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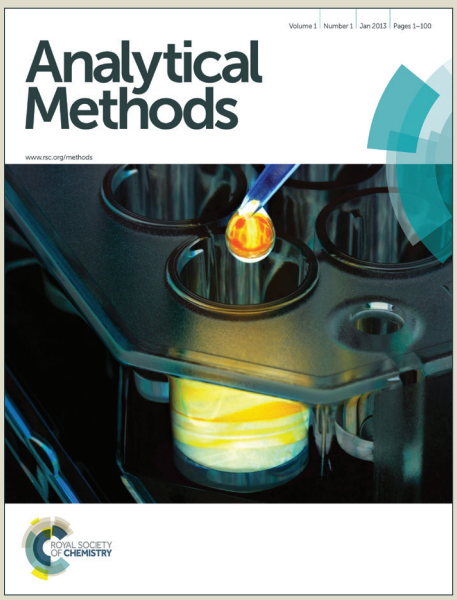

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Enantioseparation Characteristics of Chiral Stationary Phases Based on Derivatives of Cellulose and Chitin

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Abstract

It is well known that the chiral stationary phases (CSPs) of cellulose derivatives show highly chiral resolving capability towards racemates. Some CSPs have been commercialized and applied very popularly. However, cellulose derivatives can dissolve or highly swell in some organic solvents such as chloroform, ethyl acetate etc., the CSPs prepared by a coating method can only be utilized in a limited range of eluents. To compensate for this defect, chitin bis(3,5-dimethylphenylcarbamate) (Chi-DMPC) with highly solvent tolerance was blended with cellulose tris(4-methylbenzoate) (CMB) and cellulose tris(3,5-dimethylphenylcarbamate)

(CDMPC), respectively, at a ratio of 1:1 (mol/mol) of glucose unit to obtain two biselectors, and then it was coated on 3-aminopropyl silica gel respectively, to afford two biselector CSPs, expecting to enhance the stability of CSPs by blending method. For the sake of the comparison of chromatographic properties, the corresponding single selector CSPs were also prepared. The ability of enantioseparation and solvent tolerance of these biselector CSPs was systematically investigated. Owing to the low solubility of Chi-DMPC in organic solvents and the interaction between Chi-DMPC and the cellulose derivatives, the analysis with $CHCl_3$- and AcOEt-containing mobile phases could be carried out on these biselector CSPs. What's exciting is that resolution of most analytes was enhanced under the same condition after the biselector CSPs running with $CHCl_3$- and AcOEt-containing mobile phases.

Keywords

Chitin; Cellulose; Biselector; Chiral stationary phase; Enantioseparation

1. Introduction

Over the past few decades, high-performance liquid chromatography (HPLC) has been significantly advanced in the field of dealing with chiral compounds. The number of commercialized chiral stationary phases (CSPs) has greatly increased [1-4]. Among a variety of CSPs, cellulose tris(4-methylbenzoate) (CMB) and cellulose tris(3,5-dimethylphenylcarbamate) (CDMPC) have been well known for their powerful enantioseparation capability towards a wide range of chiral compounds [5,6]. Since some cellulose derivatives including CMB and CDMPC can highly swell or dissolve in some organic solvents, the conventional coating-type CSPs based on these polysaccharide derivatives can only work in commonly used organic solvent, a limited range of eluents [7]. However, some forbidden eluents such as ethyl acetate, chloroform, acetone may provide better resolution ability [8,9]. Afterwards, although the methods to prepare immobilizing-type CSPs were established [10-12], it is still desired that CSPs are prepared by a coating method because of the merits of easy preparation, excellent enantioseparation ability and reproducibility. This situation

motivated us to develop novel biselector CSPs with highly chiral recognition as well as good solvent tolerance [13].

Chitin $(C_8H_{13}O_5N)_n$ is an abundant chiral natural polymer. It consists of *N*-acetyl-*D*-glucosamine units linked by a β-(1→4) glycoside bond. Because of its comparable structure to cellulose, chitin was also used to prepare CSPs [14-16]. Early in 1996, Casset and coworkers [17] prepared two kinds of chitin bis(aryl carbamate) derivatives CSPs for HPLC. Okamoto and coworkers [18] prepared many arylcarbamates of chitin with N, N-dimethylacetamide/LiCl as the solvent, and the derivatives were then coated on 3-aminopropyl silica gel to afford CSPs. The separation results in these two works both indicated the good recognition ability of chitin derivatives CSPs. More importantly, they found the chitin derivatives difficultly dissolved in some organic solvents in which cellulose and amylose derivatives are soluble easily. The prepared CSPs, therefore, could work in the mobile phases in which the coating type CSPs of cellulose and amylose derivatives could not work. From this progress, we speculate the CSPs prepared from the blends of chitin and cellulose derivatives should exhibit a higher tolerability compared to those prepared from pure cellulose derivatives, because the solubility of the cellulose derivative in a blend should be lower than that of the pure cellulose derivative owing to the interaction between cellulose and chitin derivatives.

In addition, if the blends can be analyzed with the mobile phases containing organic additive such as chloroform or ethyl acetate etc., it remains unknown that the supramolecular structure of the blends will be altered or not. This uncertain question is interesting because enantioseparation is related to the supramolecular structure of polysaccharide derivatives [19-21], and the alteration in supramolecular structures may lead to the difference in enantioseparation.

The aim of this work is to develop novel biselector CSPs with good enantioseparation capability and satisfactory tolerability to organic solvents. The biselector CSPs were prepared by blending chitin bis(3,5-dimethylphenylcarbamate) (Chi-DMPC) with CMB and CDMPC respectively, to obtain CMB/Chi-DMPC coated CSP 4 and CDMPC/Chi-DMPC coated CSP 5. For the sake of comparison of

chromatographic properties, the corresponding single selector CSPs were also prepared. The ability of enantioseparation and solvent tolerance of these biselector CSPs was systematically investigated.

