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Journal of Chromatography A, 1002 (2003) 63–70
JOURNAL OF CHROMATOGRAPHY A
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Application of diaza-18-crown-6-capped β-cyclodextrin bonded silica particles as chiral stationary phases for ultrahigh pressure capillary liquid chromatography

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Received 8 October 2002; received in revised form 6 February 2003; accepted 25 April 2003

Abstract

Two bonded chiral stationary phases (CSPs), 8-aminoquinoline-2-ylmethyl- and 8-aminoquinoline-7-ylmethyl-diaza-18-crown-6-capped [3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy]propylsilyl silica particles (non-porous, 1.5 μm), have been prepared and evaluated using capillary liquid chromatography at high pressures (≥8000 p.s.i.). High column efficiency (up to 400 000 plates m^{-1}) was achieved for chiral separations. These CSPs with two recognition sites, i.e. substituted-diaza-18-crown-6 and β-cyclodextrin combined with high chromatographic efficiency provide good resolution of a variety of enantiomers and positional isomers in relatively short times under reversed-phase conditions. After inclusion of a Ni (II) ion from the mobile phase, the positively charged crown ether-capped β-cyclodextrin facilitates specific static, dipolar, and host–guest complexation interactions with solutes.

Keywords: Enantiomeric separations; Chiral stationary phases, LC; Diaza-18-crown-6-capped β-cyclodextrin bonded silica

1. Introduction

Enantiomeric forms of a drug can differ in potency, toxicity and behavior in biological systems [1]. Chiral separations for measuring drug impurities, degradation products, synthetic precursors, side products, and metabolites have been extensively explored over the last several decades [2]. Chromatographic methods are typically employed for chiral separations, including high-performance liquid chromatography (LC) [3], gas chromatography (GC) [4], micellar electrokinetic capillary chromatography (MECC) [5] and, more recently, capillary electrochromatography (CEC) [6].

Conventional LC cannot utilize very small particles (<2 μm) and/or long column lengths to obtain high efficiencies because of the pressure limitations of commercially available pumping systems. Although GC usually offers higher efficiencies than LC due to a larger number of available theoretical plates,

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0021-9673/03/$ – see front matter
doi:10.1016/S0021-9673(03)00732-5

most chiral compounds of interest have low volatilities. This limits the use of GC for chiral separations [2]. MECC is a fast and efficient separation technique for chiral separations, and a number of chiral selectors can be added to the run buffer [2,7]. However, chiral additives in MECC are limited to those that have sufficient solubility in water and have low UV–Vis absorption. CEC utilizes electroosmosis to significantly improve chromatographic efficiency by providing a flat mobile phase flow profile [8]. Very small particles can also be used because there is no limiting pressure drop. Therefore, CEC should be ideal for enantiomeric separations [6,9,10] if and when electroosmosis can be precisely controlled.

MacNair et al. introduced ultrahigh pressure liquid chromatography (UHPLC) to overcome the pressure limitations of conventional pumping systems [11,12]. With UHPLC, it is possible to use long capillary columns or very small particles to provide high efficiencies, even at high linear velocities [13].

Both cyclodextrins (CD) and crown ethers have proven to be effective chiral selectors [5,14]. While many chiral separations have been accomplished using CD-type stationary phases [9], Park et al. [15] reported that one drawback in utilizing CDs was the low binding constants for most guest molecules. Willner et al. [16] and Park et al. [15] reported that diaza-18-crown-6-capped β-CD exhibited high binding constants for several guest molecules due to cooperative functioning of the β-CD and the crown ether. Recently, it was shown that the combination of a crown ether and β-CD as capillary electrophoresis (CE) additives sometimes produced better enantioseparations than did either selector alone [5,17]. However, many crown ethers and derivatized CDs with high UV–Vis absorption characteristics and/or poor solubility in water are not suitable for use as CE additives for direct detection. Alternatively, they can be bonded onto silica to use as CSPs in LC [18,19]. We previously showed that crown ether-capped β-CD bonded phases gave better enantioselectivity in CEC for a number of analytes than either β-CD bonded phases or crown ether bonded phases alone under the same separation conditions [20].

