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Journal of Chromatography A, 1059 (2004) 53–59
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Enantioseparation on mono(6^A-*N*-allylamino-6^A-deoxy)permethylated β-cyclodextrin covalently bonded silica gel

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Received 24 March 2004; received in revised form 8 July 2004; accepted 9 July 2004

Abstract

A chiral selector, mono(6^A-*N*-allylamino-6^A-deoxy)permethylated β-cyclodextrin, was synthesized through a facile synthetic route and chemically immobilized onto porous silica gel via hydrosilylation to afford a cyclodextrin based chiral stationary phase MeCD-CSP. This chiral stationary phase exhibited good enantioselectivity under standard HPLC conditions. The optimal resolution of 1-(*p*-bromophenyl)ethanol and bromopheniramine was achieved under normal-phase conditions using a mobile phase comprising *n*-hexane (hexane) and 2-propanol (IPA). The enantioseparation of warfarin, suprofen and a series of flavanones under reversed-phase conditions were optimized and efficient enantioseparations for these analytes were obtained.

Keywords: Permethylated cyclodextrin; Chiral stationary phases; Liquid chromatography; Flavanone

1. Introduction

A great deal of progress has been made in the area of regulations and requirements for chiral compounds since the first international symposium on chiral separations was held in Paris in 1988 [1]. The increased demand for enantiopure compounds, especially those of pharmaceutical importance, has led to the rapid development of a variety of stereoselective separation technologies [2,3]. Direct enantiomeric separation by chiral stationary phases (CSPs) in high-performance liquid chromatography (HPLC) [4–9] remains one of the most important techniques for analysis of enantiomeric purity as well as the convenient access to enantiomerically pure materials.

Cyclodextrins (CDs) are cyclic oligomers of α-D-glucose bonded through α-(1,4) linkages; the shape of a CD molecule is similar to a truncated cone with a cavity [10,11]. To obtain cyclodextrins with desired properties, many modified CDs have been prepared from their native forms [12,13]. The native and the derivatized CDs are predominant as selectors in the majority of enantioselective separation techniques due to their special molecule structures.

In the past decade, permethylated CDs have been widely used as the chiral stationary phase in all chromatographic enantioseparation methods and good enantioseparation performance was achieved on these permethylated CD-CSPs. Schurig and co-workers studied permethylated cyclodextrins and successfully applied the derivatives as chiral stationary phases in capillary supercritical fluid chromatography (CSFC) [14,15], open capillary electrochromatography (o-CEC) [16], pressure-assisted micro-packed capillary electrochromatography (CEC) [17], gas–liquid chromatography (GLC) [18] and gas chromatography (GC) [19–21]. In 1994, Ciucanu and Konig reported a preparation method for mono-*O*-5-pent-1-enyl permethylated β-cyclodextrin and its chiral separation properties by high-performance liquid chromatography [22]. Thereafter, Ciucanu [23] and Konig and co-workers [24] reported some more applications of permethylated β-cyclodextrin in enantioselective HPLC. However, the preparation of these chiral stationary phases involved complicated purification or multistep protection/deprotection of

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doi:10.1016/j.chroma.2004.07.018

hydroxyl groups on cyclodextrin, which made the synthesis routes tedious with diminished yields of products. We report herein a facile and convenient synthetic route into MeCD-CSP, a novel CSP based on permethylated β-cyclodextrin, and the ensuing enantioseparations/optimization studies for a number of racemic compounds.

As part of a systemic program aimed to exploit CD derivatives as the chiral selectors in enantioselective HPLC, we have studied the facile synthesis of monosubstituted CDs [25–27]. Recently, Ng et al. [28] synthesized a novel methylated β-cyclodextrin chiral stationary phase, which was chemically bonded onto aminized silica via multiple urea-linkages; the methylated CD-CSP depicted good enantioseparation ability towards some well-known flavors and fragrance compounds using HPLC under reversed-phase conditions. However, the use of aminized silica in this procedure [28] would invariably result in remnant unreacted amine moieties on the silica gel surface. The presence of free amine groups on the surface may be undesirable under some conditions because they may interact with analytes through H-bonding. To solve this problem, we reported [29,30] a convenient synthesis for the CSP using mono(6^A-*N*-allylamino-6^A-deoxy)perphenylcarbamoylated β-CD as the chiral selector immobilized via hydrosilylation; this CSP exhibited accentuated enantioseparation abilities towards some racemic compounds.