2. Experimental

2.1. General materials and apparatus

Microcrystalline cellulose and chitin were purchased from Sigma-Aldrich (USA). Wide-pore spherical silica gel with a mean particle size of 7μm and a mean pore size of 1000 Å was purchased from Daiso Co., Ltd (Japan). 4-Methylbenzoyl chloride and 3,5-dimethylphenyl isocyanate were commercially available from Jiangsu Panoxi Chemical Co., Ltd (China) and Dezhou Lvbang Chemical Co., Ltd (China), respectively. 3-Aminopropyltriethoxysilane (APTES) was purchased from J&K Scientific Ltd. (China). Chiral analytes were obtained from Nhwa Pharmaceutical Co., Ltd (China), Huahai Pharmaceuticals Co., Ltd (China), Nantong General Pharmaceutical Co., Ltd (China), or synthesized according to reported literature [22]. Pyridine was refluxed with KOH and CaH_2 in sequence, and then was redistilled. Triethylamine and toluene were refluxed with P_2O_5 and sodium, respectively, and redistilled. N,N-dimethylacetamide (DMAc) was dried twice over 4Å molecular sieves. Lithium chloride was dehydrated in vacuum at 130 ^oC for 24h. The organic solvents used for chromatographic analysis and other chemicals used for CSPs preparation were purchased from Sinopharm Chemical Reagent Co., Ltd (China).

Elemental analysis (EA) measurement was conducted on an Elemental Vario EL III CHNOS analyzer (Germany). IR spectra were recorded on a Nicolet FT-IR instrument (USA) with KBr pellets. Enantioseparation was performed on an Agilent 1100 chromatographic apparatus (USA) consisting of an Agilent G1365B DAD, an Agilent G1311A quaternary pump, an Agilent G1379A degasser, and an Agilent G1313A ALS autosampler. The stainless steel HPLC hollow columns (250mm×4.6mm) were purchased from Hypersil (UK). The CSPs were packed into the empty columns with an Alltech 1666 slurry packer (USA).

2.2. Preparation of Chiral Stationary Phase

The structures of prepared polysaccharide derivatives and the preparation scheme of the CSPs are shown by Fig. 1.

CMB and CDMPC were synthesized by the reaction of an excess of 4-methylbenzoyl chloride or 3,5-dimethylphenyl isocyanate with microcrystalline cellulose in pyridine and collected as the fraction insoluble in methanol [23]. Similarly, Chi-DMPC was prepared by the reaction of excessive 3,5-dimethylphenyl isocyanate with chitin in DMAc/LiCl. The preparation procedure and characterization of these derivatives were detailed in *Supporting Information*. 3-Aminopropyl silica gel was prepared with macroporous silica gel and APTES [23].

CSPs 1-5 were prepared by a coating method using DMF as the solvent [23]. The selectors of polysaccharide polymer were modified on the silica gel by physical adsorption because of the porous structure and large specific surface area of silica gel. The molecular weight of repeating unit of CDMPC is highest among these derivatives. During the preparation of CSP 2, CDMPC was fed by 20% (wt%). In order to ensure the selectors were fed at the same mmole per gram of silica gel (0.41mmol/g), CMB, ChiDMPC, blends 1 and 2 were fed at varied ratios by weight, which were, respectively, 17.5%, 17.1%, 17.4% and 18.6%. The molar ratio of two polymers in each blend was 1:1. The blends were prepared by mixing the calculated amount of individual derivatives in DMF with stirring, and the resulting solutions were used for coating directly.

2.3. Column Packing and chromatography

The CSPs were suspended in a solution of hexane/isopropanol (20/10, v/v). The suspensions were sonicated for 2 min to form slurries that were subsequently pushed into HPLC hollow columns by an Alltech slurry packer with hexane as the displacer solvent, under a pressure no more than 5800 psi. Dead time (t_o) was determined by measuring the retention time of 1, 3, 5-tri-tert-butylbenzene as a non-retained compound. All mobile phases were filtered and degassed before use. Chiral analytes were dissolved in ethanol to obtain chiral sample solutions (1 mg/mL), and it was

filtered through 0.2 μm membranes before injection. The injection volume was 10 μL. All enantioseparations were implemented by HPLC at 25 °C with a flow rate of 1.0 mL/min. The chiral analytes were measured at the wavelength where they absorbed most.

3. Results and discussion

3.1. General enantioseparation of the CSPs

The enantioseparation capability of CSPs 1-5 was evaluated in *n*-hexane/isopropanol (90/10, v/v) with the same chiral analytes (Fig. 2). The chromatographic results are presented in Table 1, from which the enantioseparation performance of the CSPs can be compared. As shown in Table 1, there were eight, thirteen, nine, ten and eleven chiral compounds recognized by CSPs 1-5, and three, five, two, four and six compounds baseline separated, respectively. Compounds 1 and 16 were very close to baseline separation on CSP 1. In comparison with the single selector CSPs, the biselector CSPs (CSP 4 and CSP 5) also provided satisfactory chiral recognition. Most of the capacities factors and separation factors of the biselector CSP intermediated between those of their single CSPs. However, the separation performance on biselector CSPs was better than chitin-based single selector CSP 3, with the increase of resolution and recognition range towards chiral analytes. For example, compounds 10, 13, 17 can be baseline separated on biselector CSPs, while can not be recognized on individual CSP 3. This phenomenon might result from the existence of cellulose derivative on biselector CSPs, the structures of which exhibit special recognition performance towards compounds 10, 13, 17. Considering the recognition mechanism of polysaccharide-based CSPs, its chiral individual carbohydrate monomers, a long-range helical secondary structures and chiral cavities play a significant role in enantioseparation [2, 24]. These structures constitute many possible interaction sites that can resolve many chiral analytes. The type of side chain may also have some effect on the helical structure of the polysaccharide polymer. Hence, besides the backbone, the type of derivative, ester or carbamate, as well as the residue, which can form various interactions such as hydrogen bonds interactions, hydrophobic

interactions, π-π interactions with chiral analytes, also influence the enantiorecognition profiles of the polysaccharide CSPs [1, 25]. While, in our prepared biselector CSPs, except the influence factors mentioned above, the interactions between those two selectors on biselector CSPs might play an important role in chiral separation. For example, compounds 8 and 17 were not discriminated on CSP 1 and CSP 3 within a retention time of 80 minutes, but could be recognized, especially with baseline separation of compound 17 on biselector CSP 4 (Fig. 3). The reason may be attributed to the different steric structures of biselector CSPs compared with individual CSP [9, 13]. Through blending two chiral selectors, the supramolecular structure of selectors on biselector CSPs might be changed. Therefore, different steric environments result in differences in the chiral cavities on biselector CSPs [26]. The chiral cavities of biselector CSP 4 may be more suitable to fit the stereostructure of compound 8 and 17, thus chiral separation can be achieved on biselector CSP 4 rather than individual CSP.

