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Short communication

Synthesis and enantioseparation characteristics of a novel mono-6-deoxy-(2,4-dihydroxybenzimide)-β-cyclodextrin as a chiral stationary phase in high-performance liquid chromatography

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ABSTRACT

A novel chiral selector mono-6-deoxy-(2,4-dihydroxybenzimide)-β-CD (MDHB-β-CD) in which the derivatized group and the cavity of CD is linked by $CH_2–N{=}C$ group, was successfully prepared, and the structural characteristics were determined by FT-IR, ^{1}H and ^{13}C NMR, MALDITOF-MS and element analysis. The corresponding stationary phase (CSP) was used in HPLC and the enantioseparation performance was investigated using chiral 1-phenyl-2-nitroethanol derivatives as test samples in the reverse-phase mode composed of methanol/water and acetonitrile/TEAA. Better separation abilities and excellent enantioselectivities ($\alpha > 1.26$, $R_S > 1.73$) were obtained on MDHB-β-CD CSP for these chiral compounds in the methanol/water mobile phase.

1. Introduction

Cyclodextrins and their derivatives can form disatereomeric complexes with the enantiomers. So as one of the most commonly used chiral selectors, they have been widely used for high-performance liquid chromatography (HPLC) and become an efficient separation method for chiral compounds such as racemic drugs [1–3], aromatic chemicals, fragrances [4], amino acids [5] and so on. Up to now, two types of CD derivatives as the stationary phases (CSPs) are mainly used for enantioseparation: (1) multi-substituent derivatives and (2) mono-substituent derivatives. Much effort has been focused on the research of multi-substituent CD derivatives. For example, Armstrong and co-workers [6–8] prepared per-dimethylated, acetylated, hydroxy-propyl, naphthylethyl-carbamate and dimethyl-phenyl-carbamate CD derivatives. Ng and co-workers [9,10] synthesized per-methylated and per-phenylcarbamated CD derivatives by “Click” reaction. These per-substituent CD derivatives as chiral selectors have shown good ability for the enantioseparation of chiral dihydrofurocoumarins, furan derivatives, aryl alcohols, flavonoids, bendroflumethiazide and arropine. However, to our knowledge, few papers [11–15] reported mono-substituent cyclodextrin derivatives as the stationary phases in HPLC. To further broaden the application of CD derivatives in HPLC and investigate the recognition mechanism between an analyte and a chiral selector, it is necessary to synthesize novel mono-substituent cyclodextrin derivatives.

Our previous work has reported several mono(6-deoxy-imino)-β-cyclodextrin and obtained excellent enantioselectivities for certain chiral compounds [11–14]. In view of the effect of linkage groups bridging a substituent and the CD cavity on the enantiomeric separation, in the present paper, we reported the synthesis of a novel CD derivative: mono-6-deoxy-(2,4-dihydroxybenzimide)-β-CD (MDHB-β-CD) in which the substituent and the CD cavity is linked by a longer and more flexible linkage group ($CH_2–N{=}C$) compared to our previously reported CD derivatives, and bonded to silica gel to form CSP. The structural characteristics were determined by FT-IR, ^{1}H and ^{13}C NMR, MALDITOF-MS and element analysis. Chiral 1-phenyl-2-nitroethanol derivatives as test compounds were used to investigate the separation performance of MDHB-β-CD CSP.

2. Experimental

2.1. Chemicals and reagents

All the chemicals used were analytical grade. β-Cyclodextrin was purchased from the Kermel Chemical Reagent Factory of