In this paper, two novel types of crown ether-capped β-CD bonded stationary phases, 8-aminoquinoline-2-ylmethyl- and 8-aminoquinoline-7-ylmethyl-diaza-18-crown-6-capped [3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy]propylsilyl silica particles (AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS), were prepared and used as new stationary phases in UHPLC for chiral separations. The selectivities of these bonded stationary phases were examined by application to positional isomers of *o,m,p*-nitroaniline and enantiomers of selected chiral compounds.

2. Experimental

2.1. Reagents and materials

β-CD and racemic drug compounds were purchased from Sigma (St. Louis, MO, USA) and dried in vacuum at 120 °C for 12 h. Non-porous bare silica particles (1.5 μm) were purchased from Micra Scientific (Darien, IL, USA). Water was processed with a Milli-Q ultrapure water system (Millipore, Bedford, MA, USA). HPLC-grade acetonitrile, triethylamine and isopropyl alcohol (IPA) were all purchased from Fisher Scientific (Fair Lawn, NJ, USA). Analytical-grade phosphoric acid and potassium hydroxide were obtained from Aldrich (Milwaukee, WI, USA). SFC grade carbon dioxide and compressed nitrogen were obtained from Airgas (Salt Lake City, UT, USA). Fused-silica tubing with an internal diameter of 75 μm and an outer diameter of 360 μm was purchased from Polymicro Technologies (Phoenix, AZ, USA).

2.2. Apparatus

Our UHPLC system was previously described in detail [21]. Briefly, a double-head air-driven liquid pump (Model DSHF-32, Haskel, Burbank, CA, USA) was used to generate the necessary liquid pressures. The maximum nitrogen gas supply pressure was 150 p.s.i. (10 atm), resulting in a pump pressure limit of 52 K p.s.i. (3600 atm). The outlet of this pump was connected to a home-built injection system. A static-split injection technique was employed for sample introduction [21,22]. A Model UV3000 scanning detector from Thermo Separations (Sunol, CA, USA) was used to monitor UV absorbance on-column. Data were acquired with

ChromQuest 2.5.1 (ThermoQuest, Sunol, CA, USA). A Model DSF-150-C1 air-driven liquid pump (Haskel) was used for packing capillary columns.

2.3. Preparation of bonded stationary phases

The syntheses of AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS were previously described in detail [18]. Briefly, β-CD, which was primarily derivatized at its more reactive and less sterically hindered C(6) position [23,24] by treatment with seven equivalents of bromoacetyl bromide, was anchored onto silica particles at its C(2) position to form bromoacetate-substituted [3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy]propylsilyl silica particles (BACD-HPS). Finally, BACD-HPS was reacted with excess 8-aminoquinoline-2-ylmethyl- or 8-aminoquinoline-7-ylmethyl-diaza-18-crown-6 to form AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS. The amounts of anchored β-CD and substituted bromoacetate moieties in BACD-HPS were 30 and 137 μmol g^{-1}, respectively, as determined by elemental analysis. The amounts of diaza-18-crown-6 moieties in AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS were 41.84 and 27.30 μmol g^{-1}, respectively. The degree of substitution of crown ether was calculated to be 1.4 for AQ2D18C6-CD-HPS and 0.91 for AQ7D18C6-CD-HPS. The structures of AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS are shown in Fig. 1.

2.4. Chromatography

The bonded stationary phases were packed into capillary tubing using the carbon dioxide slurry packing method [21] to fabricate a 23 cm×75 μm I.D. column packed with AQ2D18C6-CD-HPS and an 18 cm×75 μm I.D. column packed with AQ7D18C6-CD-HPS. The internal column frits and on-column UV detection windows were fabricated using a resistive heating device (Innova Tech, UK). The mobile phases used were mixtures of acetonitrile–phosphate buffer. The buffer was prepared by dissolving the desired amount of phosphoric acid in water and then adding potassium hydroxide solution to achieve the desired pH. The sample concentration in acetonitrile was ~0.5–5 m*M*. Acetonitrile and buffers were filtered through 0.22-μm Durapore membrane filters (Millipore, Bedford, MA, USA) before use. Similarly, samples were filtered through 0.2-μm polytetrafluoroethylene (PTFE) syringe filters (Chromacol, Trumbull, CT, USA). Chromatography was carried out at room temperature. Supporting evidence for chiral separation was supplied by