Given the impetus stated above, we proceeded to investigate a facile synthesis of mono(6^A-*N*-allylamino-6^A-deoxy)permethylated β-CD to afford the target chiral stationary phases MeCD-CSP, which is anticipated to depict complementary enantioseparation ability in comparison to the perphenylcarbamate analogue [30,31]. Efficient chiral separations for a wide range of analytes on this CSP were demonstrated and the separation conditions optimized under normal phase as well as reverse-phase mode.

2. Experimental

2.1. Apparatus and reagents

FT-IR spectra were performed on a Bio-Rad TFS156 instrument using KBr pellets. Elemental analysis was measured on a Perkin-Elmer 2400 CHN analyzer. The employed HPLC system comprised of a Perkin-Elmer series 200 LC pump and a Perkin-Elmer 785A UV–vis detector connected to a computer via Perkin-Elmer Nelson 900 series interface and 600 series link. The CSP was packed into the empty column by following a conventional high-pressure slurry packing procedure using an Alltech air compression pump (Alltech, USA). All the chromatograms were obtained at ambient temperatures.

Silica gel used for HPLC was supplied by Kromasil with a mean pore size of 100 Å, particle size of 5 μm and surface area of 300 m^2/g. Empty stainless steel HPLC columns (250 mm × 4.6 mm) were purchased from Phenomenex (USA). Triethylamine and β-cyclodextrin were obtained from Fluka (Buchs, Switzerland). Allylamine was purchased from TCI, Tokyo. Tetrakis(triphenylphosphine)platinum(0) and sodium hydride were purchased from Alfa Aesar (Germany). Triethoxysilane was supplied by Aldrich (USA) and iodomethane was purchased from Merck. Tetrahydrofuran (THF) was dried by refluxing over sodium for 4 h before use, cyclodextrin and all other reagents were used without further purification. 1-(*p*-Bromophenyl)ethanol was prepared by reducing the corresponding ketone with $LiAlH_4$ in anhydrous THF, the other racemic samples were obtained from Sigma–Aldrich.

2.2. Packing the column

The slurry method (using CCl_4–dioxane) was applied to pack the derived CSP into HPLC columns using methanol as the packing solvent. After suspending the CSP (3.5 g) in CCl_4–dioxane (20/10 ml) and sonication for 20 min, the CSP slurry was packed into the stainless steel column at a pressure of 7800 psi maintained for 20 min before gradual release of the pressure (1 psi = 6894.76 Pa). The column was conditioned with mobile phase before use.

2.3. Preparing the buffer system

Triethylammonium acetate (TEAA) buffer, prepared using 1% aqueous triethylamine, was adjusted by addition of glacial acetic acid to the desired pH. The mobile phase, comprising of triethylammonium acetate buffer and the appropriate amount of the organic modifier, methanol or acetonitrile, was freshly prepared, filtered and sonicated for 30 min before use and degassed on-line using a Degasys DG-2410 degasser during HPLC running.

2.4. Synthesis of mono(6^A-*N*-allylamino-6^A-deoxy)permethylated β-cyclodextrin covalently bonded silica gel

Fig. 1 depicts the synthetic route applied in this paper. As we have reported previously [32], mono-tosylated β-CD (**1**) can be conveniently permethylated to afford the key intermediate mono[6^A-*O*-(*p*-tolylsulfonyl)]permethylated β-CD (**2**). Thereafter, the *p*-tolylsulfonyl-oxy group of **2** was substituted by allylamine to afford the desired compound **3** under mild conditions in good yields. Hydrosilylation of **3** with $(EtO)_3SiH$ in the presence of catalytic amount of tetrakis(triphenylphosphine)platinum(0) gave the reactive siloxane **4**, which was directly immobilized onto the surface of silica gel to afford the target MeCD-CSP.