As shown in Table 1, elution order of some same chiral analytes on individual CSPs was different. The reversed elution orders on individual CSPs impaired the resolution on biselector CSPs, while the same elution orders improved the resolution [13]. It might result from the positive coordinate effect of the same elution orders and negative coordinate effect of the reversed elution orders. For example, the elution order of compound 12 on individual CSP 1 and CSP 3 were reversed, which resulted in unrecognized on its corresponding biselector CSP 4; while the elution order on individual CSP 2 and CSP 3 were the same, the resolution of compound 12 on biselector CSP 5 was increased. However, there are exceptions to this, such as the enantioseparation of compound 15. It can be separated on CSP 2 and CSP 3 with the same elution order, but was not recognized on biselector CSP 5. Taking the above results into account, the enantioseparation of biselector CSPs might not only rely on the elution order of chiral analytes on the individual CSP, but also the chiral cavities changed by supramolecular structure in steric environments through the interaction between two selectors in a blend. Therefore, together with the improvement in resolution due to the same elution orders, biselector CSPs hold a potential that is

complementary with single CSPs in enantioseparation.

3.2. Effect of organic additives on enantioseparation

It is well known that some cellulose derivatives such as CMB and CDMPC etc., can dissolve or highly swell in chloroform and ethyl acetate. Thus, these solvents can not be applied as the additives for coating-type CSPs of the derivatives. In this work, CSP 4 and CSP 5 were running with the mobile phase containing chloroform and ethyl acetate as additives. The enantioseparation results are summarized in Tables 2 and 3. In order to conveniently compare the results with/without additives, the separation results in the mobile phase of *n*-hexane/isopropanol are also listed in the tables. As shown in Tables 2 and 3, the elution orders remained the same in the presence/absence of additives, however, some resolutions on these two biselector CSPs were significantly changed. For example, compound 11 was separated by CSP 4 with a resolution of 1.36 in *n*-hexane/isopropanol (90/10, v/v), and that was improved to 1.75 in *n*-hexane/$CHCl_3$/isopropanol (90/5/5, v/v/v) (Fig. 4). Compounds 7, 18 and 19 were baseline separated both on CSP 4 and CSP 5 in *n*-hexane/$CHCl_3$/isopropanol (80/10/10, v/v/v) with long retention time above 80 min. Compound 8 was not baseline separated by CSP 5 in *n*-hexane/isopropanol (90/10, v/v), however, a baseline separation was achieved in *n*-hexane/$CHCl_3$/isopropanol (90/5/5, v/v/v). In addition, the resolution of compound 16 was further enhanced when the additives were employed. In short, the utilization of organic additives can improve the resolution of some compounds by CSP 4 and CSP 5, while these additives may not be adopted for coating type CSPs of cellulose derivatives. The change of separation performance on biselector CSPs by the organic additives might attribute to the suprastructural variation of biselector [27-30].

3.3 Enantioseparation of biselector CSPs after running with additive

Another question arose which was whether the enantioseparation capability of CSP 4 and CSP 5 decreased or not after they were running with $CHCl_3$- and AcOEt-containing mobile phases. Thus, the two CSPs were subjected to the

enantioseparation again with the same sample and the same mobile phase that was initially used to evaluate their enantioseparation capability. As can be seen in Table 4, most latter resolutions on CSP 4 and CSP 5 were higher than the former ones, especially for those on CSP 4. Typically, compound 11 for example, was initially separated on CSP 4 with a resolution of 1.36, and the latter resolution, however, amounted to 2.02; the initial resolution of compound 13 resolved on CSP 5 was 1.38, and the latter one reached 2.19 (Fig. 5). In other words, the enantioseparation capability of CSP 4 and CSP 5 generally increased although they had been analyzed with $CHCl_3$- and AcOEt-containing mobile phases. These two CSPs can be analyzed with an additive-containing mobile phase to improve the resolution for a specific chiral compound.

3.4 Reproducibility of biselector CSPs

The reproducibility of biselector CSP is an important indicator to evaluate the usability of a column. In this experiment, chiral compound 10 as target analyte was chosen to investigate the reproducibility of two biselector CSP 4 and CSP 5. The reproducibility was calculated on the basis of the relative standard deviations (RSDs) of migration time and peak area obtained from compound 10 for five replicate analysis. The intra-day and inter-day RSDs shown in Table 5 were all below 2%, which indicated that two biselector CSP 4 and CSP 5 have a good reproducibility. Slight change in the separation performance was observed after 100 consecutive runs. All these results demonstrate that biselector CSP 4 and CSP 5 possesses good stability.

4. Conclusion

The biselector CSPs prepared from ChiDMPC/CMB and ChiDMPC/CDMPC bear powerful chiral recognition capability compared to the corresponding single selector CSPs. Other than the coating type CSPs of cellulose derivatives, these biselector CSPs could be analyzed with $CHCl_3$- and AcOEt-containing mobile phases, in which resolution of analytes were improved. This result initiates a consideration that the coating type biselector CSPs prepared from chitin bis(arylcarbamate) and cellulose

tris(arylcarbamate) or tris(arylformate) might be used with the mobile phases in the presence of organic additives. Thus, these CSPs can work in a wider range of mobile phases. Besides, the supramolecular structure of polysaccharide derivatives in different mobile phases was variable, resulting in changeable enantioseparation ability. The change of supramolecular structure was favorable to enantioseparation, which was evidenced by the increased resolutions on CSPs 4 and 5 after they were analyzed with $CHCl_3$- and AcOEt-containing mobile phases. It is interesting and worthy to be further investigated how to control or adjust the supramolecular structure of polysaccharide derivatives that are used as chiral selectors.

