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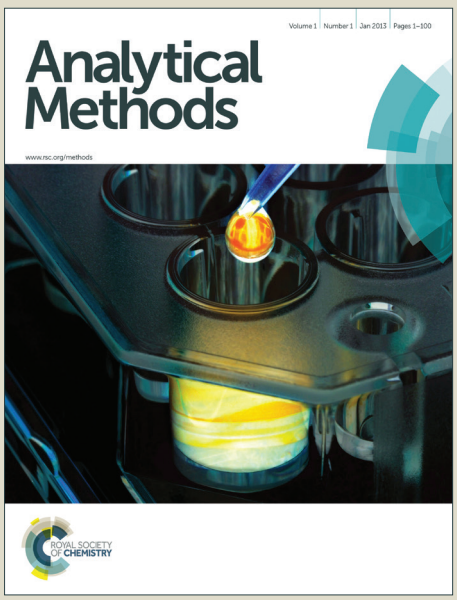

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ARTICLE TYPE

Exploration of β-cyclodextrin clicked chiral stationary phase in high-performance liquid chromatography

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The baseline enantioseparation of 19 racemic drugs including ondansetron, citalopram, galanthamine base, pregabalin, timolol, atenolol, tropicamide, ephedrine hydrochloride, flavanoids and aryl alcohols has been successfully achieved with a cyclodextrin clicked chiral stationary phase in high performance liquid chromatography. The best separation results were presented under optimized conditions. The effect of organic modifiers including methanol and acetonitrile on the enantioseparation of racemates was also studied. Chiral resolutions of 4.19 and 4.42 were achieved for citalopram and tropicamide, respectively.

Chiral separation has attracted extensive research interest in academia and industry, especially for those compounds with biological or pharmaceutical interest.[1,2] High performance liquid chromatography (HPLC) is one of the most important chromatography techniques for enantioseparations. The chiral analysis in HPLC is strongly dependent upon the chiral stationary phases (CSPs) used. A great variety of CSPs have been developed for chiral HPLC, either by chemical bonding of chiral selectors (cellulose, cyclodextrin, protein etc) or by physical coating to supporting materials (ca. silica gel).[1] Among them, the chemical bonding via click chemistry is proven to be an efficient approach in the development of robust CSPs,[3-7] which have been successfully explored for enantioseparations in various chromatography techniques.[8,9]

It is generally acknowledged that there are many factors accounted for the mechanism of enantioseparations in HPLC such as inclusion complexation, π-π interactions, dipole interactions, steric repulsion interactions and hydrogen bonding. For enantioseparation with cyclodextrin (CD) as chiral selector, the capability of forming inclusion complexes with its hydrophobic cavity is regarded as superiority,[10] especially for the racemic compounds containing aromatic rings. The β-CD clicked CSP developed by Zhang et al. exhibited excellent resolutions for flavanone compounds.[11] The improved chiral recognition was attributed to the hydrogen bonding interactions formed between the hydroxyl of CD and oxygen of flavanone. For the separation of racemic amides, four π-acidic CSPs were developed.[12] The resolution was found to be fine-tuned by hydrogen bonding, π-π interaction and steric repulsion interaction besides inclusion complexation. For CD covalently bonded CSPs, the linker between CD and silica support[13] as well as CD type[4] was found to play important role for their enantioselectivities. Mono[6^{A}-N-(ω-alkenylamino)-6^{A}-deoxy]-perphenylcarbamoylated β-CD CSPs were developed with different length spacers via click chemistry. Their enantioseparation results indicated butyl or hexyl chain as linker led to higher possibilities for interactions (ca. hydrogen bonding, π-π interaction or probably dipole interaction) and thus better enantioselectivities. By using click chemistry, a β-CD clicked CSP with triazolyl linkage was developed and exhibited good enantioselectivity towards a variety of racemate.[14]