The carbon content in the elemental analysis (C, 8.01%; H, 1.91%) of MeCD-CSP shows that the cyclodextrin moiety has been successfully immobilized onto the surface of the porous silica gel. According to the microanalysis data, the surface concentration [33] of the cyclodextrin derivative on the silica gel is determined to be 0.4 μmol/m^2. The resultant

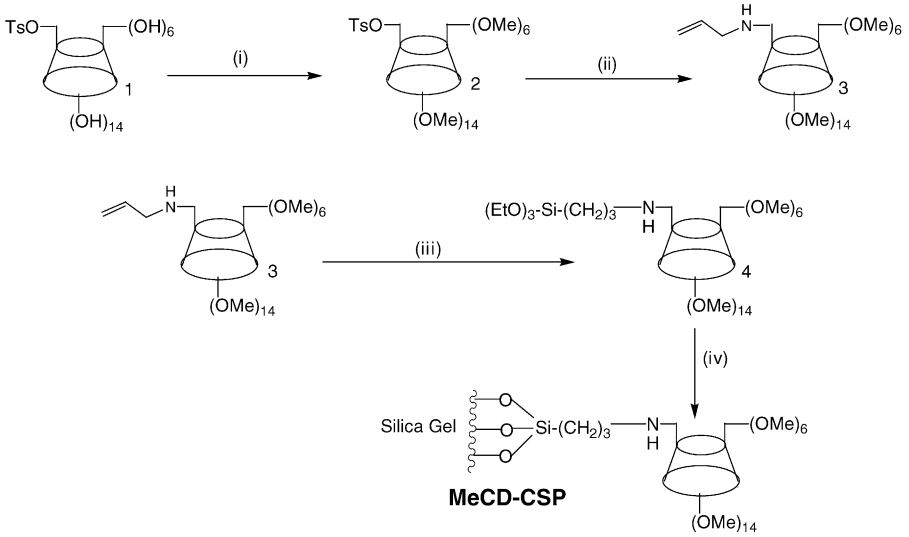


Fig. 1. Synthesis of the target chiral stationary phase MeCD-CSP. *Reagents and conditions*: (i) MeI/DMF/NaH; (ii) $CH_2{=}CHCH_2NH_2/\Delta$; (iii) $(EtO)_3SiH$/cat. $Pt(PPh_3)_4$/THF/Δ; (iv) silica gel/$CH_3C_6H_5/\Delta$.

column gave efficiency of 39 000 plates/m using biphenyl as testing sample under normal-phase condition (*n*-hexane (hexane) and 2-propanol (IPA) in 90/10 (v/v) ratio, 0.8 ml/min).

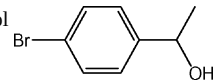
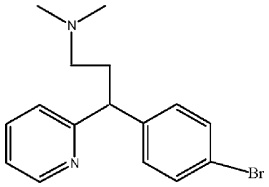
3. Results and discussion

3.1. Influence of flow rate and mobile phase composition on the enantioselectivity under normal-phase conditions

Table 1 depicts the enantioseparation results for 1-(*p*-bromophenyl)ethanol and bromopheniramine on MeCD-CSP under normal-phase conditions. The volume ratio of hexane–IPA was varied from 90:10 (conditions a and b) to 97:3 (conditions c and d). Regarding the enantiomeric discrimination of 1-(*p*-bromophenyl)ethanol, it is remarkable that better separation (α) and resolution (R_s) were achieved under condition c than that under condition b (see Table 1). This indicates that suitable adjustment of the content of polar modifier in the mobile phase may improve the chiral separation. Similar phenomenon was observed in the enantioseparation of bromopheniramine. Satisfactory enantioselectivity of bromopheniramine was effected under conditions c and d while no separation was observed under conditions a and b.

It is further of interest to note that the mass transport of the solutes between the mobile and the stationary phases is different under different conditions. Fig. 2 depicts the chromatograms of 1-(*p*-bromophenyl)ethanol under different separation conditions. Comparison of the chromatograms under conditions c and d, obvious peak aberrance occurred under flow rate of 0.8 ml/min while improved peak shape was afforded under flow rate of 1.0 ml/min. Improved enantioselectivity was also observed for bromopheniramine under flow rate of 1.0 ml/min (see Table 1). This suggests that the condition d may be an optimum separation condition for MeCD-CSP under normal-phase conditions.