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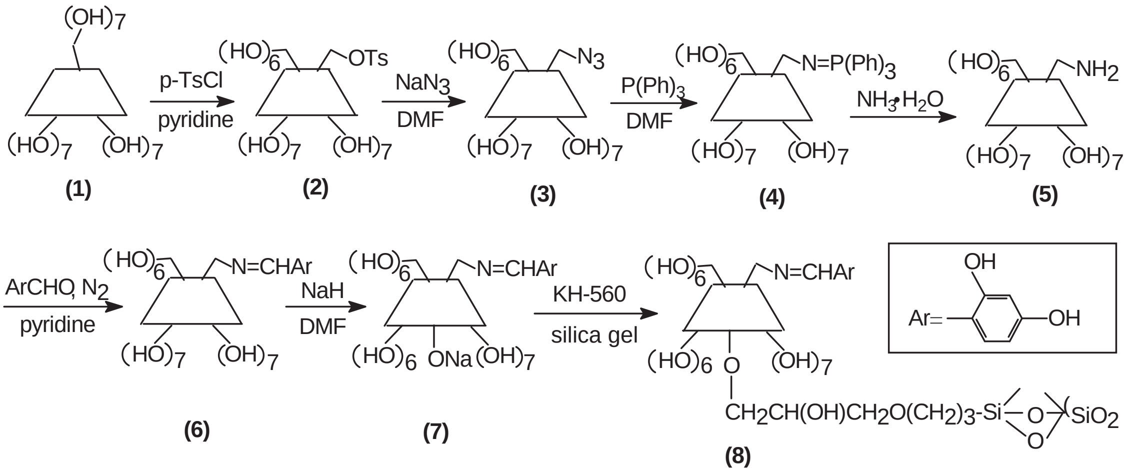


Fig. 1. Synthesis route for MDHB-β-CD chiral stationary phase.

Tianjin (Tianjin, China) and recrystallized three times with water before use. *p*-Toluenesulfonyl chloride was obtained from the Fuchen Chemical Reagent Factory of Tianjin (Tianjin, China) and purified by dry methylene chloride. Sodium azide, triphenylphosphine and 3-glycidoxypropyltrimethoxysilane were purchased from the Tianjin Chemical Reagent Factory No. 3 (Tianjin, China), Sinopharm Chemical Reagent Co. Ltd (Shanghai, China) and the Chemical Plant of Wuhan University (Wuhan, China), respectively, and used directly. DMF and pyridine obtained from the Fuchen Chemical Reagent Factory of Tianjin (Tianjin, China) were dried and redistilled before use. Silica gel (5 μm, 100 Å) purchased from Fuji (Japan) was heated at 160 °C for 12 h before use. Methanol, acetonitrile and water used as the mobile phase were HPLC grade and purchased from Carmichael Fisher Limited (USA), and other reagents (i.e., triethylamine, acetic acid, acetone and ammonia) were analytical grade and were used directly. Chiral 1-phenyl-2-nitroethanol derivatives were prepared by Henry reaction between benzaldehyde derivatives and nitromethane in our laboratory.

2.2. Instruments

The melting points and fourier-transform infrared (FT-IR) spectra were obtained on a XT4-100 purchased from Beijing Keyi Electric Light Instrument Co., Ltd (China) and in KBr on a Nicolet Magna 560 instrument purchased from Nicolet Instrument Corporation (USA), respectively. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and ^{13}C–^{1}H correlation spectroscopy (COSY) spectra performed in DMSO-d_6, matrix-assisted laser-desorption/ionization time-of-flight mass spectra (MALDI-TOF-MS) and element analyse were determined on an ARX-300, a BRFLEX MALDITOF system and an Elementar Vario EL, respectively, which were all purchased from Bruker-Spectrospin Ag (Swiss).

2.3. Preparation of MDHB-β-CD CSP

MDHB-β-CD CSP as a chiral selector was prepared through the following procedures, which are shown in Fig. 1. Compound (**2**) was prepared according to the method reported previously [15], product yield was 37.5%. Reaction of compound (**2**) and NaN_3 in DMF for 2 h at 105–110 °C gave compound (**3**) with 97.07% yield [16]. Compound (**3**) reacted with triphenyl phosphine for 3 h at room temperature to generate compound (**4**) in a yield of 84.01%. Through hydrolyzing compound (**4**) with ammonia water, compound (**5**) was obtained as a white solid with a yield of 88.69%. Table 1 shows the characteristic data for compounds (**2**), (**3**), (**4**) and (**5**). Mono-6-deoxy-(2,4-dihydroxybenzimide)-β-cyclodextrin (**6**) was prepared by the condensation of compound (**5**) and 2,4-dihydroxybenzaldehyde in a yield of 89.51%. All the above products were treated with acetone to remove the excess reactants. Thereafter, MDHB-β-CD CSP (**8**) was synthesized by bonding CD derivative to silica gel according to previous report [11]. The calculated concentration is 0.54 mol/m^2 according to the formula [17]:

$$\frac{\mu\text{mol}}{\text{m}^2} = \frac{\%N \times 10^6}{S(1400 \times n_N - \%N \times M_r)}$$

Where N is the percentage of nitrogen in the sample, as determined by elemental analysis (C: 7.63%, H: 1.50%, N: 0.20%). M_r and n_N are the molecular weight of the cyclodextrin derivative and the number of nitrogen atoms in the chiral selector [18], respectively. S is the special surface area of silica gel, which is 320 m^2/g according to the manufacturer.