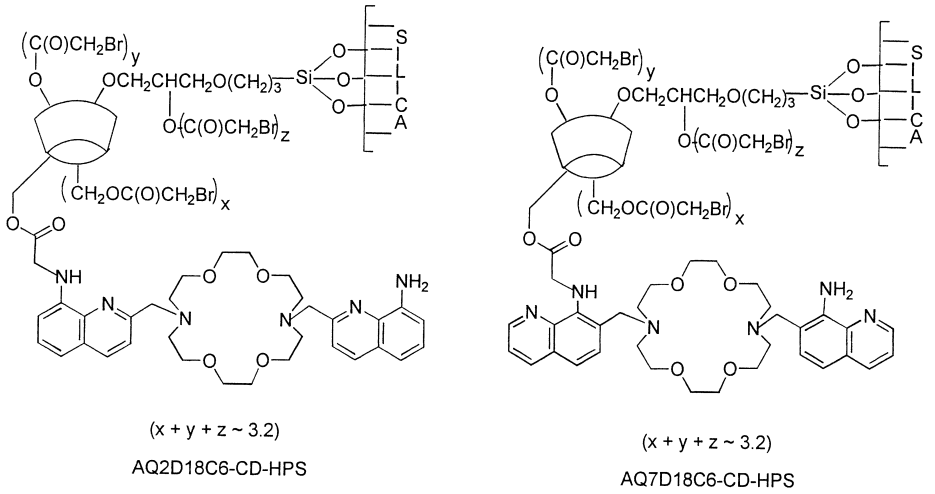

Fig. 1. Structures of the crown ether-capped β-CD bonded silica particles.

repeating the separation with detection at different UV wavelengths (e.g. 215, 230, 254 nm, etc.).

3. Results and discussion

3.1. Separation of o-, m-, and p-nitroaniline

The separation of *o*-, *m*-, and *p*-nitroaniline was performed using acetonitrile–phosphate buffer as mobile phase. The influence of acetonitrile content in the mobile phase on retention of solutes on the AQ2D18C6-CD-HPS column is shown in Table 1. The retention of solutes increases when acetonitrile content decreases, demonstrating that AQ2D18C6-CD-HPS has some hydrophobic interaction with the solutes.

Nitroanilines are useful test compounds for β-CD columns [25]; the difference in the relative retention of the *para* and *ortho* isomers can be directly correlated to the amount of β-CD bonded onto the silica support. If the β-CD loading is very low to zero, *p*-nitroaniline will elute first instead of last. This is because of the larger binding constant of *p*-nitroaniline that results from its linear geometry, which allows optimal penetration into the CD cavity to form a more stable inclusion complex. As shown in Table 1, *p*-nitroaniline always elutes last on the AQ2D18C6-CD-HPS column. This indicates that there is a significant amount of β-CD anchored onto the bonded silica. Compared with a reported aza-18-crown-6 bonded stationary phase [26], the selectivity for the three nitroaniline isomers is higher for AQ2D18C6-CD-HPS.

Table 1
Retention factors (k) for nitroanilines[a]

Solutes	Acetonitrile–phosphate buffer (v/v)			
	80:20	60:40	40:60	20:80
o-Nitroaniline	0.62	0.73	0.80	0.93
m-Nitroaniline	0.54	0.68	0.73	0.81
p-Nitroaniline	0.76	0.82	1.32	2.16

[a] Column: 23 cm×75 μm I.D. fused-silica column packed with non-porous 1.5-μm silica bonded with QA2D18C6-CD-HPS, 5 m*M* H_3PO_4-KOH (pH 7.5), ~1.8 mm s^{-1} mobile phase flow rate (vitamin C as t_0 marker), 254-nm UV detection.

