water produced longer retention, higher enantioselectivity, and higher enantiomeric resolution. Therefore, the main chiral recognition mechanism appears to be the formation of an inclusion complex in which the hydrophobic portion of the solute is included in the capped β-CD cavity and the rifamycin unit provides further interactions with the solute. Baseline enantioseparation for 1-phenyl-1-propanol was easily obtained when the methanol content was reduced to 20% (or less) when using the RCD-HPS-packed column.

Table 2 lists the retention factors (k'), selectivity factors (α) and separation resolution (R_S) for the chiral drugs resolved on the RCD-HPS-packed column. Typical chromatograms of the chiral separation of the drug compounds on the RCD-HPS-packed column are shown in Fig. 4. The bonded phase RCD-HPS has a chiral selector with two recognition sites: rifamycin and β-CD. Rifamycin contains nine chiral centers and five hydroxyl groups and two aromatic rings [17,18] which are capable of providing multiple interactions with chiral solutes to enhance chiral recognition. Compared to the BACD-HPS-packed column [1], better enantioselectivities and higher resolutions for some chiral compounds, e.g., α-methylbenzylamine ($\alpha = 2.49$ and $R_S = 2.50$ on BACD-HPS and $\alpha = 3.48$ and $R_S = 3.36$ on RCD-HPS) and pindolol (no separation on BACD-HPS and $\alpha = 1.64$ and $R_S = 1.75$ on RCD-HPS), were achieved on RCD-HPS-packed column. This suggests that the rifamycin moiety in RCD-HPS does play an important role in improving chiral recognition for a higher coverage of chiral compounds.

Compared to commercial Cyclobond I (β-CD-bonded CSP) column [14,15] and Chirobiotic V (vancomycin-bonded CSP) column [9], RCD-HPS exhibited better enantioselectivity for some chiral compounds. For example, under reversed-phase conditions, better enantioselectivties for warfarin ($\alpha = 1.17$ on Cyclobond I [19] and $\alpha = 1.22$ on RCD-HPS) and propanolol ($\alpha = 1.04$ on Cyclobond I [14] and $\alpha = 1.18$ on RCD-HPS) were obtained on the RCD-HPS-packed column in HPLC. Under similar mobile phase conditions, better enantioselectivity for propanolol ($\alpha = 1.04$ on Chirobiotic V [9] and $\alpha = 1.18$ on RCD-HPS) was achieved on RCD-HPS-packed

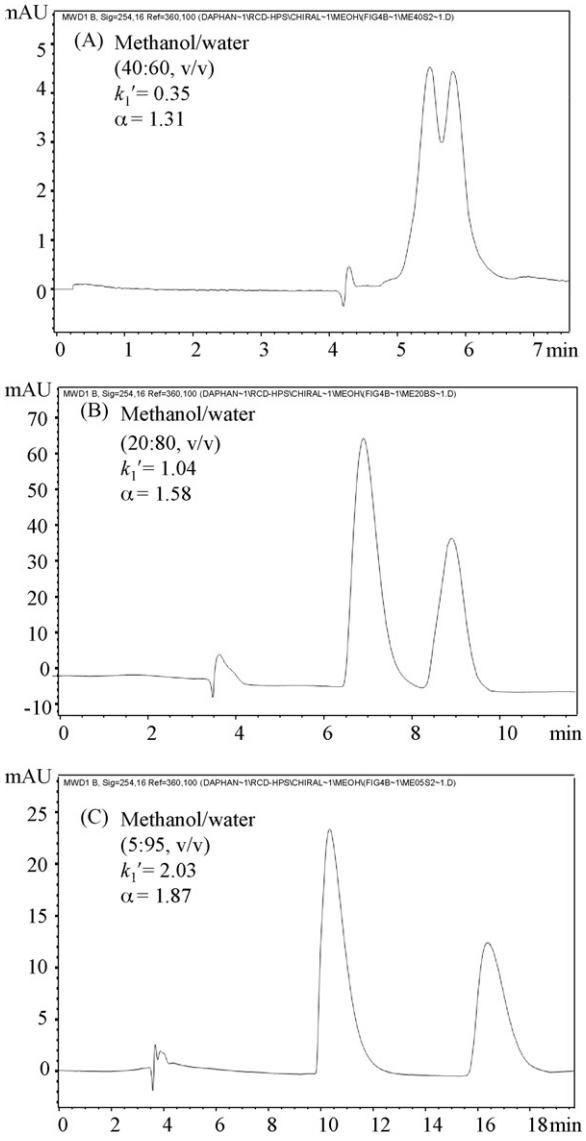

Fig. 3. Progressive enantioseparation of 1-phenyl-1-propanol on the RCD-HPS-packed column as composition of mobile phase is varied.