Table 1
Enantioseparation under normal-phase conditions on MeCD-CSP

Compound	Chromatographic result						
	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s	Condition
1-(*p*-Bromphenyl)ethanol 	9.8	11.0	0.66	0.86	1.30	1.33	a
	6.2	7.0	0.63	0.84	1.33	1.44	b
	9.8	12.2	1.39	1.98	1.42	2.19	c
	8.0	9.9	1.42	2.0	1.41	2.27	d
Bromopheniramine 	10.7	10.7	0.81	0.81	∼1	/	a
	6.8	6.8	0.79	0.79	∼1	/	b
	8.0	9.6	0.95	1.34	1.41	2.44	c
	6.5	7.90	0.97	1.39	1.43	2.45	d

Conditions: (a) hexane–IPA (90:10), flow rate 0.5 ml/min; (b) hexane–IPA (90:10), flow rate 0.8 ml/min; (c) hexane–IPA (97:3), flow rate 0.8 ml/min; (d) hexane–IPA (97:3), flow rate 1.0 ml/min. (/) No separation was observed under the specified condition.

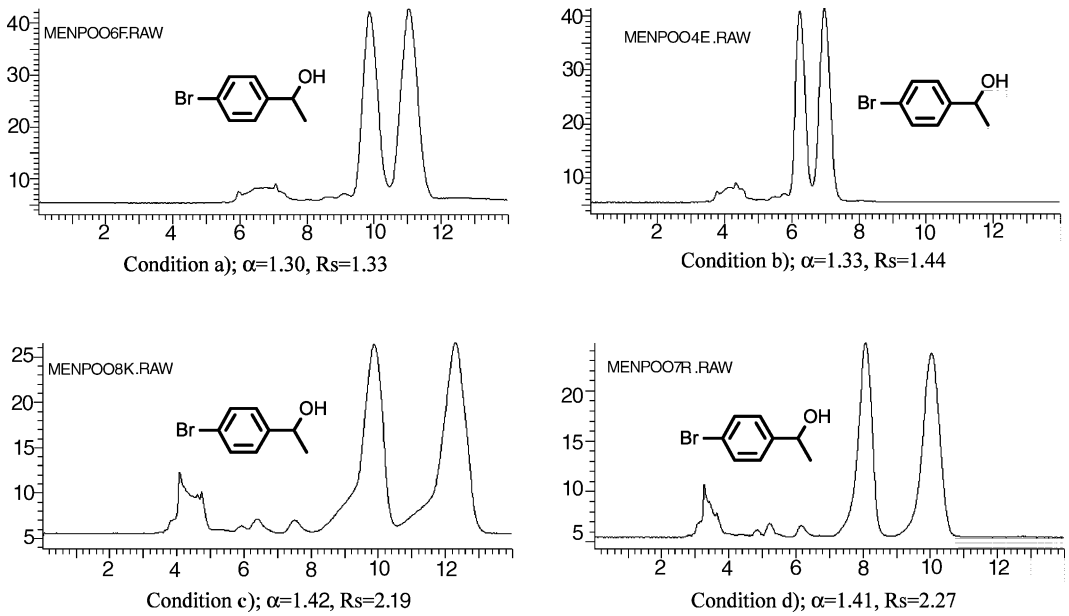


Fig. 2. Chromatograms of 1-(*p*-bromophenyl)ethanol on MeCD-CSP under normal-phase conditions (for separation conditions refer to Table 1).

3.2. Influence of methanol content in mobile phase on the enantioselectivity of warfarin and suprofen under reversed-phase conditions

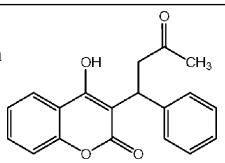
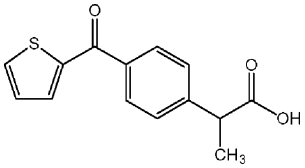
The choice of an appropriate eluent for a certain solute is an important part of method development and optimization. Table 2 demonstrates that warfarin and suprofen can be successfully resolved into their respective enantiomers on MeCD-CSP under reversed-phase conditions with different eluent compositions.

It also can be seen that, when methanol/aqueous buffer mixture was used as eluent on MeCD-CSP column, the methanol concentration dramatically influences the enantioseparation of warfarin and suprofen on this column (see Table 2). For warfarin, decreased α and R_s values were observed when increasing the methanol content. For suprofen, however, α and R_s values increase as methanol content increases. This means that the enantioselectivity of MeCD-CSP is distinctly influenced by the concentration of organic modifier under reversed-phase conditions; different changing trends may occur for different solutes.