3.2. Effect of sample injection amount on enantiomeric resolution

Non-porous particles have often been used in UHPLC because they provide better efficiencies than porous silica particles, especially at high mobile phase linear velocities [27]. Since the concentration of silanol groups on the non-porous silica surface is less than that for porous silica, the concentration of bonded functional groups is, therefore, low. In our work, the substituted crown ether-capped β-CD bonded particles were prepared using 1.5-μm non-porous silica. Therefore, the concentration of chiral selectors was not high. According to elemental analysis, the average concentration of β-CD was 30.0 μmol g^{-1} and the average concentrations of crown ether were 41.8 μmol g^{-1} for AQ2D18C6-CD-HPS and 27.3 μmol g^{-1} for AQ7D18C6-CD-HPS. Therefore, the sample capacity was low, and the sample injection amount, likewise, was kept relatively low; otherwise, the column became overloaded. For the static-split injection, the sample injection amount is determined by injection pressure and injection time. Generally, an injection pressure of 800 p.s.i. and an injection time of 3 s were used in this work. Reduction in injection amount can be realized by either decreasing the injection pressure or the injection time. It was found that when the injection pressure was decreased from 800 to 400 p.s.i. and the injection time was reduced to 2 s, baseline separation of the two enantiomers of indapamide (R_S = 2.29, α = 1.11, t_{R1} = 6.90 min, t_{R2} = 7.31 min) was obtained (Fig. 2). Compared with the results reported by Wu et al. [28], the enantioselectivity and resolution values for the enantiomers of indapamide were higher in UHPLC using AQ2D18C6-CD-HPS as chiral stationary phase than in capillary zone electrophoresis (CZE) with β-CD as chiral additive (R_S = 1.50, t_{R1} = 24.08 min, t_{R2} = 24.96 min). The separation speed in UHPLC was also much faster than CZE.

3.3. Chiral separations on columns packed with AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS

Using acetonitrile–phosphate buffer mobile phase, the substituted-diaza-18-crown-6 can include a metal

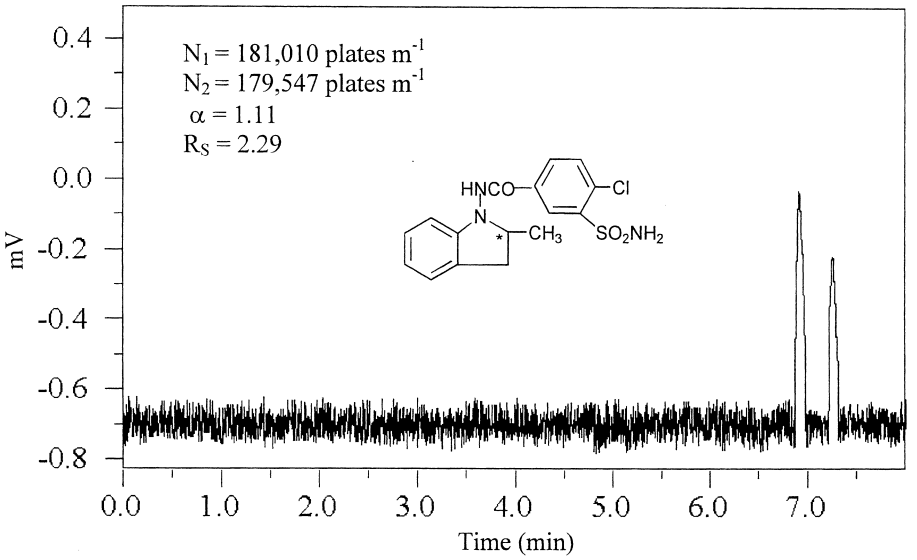

Fig. 2. Separation of enantiomers of indapamide. Conditions: 23 cm×75 μm I.D. fused-silica column packed with non-porous 1.5-μm silica bonded with AQ2D18C6-CD-HPS; 5 m*M* phosphate buffer (pH 7.5)–acetonitrile (80:20, v/v); 8000-p.s.i. column inlet pressure; 215-nm UV detection.

ion from the buffer to form a positively-charged inclusion complex [18,29]. This positively-charged complex provides static interaction with ionizable solutes and enhances dipolar interaction with polar neutral solutes. This increases both retention and selectivity.