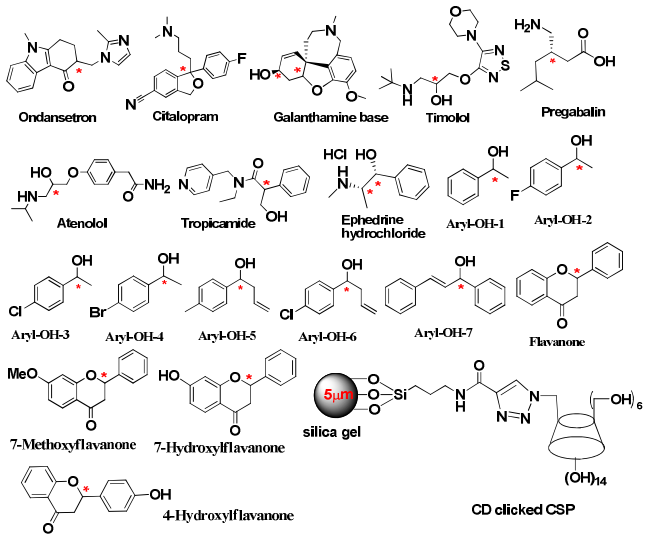

Fig. 1 Structures of the analytes studied and CD clicked CSP.

To explore the potential of the CD clicked CSP for enantioseparation in HPLC, 19 racemic drugs (eight pharmaceutical drugs, four flavanoids and seven aryl alcohols) were selected as model racemates. Chromatographic conditions were tried for each enantiomers in order to achieve baseline separation by adjusting eluent composition, UV detection

wavelength and concentration of organic additives.

The molecular structures of these enantiomers are presented in Fig.1. The HPLC column was packed with the CD clicked CSP, which was synthesized according to the previous literature.[14] The column efficiency was measured to be 9,800 plates/m under normal phase (hexane/isopropanol, 90/10 (v/v), 0.70 mL/min) using biphenyl as the testing compound. The baseline enantioseparation of the selected racemates on the CD clicked CSP was explored by adjusting the eluent composition with organic solvent including methanol (MeOH), acetonitrile (ACN), *n*-hexane and isopropanol (IPA). The baseline separations for 8 medicine racemates were achieved, with separation data listed in Table 1.

Table 1. Enantioseparation data on CD clicked CSP

Racemates	Retention factors (k)	α	R_s	Con.
Ondansetron	k_1=6.104; k_2=6.493	1.06	1.13	I
Citalopram	k_1=1.596; k_2=2.234	1.40	4.19	II
Galanthamine base	k_1=1.766; k_2=2.226	1.26	3.06	III
Pregabalin	k_1=2.057; k_2=2.281	1.11	1.42	IV
Timolol	k_1=1.760; k_2=2.170	1.23	1.30	V
Atenolol	k_1=5.144; k_2=5.868	1.14	1.08	VI
Tropicamide	k_1=2.286; k_2=4.417	1.93	4.42	VII
Ephedrine Hydrochloride	k_1=1.959; k_2=2.480	1.27	2.13	VIII

Conditions: (I) UV 308 nm, MeOH/H_2O=60/40(v/v); (II) UV 240 nm, MeOH/H_2O=80/20(v/v); (III) UV 280 nm, ACN/H_2O=60/40(v/v); (IV) UV 280 nm, MeOH/H_2O=80/20(v/v); (V) UV 303 nm, ACN/MeOH=95/5(v/v); (VI) UV 275 nm, ACN/MeOH/H_2O 2/2/96(v/v/v); (VII) UV 254 nm, MeOH/H_2O=65/35(v/v); (VIII) UV 254 nm, *n*-hexane/IPA=60/40(v/v).