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Figure captions

Fig. 1. Preparation scheme of CSPs 1-5.

Fig. 2. Structures of the chiral compounds separated by CSPs 1-5.

Fig. 3. The separation chromatogram of chiral compound 17 resolved by CSPs 1, 3 and 4. Eluent: n-hexane/isopropanol (90/10, v/v); detection temperature: 25oC; flow rate: 1ml/min.

Fig. 4. The separation chromatogram of chiral compound 11 resolved by CSP 4. Eluent: I: n-hexane/isopropanol (90/10, v/v); II: n-hexane/$CHCl_3$/isopropanol (90/5/5, v/v/v).

Fig. 5. The separation chromatograms of chiral compound 13 resolved by CSP 5. Eluent: n-hexane /isopropanol (90/10, v/v); I: analyzing before using additives; II: analyzing after using additives.

Table 1. The comparison of the enantioseparation of chiral compounds resolved by CSPs 1-5

Analytes	CSP **1**			CSP **2**			CSP **3**			CSP **4**			CSP **5**		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
1	$^{-}$1.45	1.23	1.45	0.64	1.00	0	0.20	1.00	0	$^{-}$0.93	1.22	1.08	0.41	1.00	0
2	5.42	1.00	0	2.86	1.00	0	^{S}1.88	1.04	0.23	3.59	1.00	0	2.90	1.00	0
3	6.30	1.00	0	^{S}6.56	1.11	0.51	^{R}6.30	1.25	1.58	^{R}6.64	1.14	0.95	^{R}7.44	1.07	0.90
4	^{S}1.67	1.15	0.20	^{R}1.31	1.36	1.40	1.64	1.00	0	1.62	1.00	0	^{R}1.55	1.09	0.60
5	5.25	1.00	0	^{R}3.08	1.43	2.28	^{R}1.23	1.07	0.32	3.04	1.00	0	^{R}2.27	1.25	1.81
6	3.79	1.00	0	^{R}5.41	1.23	1.19	2.92	1.00	0	4.11	1.00	0	^{R}4.49	1.09	0.71
7	Retention time>80min			Retention time>80min			Retention time>80min			Retention time>80min			Retention time>80min		
8	4.86	1.00	0	^{R}2.94	1.21	1.11	1.12	1.00	0	^{R}2.74	1.09	0.60	^{R}2.13	1.16	1.41
9	^{S}3.44	1.20	0.78	^{R}6.18	1.67	2.91	2.01	1.00	0	^{S}2.80	1.16	0.77	^{R}4.11	1.32	2.56
10	$^{+}$0.85	7.68	7.66	$^{+}$0.90	1.35	1.56	0.18	1.00	0	$^{+}$0.53	5.98	6.43	$^{+}$0.49	1.36	1.53
11	$^{-}$2.92	1.34	2.07	$^{-}$1.90	1.04	0.24	0.59	1.00	0	$^{-}$1.54	1.14	1.36	1.24	1.00	0
12	$^{-}$2.19	1.07	0.56	$^{+}$2.79	1.15	0.96	$^{+}$1.36	1.10	0.60	2.04	1.00	0	$^{+}$2.34	1.10	1.15
13	$^{+}$1.08	1.40	1.97	$^{-}$0.70	1.76	2.80	0.17	1.00	0	$^{+}$0.60	1.33	1.97	$^{-}$0.39	1.42	1.38
14	11.67	1.00	0	^{R}14.75	1.26	0.62	^{S}10.91	1.08	0.45	^{S}10.11	1.10	0.58	12.77	1.00	0
15	2.74	1.00	0	^{R}1.75	1.12	0.53	^{R}0.57	1.25	0.70	1.52	1.00	0	1.45	1.00	0
16	^{2R,3S}6.81	1.36	1.45	15.20	1.00	0	^{2S,3R}3.81	3.50	9.34	^{2S,3R}5.78	1.93	5.99	^{2S,3R}10.25	1.53	5.10
17	7.04	1.00	0	^{1S,2R} 3.02	4.66	7.57	0.81	1.00	0	^{1S,2R}2.73	1.65	1.99	^{1S,2R} 1.97	3.08	10.24
18	Retention time>80min			Retention time>80min			$^{+}$14.14	1.19	0.98	Retention time>80min			Retention time>80min		
19	Retention time>80min			Retention time>80min			$^{+}$14.91	1.20	1.15	Retention time>80min			Retention time>80min		

Eluent: *n*-hexane/isopropanol (90/10, v/v); k_1: retention factor of the first enantiomer; α: separation factor; Rs, resolution factor. "R" or "S", or "+", or "–", or "1S,2R", or "2R,3S" or "2S,3R" marked in the left superscript of k_1 values refers to the first-eluted enantiomer.

Table 2. The comparison of the enantioseparation of chiral compounds resolved by CSP 4 analyzed with various mobile phases