Fig. 3 shows enantioseparations of 2-phenylpropionaldehyde on the AQ2D18C6-CD-HPS-packed column as the mobile phase composition was varied from 20 to 90% buffer. Increasing the proportion of buffer produced longer retention 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 positively-charged crown ether-metal ion inclusion complex provides further H–H interaction, static interaction and/or dipolar interaction with the solute. The two side arms also supply two ligand sites for solutes. As shown in Fig. 3B, the two enantiomers of 2-phenylpropionaldehyde are separated in 6 min with high resolution ($R_S = 24.5$) and high selectivity ($\alpha = 3.06$). Such high selectivity and high resolution using either β-CD type stationary phases or crown ether type stationary phases independently for chiral liquid chromatography have never been previously reported in the literature.

As shown in Fig. 4, a column efficiency of 376 546 plates m^{-1} was achieved for one enantiomer of trans-2-phenyl-cyclohexanol on the AQ2D18C6-CD-HPS-packed column. Fig. 5 shows a separation of α-methyl-1-naphthalene-methanol on the AQ7D18C6-CD-HPS-packed column. A column efficiency of 419 254 plates m^{-1} was achieved. Data for chiral separations of some racemic drugs are given in Table 2. It was found that pH had no observable effect on retention and selectivity for the studied chiral solutes on the AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS packed columns when the pH of the buffer changed from 7.5 to 4.5.

4. Conclusions

AQ2D18C6-CD-HPS and AQ7D18C6-CD-HPS are novel bonded chiral stationary phases suitable for fast UHPLC. They demonstrated high column ef-