As listed in Table 1, the CD clicked CSP exhibited good enantioresolution ability for citalopram and tropicamide, whose R_s value is as high as 4.19 and 4.42, respectively. As known to us, the inclusion complexation is the main driving force for CD's enantioseparation under reverse phase. The cavity of β-CD fit best with naphthalene-like aromatic rings. Hence, the CD clicked CSP demonstrated better enatioseparation for citalopram, galanthamine base, ephedrine and tropicamide. The high R_s was comparable to those achieved on commercial available CSPs.[1] Relatively low R_s values were observed for single aromatic-ring containing racemates like timolol, atenolol and ephedrine. For atenolol, its enantioseparation on CD clicked CSP was inferior to that on chiralpak OD-H,[15] where a R_s of 4.7 was achieved with tris(3,5-dimethylphenylcarbamate)cellulose based CSP. The better resolution is probably attributed to the formation of strong π-π interactions between dimethylphenylcarbamate groups in chiralpak OD-H and atenolol, together with hydrogen bonding between the -NH, -NH_2 and -OH groups of atenolol and the urea bond on spacer arm of Chiralpak CSP. The typical separation chromatograms for six racemates are shown in Fig. 2. It can be seen that CD clicked CSP demonstrated enantioselectivity in the separation of most the tested compounds under reverse phase conditions except ephedrine hydrochloride.

For ephedrine, different reverse phase conditions were explored but no satisfactory R_s was achieved. However, a R_s of 2.13 was obtained under normal phase with *n*-hexane/IPA (60/40, v/v) as eluent. Since inclusion complexation was absent under normal phase condition, the enantioseparation was probably partially attributed to the hydrogen bonding formed between hydroxyl group of ephedrine and amide group of CD clicked CSPs, besides other driving forces.

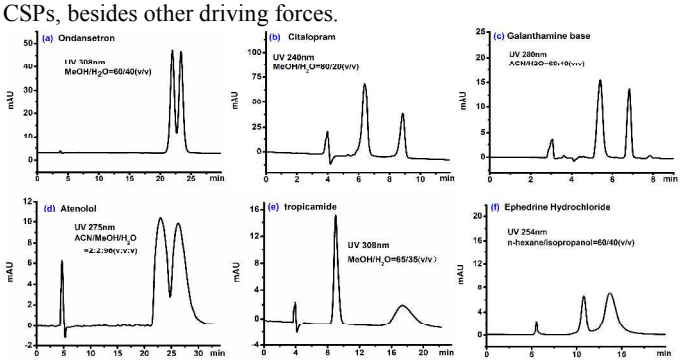

Fig. 2 Chromatograms for six selected racemates on the column packed with CD clicked CSP.

As shown in Fig. 3, the CD clicked CSP could afford good resolution to flavanoids, that may be due to the function of CD cavity and the hydrogen bonding between CSP and flavanoids molecular.[3,11] A close look at the enantioseparation of four flavanoids, the resolutions are 3.16 for flavanone, 2.65 for 7-hydroxylflavanone, 1.87 for 4-hydroxylflavanone and 4.03 for 7-methoxyflavanone, respectively. Because polarity may influence the result of enatioseparation, a flavanoid bearing hydroxy moiety may get lower resolutions while better resolutions can be obtained for flavanoids containing methoxy group. It indicates that the higher polarity may bring a worse resolution for chiral recognition.

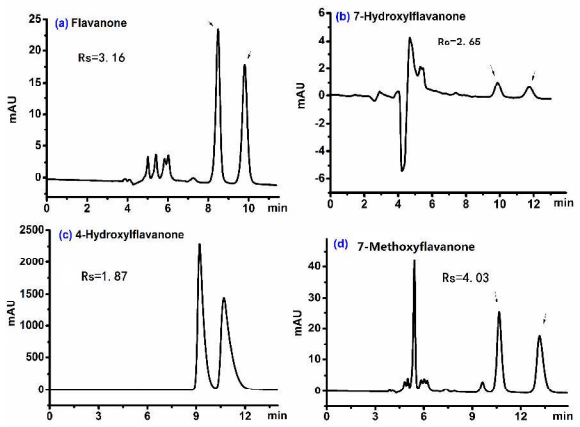

Fig. 3 Chromatograms and resolution values for four flavanoids on the column packed with CD clicked CSP. Mobile phase: MeOH/H_2O=80/20, UV wavelength is at 254nm.