Analytes	*n*-Hexane/isopropanol(90/10)			*n*-Hexane/$CHCl_3$/isopropanol			*n*-Hexane/AcOEt/isopropanol			Composition
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	proportion
1	-0.93	1.22	1.08	-0.57	1.16	0.64	-0.66	1.14	0.68	90/5/5
				0.29	1.00	0	0.37	1.00	0	80/10/10
2	3.59	1.00	0	4.03	1.00	0	3.64	1.00	0	90/5/5
				1.07	1.00	0	1.02	1.00	0	80/10/10
3	R6.64	1.14	0.95	8.92	1.00	0	R8.62	1.10	0.85	90/5/5
				1.39	1.00	0	3.00	1.00	0	80/10/10
4	1.62	1.00	0	2.15	1.00	0	1.98	1.00	0	90/5/5
				0.91	1.00	0	0.81	1.00	0	80/10/10
5	3.04	1.00	0	R2.52	1.06	0.38	2.21	1.00	0	90/5/5
				R0.84	1.07	0.38	0.72	1.00	0	80/10/10
6	4.11	1.00	0	6.67	1.00	0	4.90	1.00	0	90/5/5
				1.77	1.00	0	1.27	1.00	0	80/10/10
7	Retention time>80min			Retention time>80min			S20.75	1.23	1.50	90/5/5
				S15.60	1.30	2.12	S4.36	1.16	1.08	80/10/10
8	R2.74	1.09	0.60	R2.18	1.08	0.52	R2.12	1.04	0.33	90/5/5
				R0.71	1.06	0.35	0.67	1.00	0	80/10/10
9	S2.80	1.16	0.77	S4.14	1.17	0.85	S3.06	1.17	0.97	90/5/5
				S1.21	1.09	0.56	S0.94	1.08	0.30	80/10/10
10	+0.53	5.98	6.43	+0.30	5.17	6.76	+0.42	4.47	7.02	90/5/5
				+0.15	3.75	4.15	+0.27	3.39	5.43	80/10/10
11	-1.54	1.14	1.36	-2.01	1.20	1.75	-1.57	1.18	1.39	90/5/5
				-0.84	1.18	1.14	-0.64	1.15	0.76	80/10/10
12	2.04	1.00	0	1.59	1.00	0	+2.25	1.05	0.45	90/5/5
				0.59	1.00	0	+0.98	1.06	0.47	80/10/10
13	+0.60	1.33	1.97	+0.39	1.44	1.84	+0.41	1.38	1.77	90/5/5
				+0.25	1.33	1.10	+0.28	1.29	1.07	80/10/10
14	S10.11	1.10	0.58	S11.97	1.12	0.65	S15.09	1.14	0.88	90/5/5
				S2.62	1.13	0.65	S4.40	1.18	1.01	80/10/10
15	1.52	1.00	0	1.16	1.00	0	1.06	1.00	0	90/5/5
				0.48	1.00	0	0.42	1.00	0	80/10/10
16	2S,3R5.78	1.93	5.99	2S,3R4.96	2.49	9.04	3S,3R5.06	2.51	8.70	90/5/5
				2S,3R1.41	2.47	7.36	2S,3R1.79	2.54	7.78	80/10/10
17	1S,2R2.73	1.65	1.99	1S,2R1.49	1.74	2.53	1S,2R2.14	1.68	2.61	90/5/5
				1S,2R0.36	1.52	1.50	1S,2R0.82	1.47	1.87	80/10/10
18	Retention time>80min			Retention time>80min			Retention time>80min			90/5/5
				+7.36	1.61	3.50	+7.25	1.49	3.21	80/10/10
19	Retention time>80min			Retention time>80min			Retention time>80min			90/5/5
				+7.60	1.61	4.57	+7.40	1.50	3.86	80/10/10

Table 3. The comparison of the enantioseparation of chiral compounds resolved by CSP 5 analyzed with various mobile phases

Analytes	n-Hexane/isopropanol(90/10)			n-Hexane/$CHCl_3$/isopropanol			n-Hexane/AcOEt/isopropanol			Composition
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	proportion
1	0.41	1.00	0	0.32	1.00	0	0.35	1.00	0	90/5/5
				0.18	1.00	0	0.24	1.00	0	80/10/10
2	2.90	1.00	0	^{S}3.96	1.05	0.25	4.01	1.00	0	90/5/5
				1.09	1.00	0	1.18	1.00	0	80/10/10
3	^{R}7.44	1.07	0.90	10.01	1.00	0	^{R}9.54	1.05	0.58	90/5/5
				3.55	1.00	0	3.53	1.00	0	80/10/10
4	^{R}1.55	1.09	0.60	^{R}2.39	1.08	0.56	2.22	1.00	0	90/5/5
				^{R}0.92	1.10	0.62	0.96	1.00	0	80/10/10
5	^{R}2.27	1.25	1.81	^{R}2.03	1.31	1.77	^{R}1.86	1.29	1.65	90/5/5
				^{R}0.75	1.31	1.72	^{R}0.73	1.27	1.02	80/10/10
6	^{R}4.49	1.09	0.71	^{R}8.13	1.06	0.45	6.03	1.00	0	90/5/5
				^{R}2.23	1.07	0.55	1.77	1.00	0	80/10/10
7	Retention time>80min			Retention time>80min			^{S}20.65	1.09	0.78	90/5/5
				^{S}14.91	1.18	1.55	^{S}5.69	1.05	0.49	80/10/10
8	^{R}2.13	1.16	1.41	^{R}1.84	1.24	1.60	^{R}1.79	1.20	1.09	90/5/5
				^{R}0.66	1.23	1.39	^{R}0.72	1.20	0.73	80/10/10
9	^{R}4.11	1.32	2.56	^{R}7.27	1.17	1.25	^{R}4.94	1.19	1.45	90/5/5
				^{R}1.92	1.04	0.30	^{R}1.62	1.06	0.34	80/10/10
10	$^{+}$0.49	1.36	1.53	$^{+}$0.37	1.31	1.57	$^{+}$0.41	1.34	1.44	90/5/5
				$^{+}$0.19	1.38	1.18	$^{+}$0.31	1.31	1.11	80/10/10
11	1.24	1.00	0	1.79	1.00	0	1.45	1.00	0	90/5/5
				0.81	1.00	0	0.70	1.00	0	80/10/10
12	$^{+}$2.34	1.10	1.15	$^{+}$1.96	1.09	0.96	$^{+}$2.61	1.13	1.42	90/5/5
				$^{+}$0.76	1.06	0.53	$^{+}$1.25	1.13	1.14	80/10/10
13	$^{-}$0.39	1.42	1.38	$^{-}$0.35	1.47	2.82	$^{-}$0.34	1.25	1.45	90/5/5
				$^{-}$0.24	1.57	1.92	$^{-}$0.27	1.23	0.66	80/10/10
14	12.77	1.00	0	^{S}14.68	1.02	0.21	^{S}18.68	1.08	0.64	90/5/5
				3.30	1.00	0	^{S}5.54	1.09	0.65	80/10/10
15	1.45	1.00	0	1.34	1.00	0	1.25	1.00	0	90/5/5
				0.51	1.00	0	0.57	1.00	0	80/10/10
16	^{2S,3R}10.25	1.53	5.10	^{2S,3R}10.73	1.65	6.37	^{2S,3R}13.56	1.62	6.29	90/5/5
				^{2S,3R}3.48	1.57	4.84	^{2S,3R}5.37	1.48	4.64	80/10/10
17	^{1S,2R} 1.97	3.08	10.24	^{1S,2R} 1.40	3.99	10.31	^{1S,2R} 2.00	2.85	8.88	90/5/5
				^{1S,2R} 0.40	4.00	6.74	^{1S,2R} 0.80	2.46	6.00	80/10/10
18	Retention time>80min			Retention time>80min			Retention time>80min			90/5/5
				$^{-}$7.66	1.35	2.41	$^{-}$7.00	1.22	1.60	80/10/10
19	Retention time>80min			Retention time>80min			Retention time>80min			90/5/5
				$^{-}$8.00	1.34	3.06	$^{-}$7.22	1.21	1.97	80/10/10