3.3. Optimization of the chromatographic conditions for enantioseparation of flavanones under reversed-phase conditions

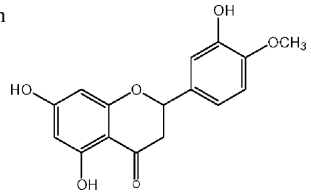
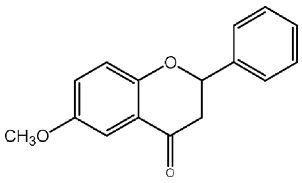
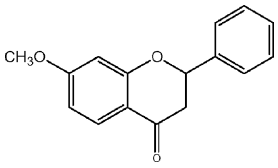
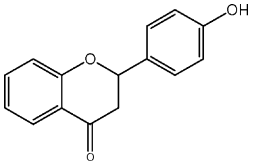
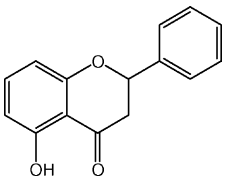
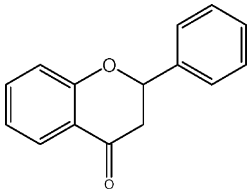
Flavanones are natural compounds commonly occurring in plants. They belong to the large group of flavonoids widely distributed in a variety of edible plant sources such as fruits, vegetables, nuts, seeds, grains, tea, and wine. The numerous health-related properties of flavonoids world include exhibition of anti-inflammatory and antiviral activities, inhibition of human platelets aggregation, and anticancer activity [34].

Table 2
Effect of methanol content on the enantioseparation on MeCD-CSP

Compound	Chromatographic result						
	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s	Condition
Warfarin 	20.9	22.6	2.43	2.70	1.11	1.0	i
	25.4	27.6	2.91	3.25	1.12	1.06	ii
	33.2	36.9	4.11	4.68	1.14	1.2	iii
Suprofen 	37.5	45.3	5.15	6.43	1.25	1.89	i
	46.5	56.0	6.15	7.62	1.24	1.61	ii
	62.0	74.0	8.54	10.38	1.22	0.96	iii

Conditions: flow rate 0.5 ml/min, buffer (1% TEAA, pH 5.5); (i) buffer–methanol (65:35); (ii) buffer–methanol (75:25); (iii) buffer–methanol (85:15).

Table 3
Enantioseparation of flavanones on MeCD-CSP under reversed-phase conditions