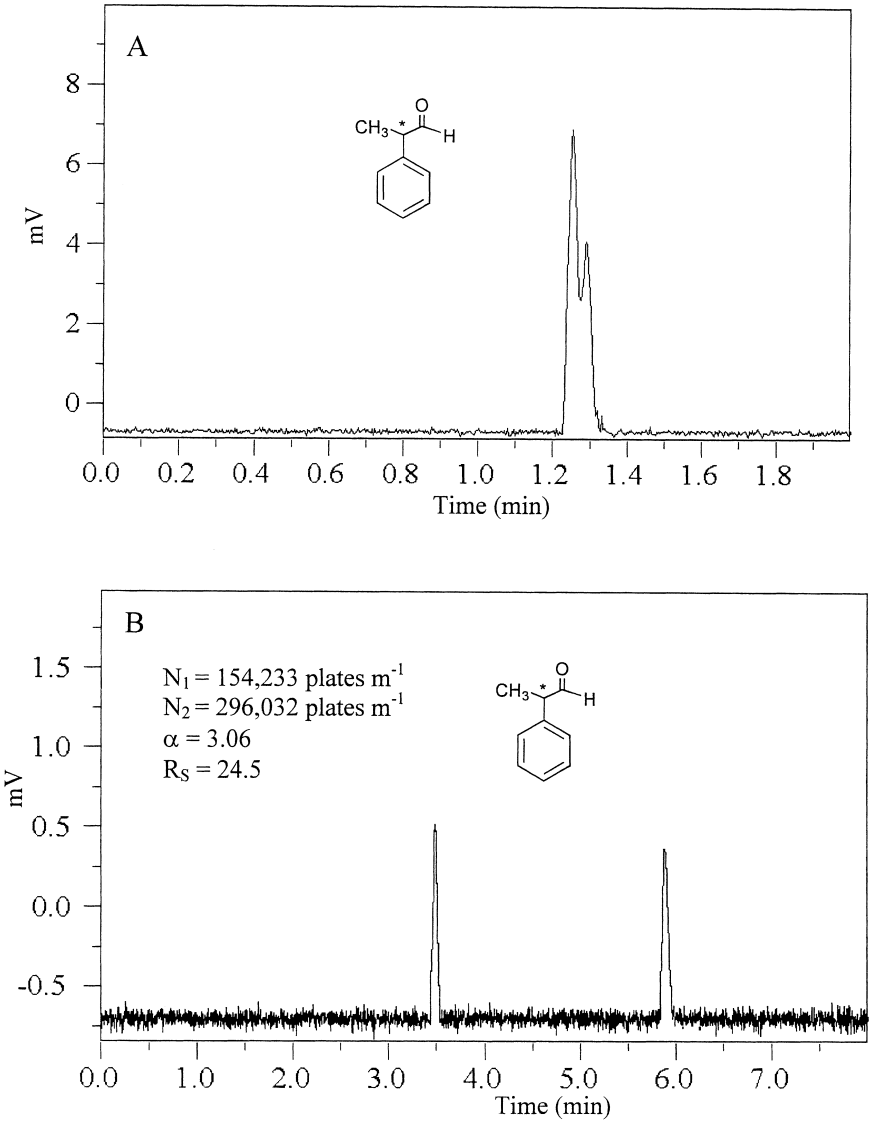

Fig. 3. Separation of enantiomers of 2-phenylpropionaldehyde with different amounts of acetonitrile in the mobile phase. Conditions: (A) 5 m*M* phosphate buffer (pH 7.5)–acetonitrile (20:80, v/v); 8000-p.s.i. column inlet pressure; (B) 5 m*M* phosphate buffer (pH 7.5)–acetonitrile (90:10, v/v); 10 000-p.s.i. column inlet pressure. Other conditions are the same as in Fig. 2.

ficiency and excellent resolution of enantiomers and positional isomers under reversed-phase conditions. Capillary columns packed with non-porous bonded silica particles demonstrate relatively low sample capacity. The sample injection amount should be carefully controlled in order to obtain the best chiral resolution.

Acknowledgements

Yinhan Gong gratefully acknowledges an Ang Kok Peng Memorial Fund Scholarship from the National University of Singapore that allowed a study leave at Brigham Young University where the described work was carried out.

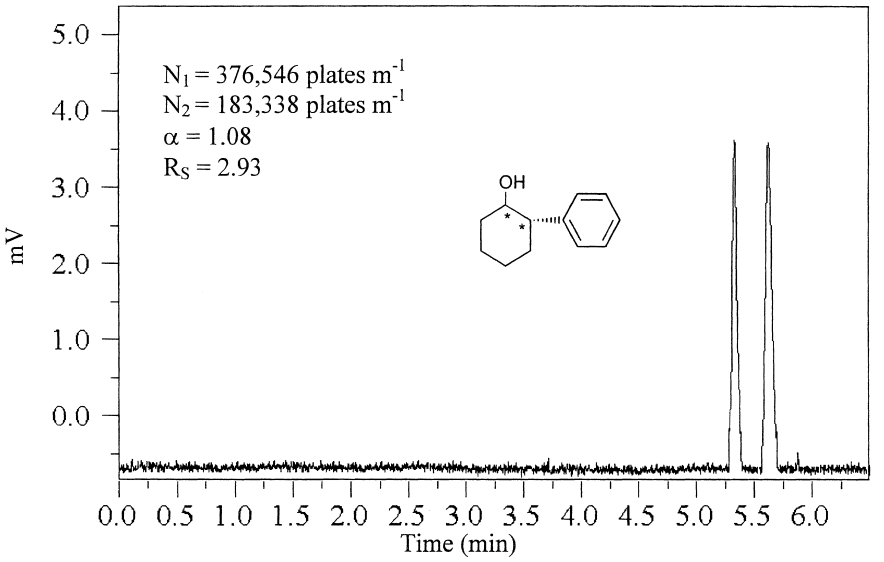

Fig. 4. Separation of enantiomers of trans-1,2-cyclohexanediol on AQ2D18C6-CD-HPS-packed column. Conditions: 10 000-p.s.i. column inlet pressure; other conditions are the same as in Fig. 2.