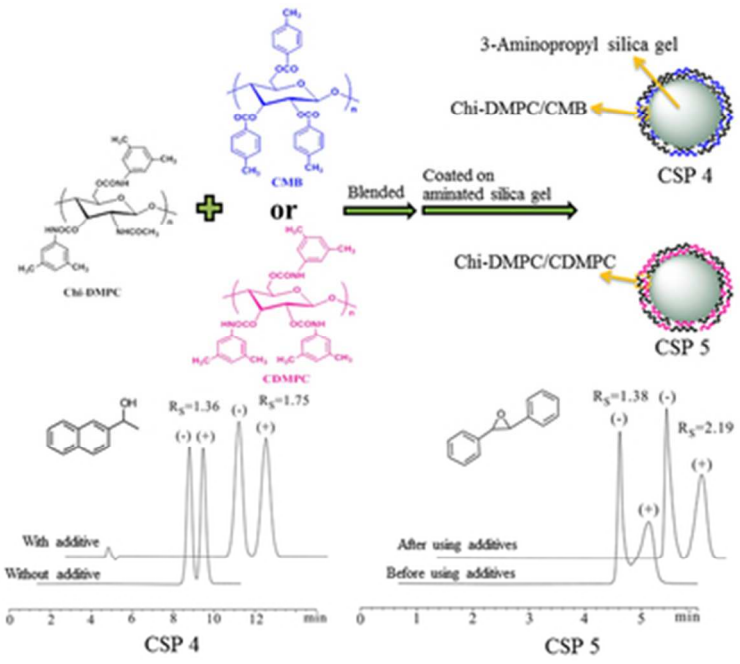
Table 4. Comparison in resolution of compounds separated twice by CSPs 4 and 5 analyzed with the same mobile phases

Analytes	CSP 4						CSP 5					
	Before analyzing with additives			After analyzing with additives			Before analyzing with additives			After analyzing with additives		
	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s	k_1	α	R_s
1	$^{-}$0.93	1.22	1.08	$^{-}$0.95	1.23	1.33	0.41	1.00	0	0.38	1.00	0
2	3.59	1.00	0	4.70	1.00	0	2.90	1.00	0	3.40	1.00	0
3	^{R}6.64	1.14	0.95	^{R}6.54	1.17	1.40	^{R}7.44	1.07	0.90	^{R}7.11	1.11	0.91
4	1.62	1.00	0	1.78	1.00	0	^{R}1.55	1.09	0.60	^{R}1.56	1.08	0.59
5	3.04	1.00	0	3.02	1.00	0	^{R}2.27	1.25	1.81	^{R}2.20	1.27	1.95
6	4.11	1.00	0	4.13	1.00	0	^{R}4.49	1.09	0.71	^{R}4.65	1.05	0.50
7	Retention time>80min			Retention time>80min			Retention time>80min			Retention time>80min		
8	^{R}2.74	1.09	0.60	^{R}2.76	1.09	0.61	^{R}2.13	1.16	1.41	^{R}2.08	1.17	1.54
9	^{S}2.80	1.16	0.77	^{S}2.87	1.16	0.82	^{R}4.11	1.32	2.56	^{R}4.37	1.29	2.47
10	$^{+}$0.53	5.98	6.43	$^{+}$0.53	6.72	7.84	$^{+}$0.49	1.36	1.53	$^{+}$0.47	1.34	1.46
11	$^{-}$1.54	1.14	1.36	$^{-}$1.60	1.25	2.02	1.24	1.00	0	1.22	1.00	0
12	2.04	1.00	0	2.01	1.00	0	$^{+}$2.34	1.10	1.15	$^{+}$2.16	1.11	1.36
13	$^{+}$0.60	1.33	1.97	$^{+}$0.60	1.33	2.02	$^{-}$0.39	1.42	1.38	$^{-}$0.36	1.54	2.19
14	^{S}10.11	1.10	0.58	^{S}10.24	1.09	0.60	12.77	1.00	0	11.99	1.00	0
15	1.52	1.00	0	1.82	1.00	0	1.45	1.00	0	1.51	1.00	0
16	^{2S,3R}5.78	1.93	5.99	^{2S,3R}6.04	1.99	6.47	^{2S,3R}10.25	1.53	5.10	^{2S,3R}10.14	1.59	5.91
17	^{1S,2R}2.73	1.65	1.99	^{1S,2R}2.69	1.72	2.62	^{1S,2R}1.97	3.08	10.24	^{1S,2R}1.89	3.34	10.40
18	Retention time>80min			Retention time>80min			Retention time>80min			Retention time>80min		
19	Retention time>80min			Retention time>80min			Retention time>80min			Retention time>80min		

Eluent: hexane/isopropanol 90/10, v/v;

Table 5. Reproducibility of CSP 4 and CSP 5

CSP	Compound 10	Time (RSD%, n=5)		Peak area (RSD%, n=5)	
		Intra-day	Inter-day	Intra-day	Inter-day
CSP 4	+	0.16%	0.46%	1.09%	1.85%
	−	0.61%	1.47%	1.71%	1.71%
CSP 5	+	0.05%	0.10%	0.75%	1.38%
	−	0.04%	0.18%	0.96%	1.44%