2.4. Chromatographic measurements and parameters

MDHB-β-CD CSP was slurry-packed into 250 mm × 4.6 mm i.d. stainless-steel LC column. Its chromatographic performance was measured by the HPLC system consisting of two Wellchrom HPLC pumps (K-501), a manual injection valve (model 7725i), a Wellchrom spectrophotometer (K-2501) and a dynamic mixing chamber. The mobile phases were methanol/water and acetonitrile/TEAA (triethylammonium acetate buffer solution). UV detection was carried out at 254 nm, and the flow rate of the mobile phase was 0.6 mL min^{-1}. All the samples were dissolved with methanol and separated at room temperature.

The chromatographic parameters k' (retention factor), α (selectivity factor) and R_S (resolution factor) are calculated according to the equations $k' = (t_R - t_0)/t_0$, $\alpha = k'_2/k'_1 = (t_{R2} - t_0)/(t_{R1} - t_0)$ and $R_S = 2 \times (t_{R2} - t_{R1})/(W_1 + W_2)$, respectively. Where t_0 is the dead time for the mobile phase to pass through the column, and t_R (retention time) is the peak time of an analyte in the chromatographic scheme. t_{R2}, t_{R1}, W_1 and W_2 represent the retention times of the second and first isomers and the corresponding base peak widths, respectively.

3. Results and discussion

3.1. Structural characterization of MDHB-β-CD

According to FT-IR spectrum of MDHB-β-CD, the peaks at 3368.47, 2929.16, 1702.56, and 1636.40, 1531.96 cm^{-1} were corresponded to the OH, C=N and C=C of aromatic ring on β-cyclodextrin

Table 1
The characteristic data for compounds (**2**), (**3**), (**4**) and (**5**).

Compound	mp (°C)	IR (KBr, ν/cm^{-1})	^{1}H NMR (500 MHz, DMSO-d_6, ppm)
(**2**)	183–185	3378.24, 2929.86, 1642.15, 1568.28, 1410.69, 1364.06, 1298.89, 1177.57, 1157.02, 1079.22, 1029.05, 947.24, 813.81, 760.38, 706.21, 668.41, 580.59	7.75 (d, 2H), 7.43 (d, 2H), 5.65–5.86 (m, 14H), 4.77–4.85 (m, 7H), 4.48–4.54 (m, 6H), 4.19–4.39 (m, 14H), 3.22–3.66 (m, 28H), 2.44 (s, 3H)
(**3**)	233–235	3367.22, 2928.19, 2104.54, 1707.43, 1639.33, 1415.58, 1367.14, 1333.00, 1300.16, 1234.64, 1156.68, 1079.35, 1029.88, 946.40, 858.36, 757.27, 705.91, 609.16, 579.86, 531.04	5.49–5.78 (m, 21H), 4.83–5.01 (m, 7H), 4.49–4.57 (m, 13H), 3.31–3.77 (m, 28H)
(**4**)	248–250	3368.17, 2926.79, 1704.40, 1638.55, 1438.65, 1412.59, 1367.72, 1333.00, 1300.79, 1257.21, 1156.03, 1120.25, 1079.52, 1030.48, 946.54, 855.50, 756.89, 724.08, 695.80, 609.16, 580.97, 541.84, 478.42	7.56–7.63 (m, 15H), 5.73–5.78 (m, 21H), 4.83 (s, 7H), 4.49–4.55 (m, 13H), 3.31–3.64 (m, 28H)
(**5**)	260–265	3389.24, 2930.26, 1705.38, 1640.99, 1412.65, 1368.00, 1333.00, 1300.79, 1259.10, 1156.75, 1079.38, 1029.99, 947.22, 856.85, 757.04, 707.85, 650.80, 609.16, 579.09, 531.50, 484.10	5.66–5.77 (m, 21H), 4.83–4.89 (d, 7H), 4.49–4.55 (m, 13H), 3.31–3.64 (m, 28H), 1.77–1.91 (t, 2H)