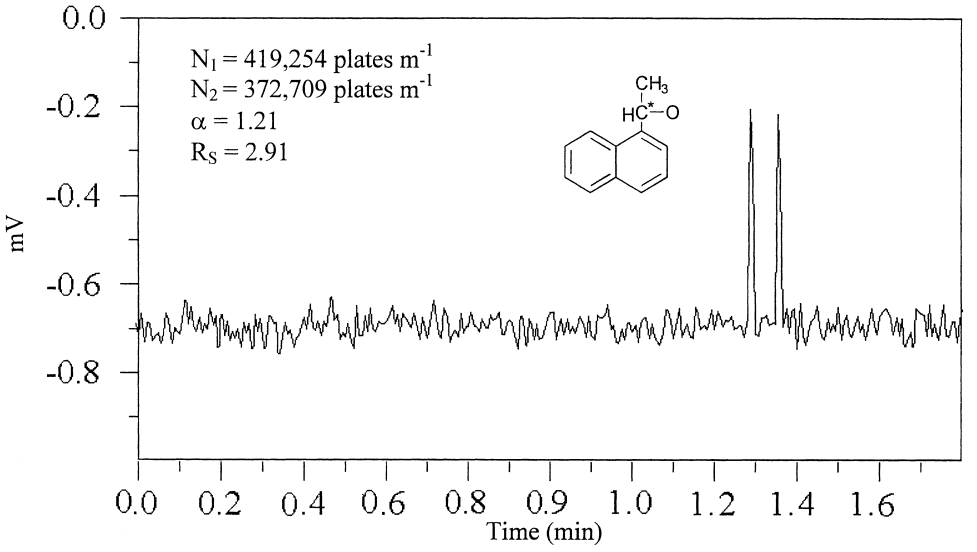

Fig. 5. Separation of enantiomers of 1-(1-naphthyl) ethanol. Conditions: 18 cm×75 μm I.D. fused-silica column packed with non-porous 1.5-μm silica bonded with AQ7D18C6-CD-HPS; 5 m*M* phosphate buffer (pH 7.5)–acetonitrile (80:20, v/v); 12 000-p.s.i. column inlet pressure; 215-nm UV detection.

Table 2
Chiral separation data for various enantiomers

Solutes	Mobile phase[a] (acetonitrile–buffer, v/v)	Column[b]	Separation data[c]		
			k_1	α	R_s
Indapamide	20:80	AQ2D18C6-CD-HPS	1.22	1.11	2.29
2-Phenylpropionaldehyde	10:90	AQ2D18C6-CD-HPS	0.46	3.06	24.50
trans-1,2-Cyclohexanediol	20:80	AQ2D18C6-CD-HPS	1.35	1.08	2.93
sec-Phenethyl alcohol	10:90	AQ2D18C6-CD-HPS	1.25	1.03	1.05
Spironolactone[d]	10:90	AQ2D18C6-CD-HPS	0.86	1.03	0.93
1-Phenyl-1,2-ethanediol	10:90	AQ2D18C6-CD-HPS	0.92	1.09	1.43
Propranolol	20:80	AQ2D18C6-CD-HPS	0.92	1.09	1.43
α-Methyl-1-naphthalene-methanol	20:80	AQ2D18C6-CD-HPS	1.09	1.12	2.91
Warfarin	10:90	AQ2D18C6-CD-HPS	1.15	1.25	2.18
trans-2-Phenylcyclohexanol	10:90	AQ2D18C6-CD-HPS	1.13	1.32	4.42

[a] Buffer: 5 m*M* H_3PO_4-KOH (pH 7.5); for *sec*-phenethyl alcohol and 1-phenyl-1,2-ethanediol, buffer contains 1 m*M* $Ni(ClO_4)_2$. 215-nm UV absorption detection.

[b] Columns: 23 cm×75 μm I.D. fused-silica column packed with 1.5-μm bonded non-porous AQ2D18C6-CD-HPS and 18 cm×75 μm I.D. fused-silica column packed with 1.5-μm bonded non-porous AQ7D18C6-CD-HPS.

[c] α, selectivity factor, k_1, retention factor for the first eluting enantiomer; R_s, resolution; t_0, marker, L-ascorbic acid [11].

[d] 7α-(Acetylthio)-17α-hydroxy-3-oxopregn-4-ene-21-caboxylic acid.

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