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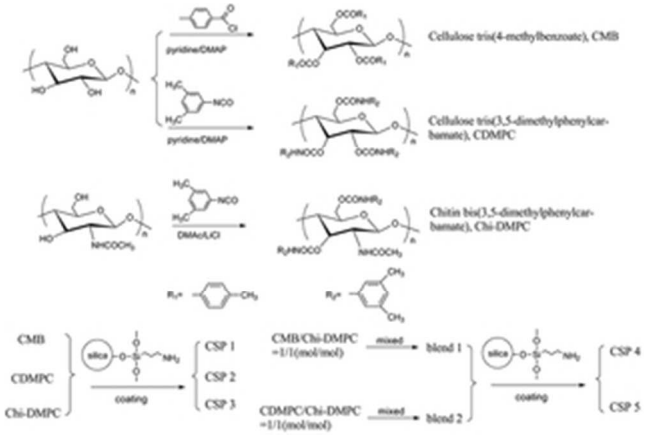

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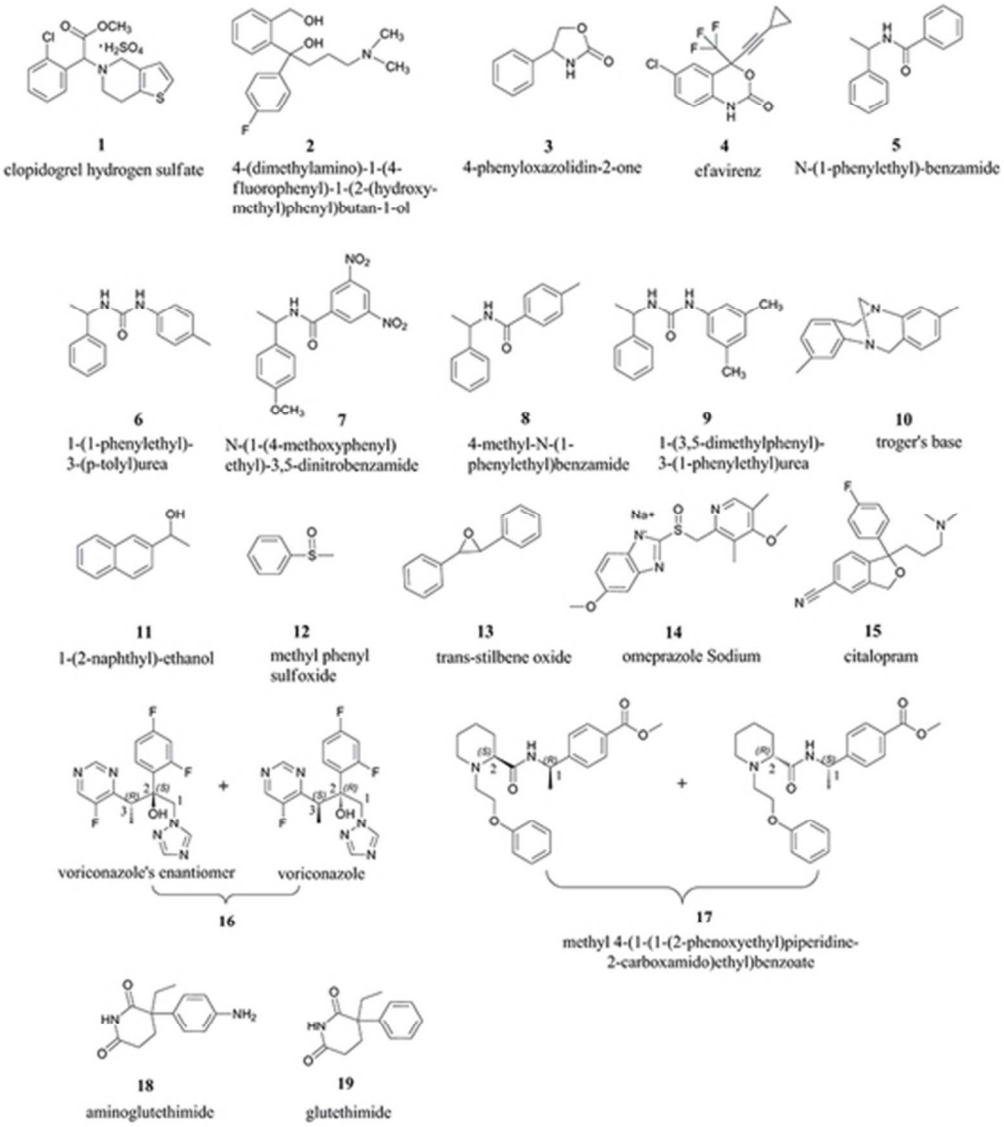

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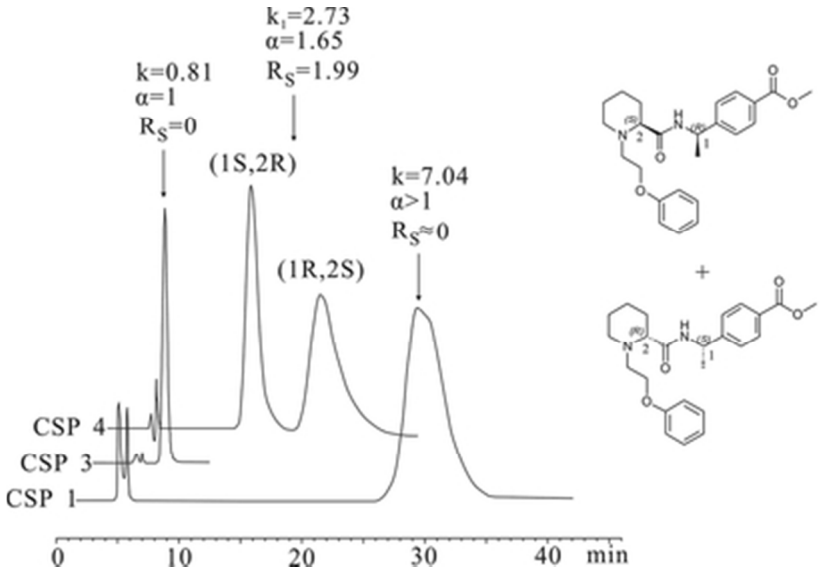