column. This is mainly due to cooperative functioning of the anchored β-CD and rifamycin in the rifamycin-capped β-CD selector of RCD-HPS. This enhanced the host–guest interaction with the solute resulting in improved chiral recognition and enantioselectivity. The rifamycins are generally easier to be available and less toxic than crown ethers [10]. Compared to diaza-18-crown-6-capped β-CD-bonded silica AQ2D18C6-CD-HPS-packed column [8], better enantioselectivity for a wider range of chiral compounds, e.g., propanolol ($\alpha = 1.09$ on AQ2D18C6-CD-HPS [8] and $\alpha = 1.18$ on RCD-HPS) and pindolol (no separation on AQ2D18C6-CD-HPS and $\alpha = 1.64$ on RCD-HPS), etc., were obtained on the RCD-HPS-packed column in HPLC. As shown in Table 2, many types of chiral drug compounds, including amino acids, beta blockers, and anti-inflammatory drugs, were resolved on RCD-HPS-packed column. This suggests that the new rifamycin-capped β-CD-bonded silica particles have significant advantages and potential wide applications for a wide range of chiral drug separations. No significant changes of chromatographic performance of the RCD-HPS-packed column have been observed for continuous usage over 6 months under the reversed-phase mobile phase conditions with addition of TEAA buffer. This suggests that the RCD-HPS phase is fairly robust.

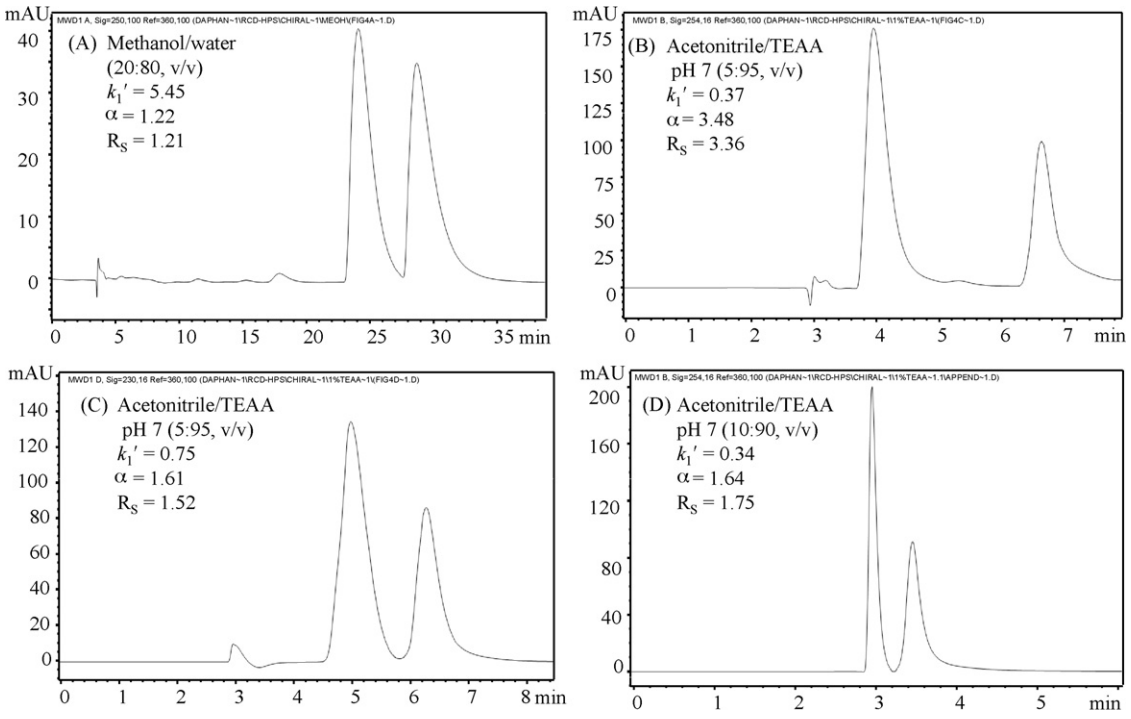

Fig. 4. Typical chromatograms for enantioseparations on RCD-HPS-packed column. (A) Warfarin, mobile phase: methanol/H_2O (20:80, v/v); (B) α-methylbenzylamine, mobile phase: acetonitrile/TEAA buffer pH 7 (5:95, v/v); (C) dichloroprop, mobile phase: acetonitrile/TEAA buffer pH 7 (5:95, v/v); and (D) pindolol, mobile phase: acetonitrile/TEAA buffer pH 7 (10:90, v/v).

4. Conclusions

A new type of rifamycin-capped (3-(2-O-β-cyclodextrin)-2-hydroxypropoxy)-propylsilyl-appended silica particles have been successfully synthesized and used as CSP to separate aromatic positional isomers and enantiomers of chiral compounds in HPLC. This new type of CSP has a chiral selector with two recognition sites: rifamycin and β-CD. The RCD-HPS has shown excellent selectivity for the separation of aromatic positional isomers and enantiomers of a wide range of chiral compounds due to the cooperative functioning of rifamycin and β-CD.

Acknowledgments

The authors thank the Ministry of Education of Singapore and National University of Singapore (NUS) for financial support of this work (Grant nos. R-174-000-110-112 and R-174-000-130-112). J.Z. is grateful for the award of a research scholarship by NUS.

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