derivative. The resonance signals at 8.29 and 7.76, 7.38, 7.13 ppm from ^{1}H NMR at 500 MHz in DMSO-d_6 indicated the protons of imine group and phenyl attached to the cyclodextrin, and the resonance signals of the corresponding carbon atoms were represented at 165.94 and 136.34, 134.07, 124.11 ppm from ^{13}C NMR at 125 MHz in DMSO-d_6. Fig. 2 presents the ^{13}C–^{1}H correlation spectroscopy (^{13}C–^{1}H COSY) of MDHB-β-CD, which was consistent with the proposed structure. The data obtained from elemental analysis for $C_{49}H_{76}O_{36}N$ were (calculated) C: 46.89%, H: 6.06%, N: 1.12%; (found) C: 46.72%, H: 5.96%, N: 1.17%, and MALDI-TOF-MS was $m/z\,(M{+}Na)^+$: 1276.3. These data indicated that 2,4-dihydroxybenzaldehyde as derivatized group has been successfully bonded to the cyclodextrin.

3.2. Column valuation of MDHB-β-CD CSP

It is well know that nitroaniline is one of the most effective compounds used to evaluate CD and CD derivatives columns. In general, *o*-nitroaniline eluted first and *p*-nitroaniline eluted last indicates that the CD loading is high, this is because the linear *p*-nitroaniline penetrates into the CD cavity and forms an inclusion complex with CD [19]. Herein, the separation of nitroaniline on MDHB-β-CD column was studied in different composition of methanol/water (v/v, 100/0, 80/20, 70/30, 60/40 and 50/50). The results showed that the retention was increased with decreasing the content of methanol in the mobile phase, and *p*-nitroaniline was eluted last. Using methanol/water (80/20) as the mobile phase, the column gave an efficiency of 30476 P/m. Besides nitroaniline, there are many other positional isomers such as aminophenol, phenylene diamine, naphthol, toluidine and nitrotoluene were separated on this column at the same chromatographic conditions. Table 2 displays the separation data for the six positional isomers at the condition of methanol/water (60/40). As can be seen from these data, high resolution values of the analytes were obtained on MDHB-β-CD column, it indicated that the MDHB-β-CD has been successfully bonded to silica gel and presented good separation performance.

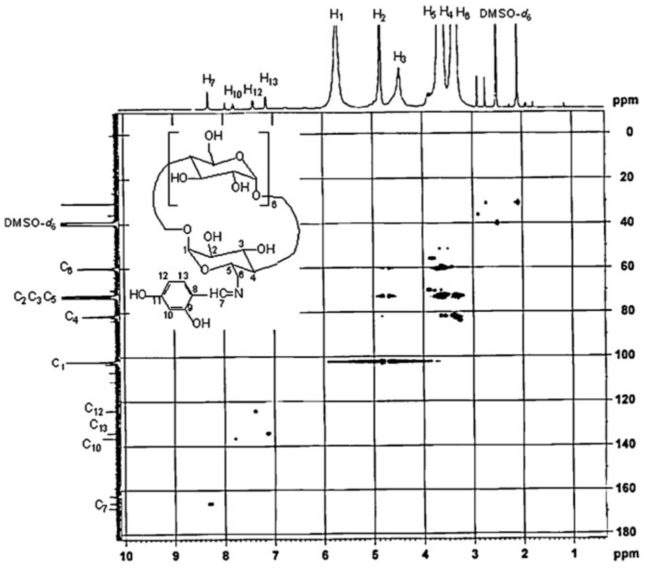


Fig. 2. ^{13}C–^{1}H correlation spectroscopy (^{13}C–^{1}H COSY) experiment for mono-6-deoxy-(2,4-dihydroxybenzimide)-β-cyclodextrin in DMSO-d_6.