Compound	Chromatographic result						
	t_1 (min)	t_2 (min)	k_1	k_2	α	R_s	Condition
Hesperetin 	112.5	134.0	16.31	19.62	1.20	1.88	1
	67.0	78.0	9.98	11.79	1.18	1.63	2
	24.8	28.0	3.20	3.75	1.17	1.33	3
	128.5	141.2	16.13	17.83	1.11	0.50	4
	75.8	84.0	9.83	11.0	1.12	0.56	5
	43.9	48.3	5.46	6.10	1.12	0.44	6
	18.4	19.7	2.17	2.40	1.11	0.49	7
	86.0	95.0	12.23	13.62	1.11	0.45	8
	26.7	29.0	3.05	3.39	1.11	0.47	9
6-Methoxyflavanone 	43.5	49.2	5.69	6.57	1.15	1.31	1
	29.5	32.5	3.84	4.33	1.13	1.15	2
	13.4	14.4	1.27	1.44	1.13	0.59	3
	46.5	54.1	5.20	6.21	1.19	1.29	4
	27.0	30.6	2.86	3.37	1.18	1.19	5
	17.4	19.0	1.56	1.79	1.15	0.97	6
	10.7	11.3	0.84	0.95	1.13	0.58	7
	36.5	41.2	4.62	5.34	1.16	0.76	8
	18.2	19.6	1.76	1.97	1.12	0.73	9
7-Methoxyflavanone 	50.3	62.8	6.74	8.66	1.28	2.28	1
	33.4	40.6	4.48	5.66	1.26	2.06	2
	14.7	16.7	1.49	1.83	1.23	1.61	3
	54.2	68.2	6.23	8.09	1.30	1.67	4
	31.5	38.6	3.50	4.51	1.29	1.66	5
	19.6	23.4	1.88	2.44	1.30	1.74	6
	11.0	12.2	0.90	1.10	1.22	1.33	7
	41.0	49.0	5.31	6.54	1.23	0.90	8
	19.3	22.0	1.92	2.33	1.21	1.02	9
4′-Hydroxyflavanone 	46.0	49.5	6.08	6.62	1.09	0.81	1
	38.1	41.0	5.25	5.72	1.09	0.93	2
	14.4	15.2	1.44	1.58	1.10	0.43	3
	53.5	57.8	6.13	6.71	1.09	0.59	4
	31.0	33.3	3.43	3.76	1.10	0.76	5
	19.9	21.1	1.93	2.10	1.09	0.65	6
	11.3	11.8	0.95	1.03	1.08	0.39	7
	35.1	37.2	4.40	4.72	1.07	0.78	8
	17.0	17.6	1.58	1.67	1.06	0.52	9
6-Hydroxyflavanone 	45.2	50.8	5.95	6.82	1.15	1.45	1
	36.4	39.5	4.97	5.48	1.10	1.20	2
	14.1	14.7	1.39	1.49	1.07	0.42	3
	47.2	54.1	5.29	6.21	1.17	1.27	4
	27.8	30.8	2.97	3.40	1.14	1.27	5
	18.4	19.8	1.71	1.91	1.12	0.94	6
	10.7	10.7	0.84	0.84	1.0	/	7
	32.0	36.5	3.92	4.61	1.18	1.21	8
	16.6	18.0	1.52	1.73	1.14	1.15	9
Flavanone 	37.5	41.4	4.77	5.37	1.13	1.31	1
	31.8	34.5	4.21	4.66	1.11	0.95	2
	13.5	14.1	1.29	1.39	1.08	0.32	3
	36.0	40.9	3.80	4.45	1.17	1.07	4
	23.2	25.7	2.31	2.67	1.16	1.29	5
	16.4	17.7	1.41	1.60	1.13	0.90	6
	10.1	10.1	0.74	0.74	1.0	/	7
	28.2	31.2	3.34	3.80	1.14	0.72	8
	16.7	17.7	1.53	1.68	1.10	0.68	9

Conditions: flow rate 0.5 ml/min, aqueous buffer (1% aqueous TEAA, pH 5.5); (1) methanol–buffer (25:75); (2) methanol–buffer (35:65); (3) methanol–buffer (50:50); (4) methanol–H_2O (15:85); (5) methanol–H_2O (25:75); (6) methanol–H_2O (35:65); (7) methanol–H_2O (55:45); (8) acetonitrile–H_2O (5:95); (9) acetonitrile–H_2O (15:85). (/) No separation was observed under the specified condition.

The enantioseparations of flavanones in HPLC system have been effected using cellulose triacetate and β-cyclodextrin (Cyclobond I) as chiral stationary phases [35,36]. However, a derivatization step was required in these separation and different flavanones could not be resolved on a certain column [35]. A direct separation of enantiomers without the derivatization step would obviously be advantageous and convenient. We have previously reported on a permethylated CD-based CSP immobilized onto aminized silica via multiple urea-linkages, which depicted good enantioseparation ability towards some well-known flavors and fragrance compounds under reversed-phase conditions [28]. To overcome the limitation of the free amino groups presented on the surface of aminized silica gel, we report herein the enantioseparation of six flavanones on MeCD-CSP under reversed-phase conditions. The chromatographic results under different separation conditions are given in Table 3.

Chromatographic separation was first attempted using methanol/aqueous buffer mobile phases. The volume ratio of methanol–buffer was varied from 25:75 (condition 1) to 35:65 (condition 2) to 50:50 (condition 3). From the data detailed in Table 3, for all the six flavanones, the respective enantiomers were resolved using conditions 1–3. Hence, we proceeded to investigate the usage of a simpler eluent: methanol–water (conditions 4–7). Except for flavanone and 6-hydroxyflavanone, which could not be separated using condition 7, resolution was achievable when using methanol–water as the mobile phase, although the retention time is quite long unless high methanol contents were applied. Consequently, acetonitrile was used to shorten the retention time (conditions 8 and 9).