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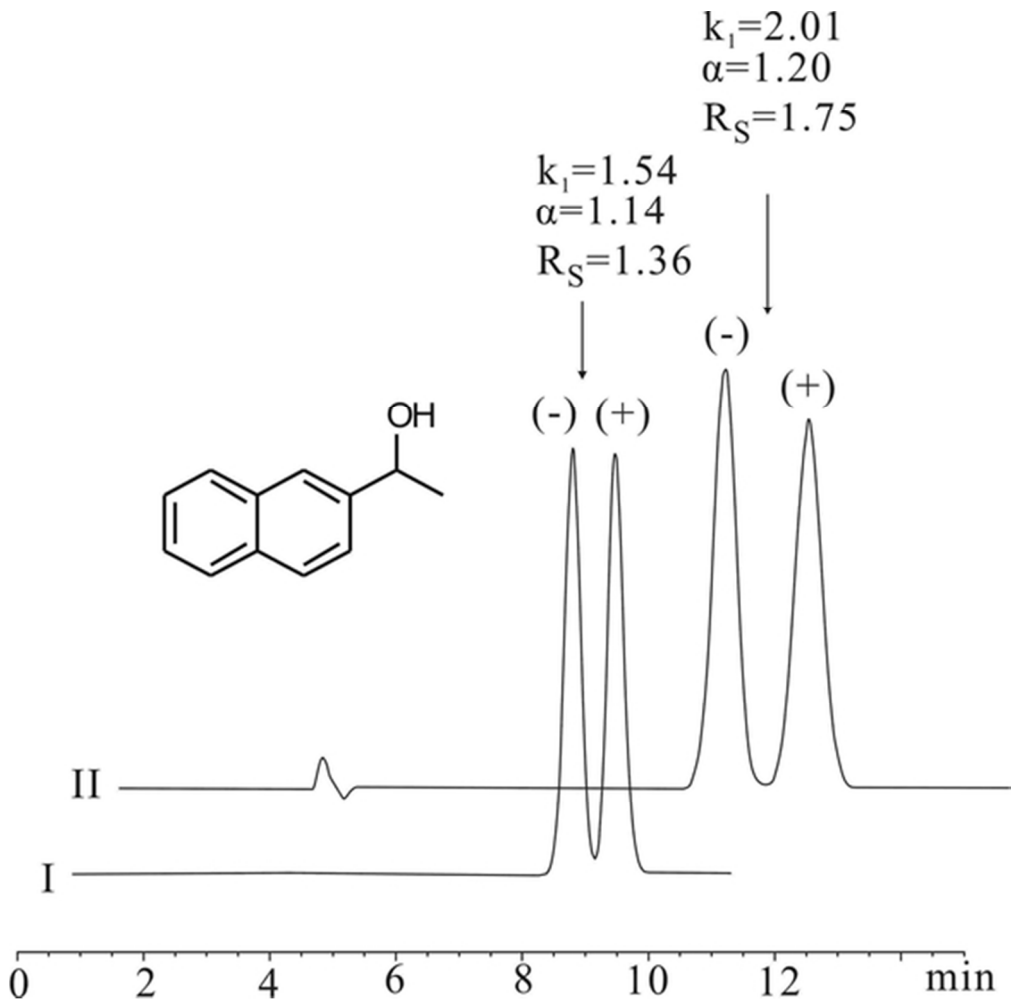

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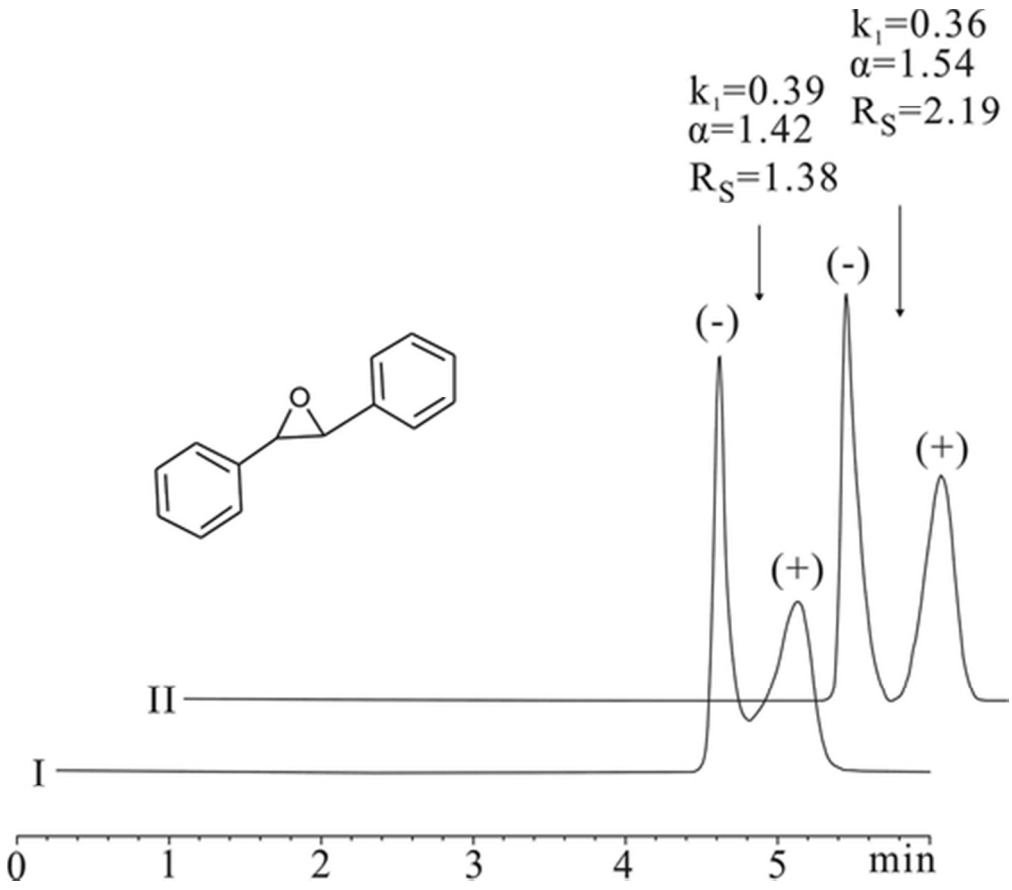

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