3.3. Separation performance of MDHB-β-CD CSP

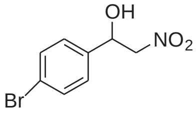
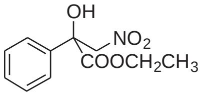
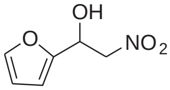
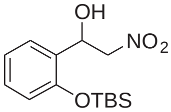
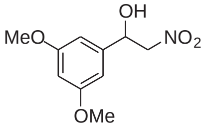
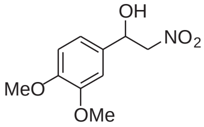
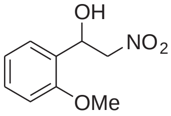
Certain chiral 1-phenyl-2-nitroethanol derivatives as test samples were chosen for evaluating the separation performance of MDHB-β-CD. The separation data for these compounds in the reverse-phase mode composed of methanol and water, and their chromatograms are shown in Table 3 and Fig. 3, respectively. Good enantioseparation results towards these chiral compounds were exhibited on this novel CSP. In experimental, the compositions of methanol and water were studied, the results showed that better enantioseparations of compounds **1** and **2** were obtained in 50/50 (v/v) of methanol/water mobile phase. For compounds **3** and **6**, better resolutions were gained under the methanol/water (v/v, 55/45) condition, however, it is beneficial for compounds **4**, **5** and **7** to separate using methanol/water (v/v, 60/40) as the mobile phase. In addition, the chemical structures of the analytes are a primary influence factor on the separation results. For example, at the same separation condition, the resolution values of compound **1**and **2** are almost the same but the retention values have a great difference. For compound **1**, this may be ascribed to the strong H-bonding interactions with the selector, however, for compound **2**, it is reasonable that ethyl formate group located at chiral center is beneficial for enhancing the enantioseparation although steric hindrance exists between the analyte and the selector. Take compounds **5**, **6** and **7** for another example, their retentions on CSP are similar although the difference of the mobile phase composition exists during the separation. This is likely due to the π–π interactions between the analytes and the MDHB-β-CD, which caused by the high electronic density of benzene ring because of the existence of methoxyl groups.

In order to further investigate the enantioseparation performance of MDHB-β-CD CSP, we also separated these chiral compounds in the reverse-phase mode composed of acetonitrile and TEAA with different pH, and the separation data are

Table 2
Separation data for the six positional isomers in the methanol/water (60/40) mobile phase.

Compound	k'	α	R_S	Elute order	Compound	k'	α	R_S	Elute order
Nitroaniline	2.74 4.59	1.68	6.08	σ,m–p	Naphthol	3.54 4.03	1.14	1.29	β–α
Aminophenol	2.07 3.92 4.27	1.89 1.09	4.89 0.97	m–p–σ	Toluidine	2.29 2.83 3.61	1.24 1.28	1.80 2.23	σ–m–p
Phenylene diamine	2.16 2.98 4.99	1.38 1.67	3.94 4.89	m–σ–p	Nitrotoluene	3.58 4.14	1.16	1.99	m,σ–p

Table 3
Separation data for chiral 1-phenyl-2-nitroethanol derivatives on MDHB-β-CD CSP at different methanol/water conditions.

No.	Chiral compounds	k'_1	k'_2	α	R_S	Conditions (methanol/water)
1		4.29	5.40	1.26	2.17	50/50
2		2.27	3.25	1.43	2.18	50/50
3		1.99	2.60	1.31	1.73	55/45
4		3.14	4.03	1.28	2.13	60/40
5		2.26	3.10	1.37	2.33	60/40
6		2.25	2.98	1.32	2.23	55/45
7		2.11	3.04	1.44	2.60	60/40

presented in Table 4. It can be seen that the resolution values obtained in the acetonitrile and TEAA mobile phase were lower than that obtained in the methanol and water mobile phase. It indicated that the adding of buffer into the reverse-phase did not enhance the enantioseparation of chiral 1-phenyl-2-nitroethanol derivatives on MDHB-β-CD CSP. This result may relate with the substituent group on MDHB-β-CD and the weak acidity of these compounds which are unfavorable to be ionized and increase the electrostatic interactions between the host and the guest. Furthermore, the optimized pH showed in Table 4 are 5.60 and 7.53, which also indicates that excessive acid or base in the mobile phase is unhelpful for the enantiomeric separation of these compounds.

Table 4
Separation data for chiral 1-phenyl-2-nitroethanol derivatives on MDHB-β-CD CSP in acetonitrile/TEAA with different pH.