Generally, MeCD-CSP depicted enantioseparation abilities toward all the six flavanones under all the nine chromatographic conditions. It should be noted that all the flavanones except 4′-hydroxyflavanone achieved the highest R_s value under condition 1 (methanol–buffer, 25:75), and three of the six racemates achieved the highest α value under condition 4 (methanol–water, 15:85). This indicates that conditions 1 and 4 might be the universal optimum conditions on MeCD-CSP for separation of flavanones under reversed-phase conditions.

3.3.1. Concentration of the organic modifier

As we have discussed in Section 3.2, the concentration of the organic modifier affected the enantioseparation significantly. Unlike the enantioseparation of warfarin and suprofen, which had opposite trend with increasing organic modifier content, the enantioseparation of flavanones had very similar trends upon changing the modifier content with a little exception. Comparing the separation of all the six flavanones under conditions 1–3, 4–7, and 8–9 (corresponding to increasing organic modifier contents in Table 3), the separation factor (α) and the resolution (R_s) generally decreases.

3.3.2. Types of the organic modifier

Comparing the chromatographic data using condition 4 (methanol–water, 15:85) and condition 9 (acetonitrile–water, 15:85), we can clearly see the influence of different organic modifiers on the enantioseparation of flavanones on MeCD-CSP. It is remarkable that the retention times were greatly reduced although the enantioselectivity (α and R_s value) also decreases when acetonitrile with same percentage was used. It is noteworthy that, for Hesperetin, the retention time is 128.5 and 141.2 min under condition 4 but falls dramatically to 26.7 and 29.0 min with almost the same α and R_s value under condition 9. A similar observation was found on 4′-hydroxyflavanone. This shows that it is feasible to achieve an acceptable resolution within short analysis time by simply changing the organic modifier.

3.3.3. Application of buffer system or water

A buffer is usually used in HPLC eluent system to adjust the ionic strength and pH value of mobile phase aiming at achieving better separation for ionizable analytes. In addition, the hydrophobic nature of analyte may be modified by the buffer composition and thus influences the inclusion-complexation with CD [37]. Perrin et al. [38] reported that ionization of analytes leads to reduced interaction of the analytes with CD-bonded CSPs because these CSPs do not possess ionic sites susceptible to interact with the charged analytes. Consequently, it is of fundamental importance to keep the analytes neutral when working with CD-bonded CSPs under aqueous mobile system.

Amongst the flavanones involved here, Hesperetin with multiple hydroxyl groups in molecular structure is a weakly acidic compound in nature whereas the other flavanones are relatively neutral compounds. As it can be expected, using buffer or water affects the enantioseparation of Hesperetin greatly while there is less significant effect on separation of the other flavanones. For Hesperetin, it is not difficult to find that the α and R_s value are obviously higher under buffer conditions (1 and 2) than that under water conditions (5 and 6). This may be due to the acidic buffer system restraining ionization of the acidic Hesperetin in aqueous mobile phase and thus improving its enantioseparation. For the other flavanones, no significant differences in their α value were observed. This indicates that similar enantioselectivity may be obtained under both water and buffer systems. However, it is also should be pointed out that better resolution (R_s) were achieved using buffer systems for these flavanones, which might imply that suitable application of buffer may improve the peak shape and provide better resolution for a given separation.

4. Conclusion

A chiral stationary phase MeCD-CSP was prepared based on the chiral selector mono(6^A-*N*-allylamino-6^A-deoxy)permethylated β-cyclodextrin. This chiral stationary phase exhibited good enantioselectivity under standard HPLC conditions. The enantioseparation conditions were optimized under normal as well as reversed-phase conditions. It was found that hexane–IPA (97:3, v/v) with a flow rate

of 1.0 ml/min may be an optimal separation condition for MeCD-CSP under normal-phase conditions. By controlling the mobile phase composition, the CSP can provide efficient enantioselectivity toward warfarin, suprofen and several flavanones under reversed-phase conditions. Different changing-trends of the enantioseparation were observed on different analytes when varying a certain parameter of the chromatographic conditions. Consequently, it may be necessary to optimize the separation conditions closely according to the target analytes.

Acknowledgements

Research funding from National University of Singapore in support of this work is gratefully acknowledged. X.-H.L. thanks NUS for awarding a research scholarship.

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