No.	k'_1	k'_2	α	R_S	Conditions (pH)
1	2.89	3.17	1.10	0.93	5.60
2	2.11	2.52	1.20	1.39	5.60
3	1.57	1.94	1.24	1.56	7.53
4	2.14	2.39	1.12	1.51	5.60
5	2.04	2.55	1.25	1.35	5.60
6	1.94	2.39	1.23	1.28	5.60
7	1.99	2.30	1.16	1.19	7.53

Through comparing the separation data for these compounds obtained on MDHB-β-CD CSP with that obtained on R-CPGCD and R-HMPGCD CSPs in our previous report [14] in the methanol/water mobile phase, better enantioseparations were obtained on the latter for compounds **1**, **3**, **5**, **6** and **7**, however, better enantioseparations were obtained on MDHB-β-CD CSP for compounds **2** and **4** which were not separated on R-CPGCD and R-HMPGCD CSPs. The results showed that the derivatized group on cyclodextrin bridged

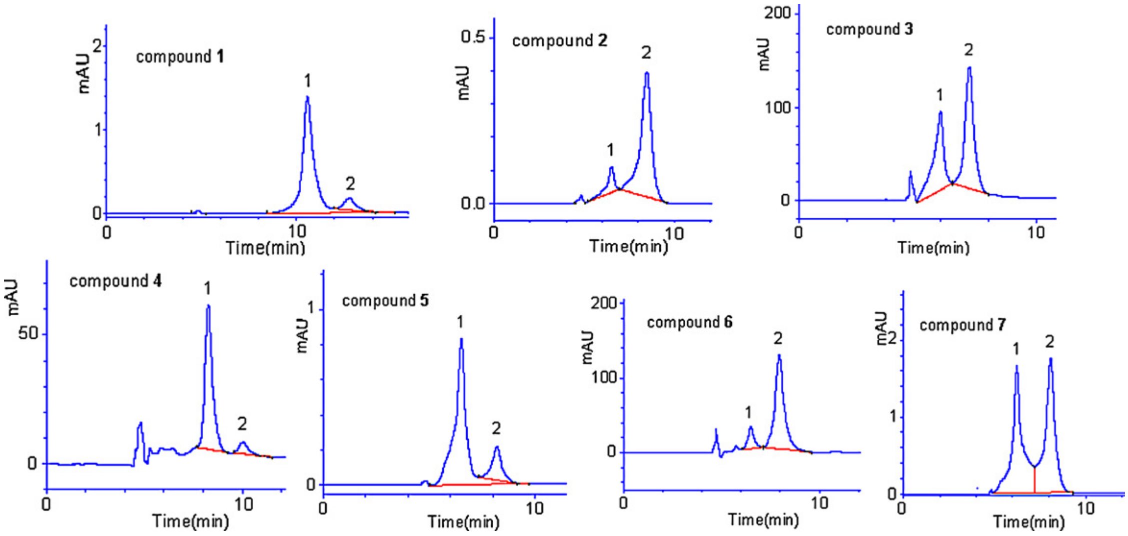


Fig. 3. Chromatograms of compounds **1**–**7** on MDHB-β-CD CSP under methanol and water mobile phase.

with a flexible linkage was easy close to the chiral carbon atom and separated the chiral compounds with flexible structures. It indicates that not only the substituent groups derivatized on CD but also the linkage groups play an important role in separating these compounds. The effects of the different linkage groups linked the same substituent group and CD on separation, and the enantioseparation of much more flexibly structural molecules will be further investigated and reported elsewhere.

4. Conclusion

The novel chiral selector MDHB-β-CD, as a mono-substituent CD derivative with a more flexible linkage group (CH_2–N=C) which linked the derivatized group and the cavity of CD, was successfully synthesized by a convenient synthetic route, and the corresponding CSP was prepared through bonding MDHB-β-CD to silica gel. The separation performance of this CSP used in HPLC were studied in the reverse-phase mode composed of methanol/water and acetonitrile/TEAA, the results showed that better enantioresolutions for these chiral 1-phenyl-2-nitroethanol derivatives were obtained in the methanol/water mobile phase. The separation factor and resolution values ($\alpha > 1.26$, $R_S > 1.73$) showed that the MDHB-β-CD CSP has excellent enantiomeric separation abilities for these compounds in the mobile phase composed of methanol and water. The chemical structures of the analytes, the derivatized group on CD and the linkage groups are the main influencing factor for the separation of these chiral compounds.

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