Because of the hydrophobic cavity of CD, the CD-clicked CSP could afford good chiral resolutions for simple aryl alcohols using methanol as modifier phase (Fig 4). The aromatic ring of aryl alcohols could enter the CD cavity and two different enantiomers may have different interactions with the CD clicked CSP. Even little difference in these interactions may result in significant difference in the retention time and chiral resolution. All aryl alcohols can be well resolved using methanol/water (50/50) except Aryl-OH-1. The resolution for Aryl-OH-1 could be improved when decreasing the concentration of methanol in the modifier from 50% to 20%, as shown in Fig. 5A. The phenomenon may be attributed to the competing complexes between Aryl-OH-2 and methanol with the CD cavity, lower concentration of methanol could thus lead a better resolution.

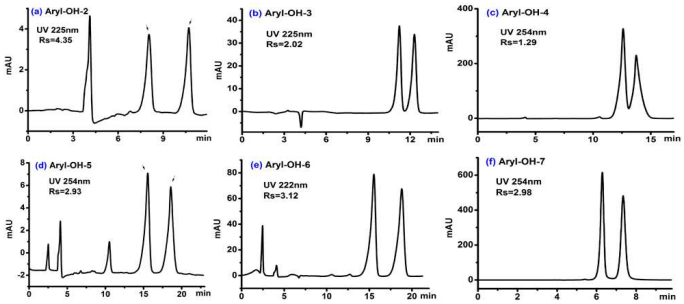

Fig.4 Chromatograms of six aryl alcohols on the column packed with CD clicked CSP. Mobile phases: (a)-(e): MeOH/H_2O=50:50; (f): MeOH/H_2O=80/20.

The screening of suitable organic modifier for enantioseparations is crucial for method development and optimization. The most commonly used organic modifiers are methanol and acetonitrile.[16-18] The chiral resolutions of enantiomers presented different behaviours towards different organic modifier. For example, ondansetron (see Fig. 5B) could not achieve successful separation with ACN/MeOH (50/50). By using MeOH/H_2O eluent, ondansetron exhibited improved resolutions when the volume content of MeOH decreased from 80% to 40%, though the elution of enantiomers was almost 1 time elongated. The separation of citailopram (Fig. 5C), however, presented a quite different behaviour as ondansetron, i.e., citailopram achieved both significantly improved R_s (4.19) and shortened elution when increasing MeOH content from 50% to 80%. This behaviour may be possibly explained by the polar nature of citalopram. For the separation of atenolol, the effect of ACN and MeOH on the chiral resolution is quite different, i.e., ACN generally led to faster elution time while MeOH rendered better chiral discrimination. When adding ACN to MeOH/H_2O in the separation of atenolol (Fig. 5D), better spike was obtained with a decrease in peak tailing. This is presumably due to stronger competition of ACN than MeOH when including into CD's hydrophobic cavity.[3]

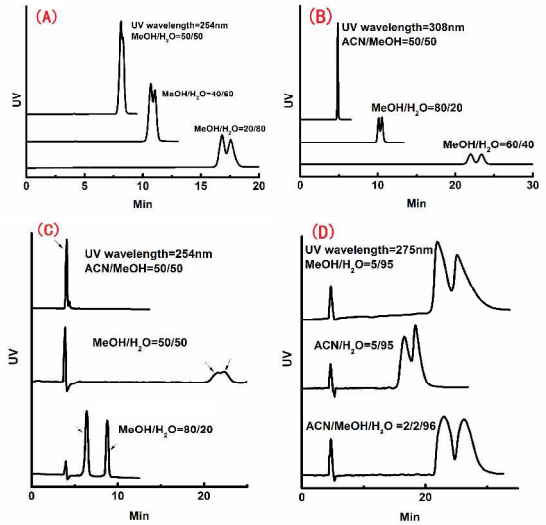

Fig.5 Influence of organic modifiers on the enantioseparation of (A) Aryl-OH-1, (B) ondansetron, (C) citalopram, and (D) atenolol on CD clicked CSP.

The separation data of five racemates under different eluent conditions with the addition of organic modifies are summarized in Table 2.

Table 2. The separate resolution of five racemates at different mobile phase conditions

Racemates	Conditions	k	α	R_s
Aryl-OH-1	UV=254nm			
	MeOH:H_2O(50/50)	k_1=1.971, k_2=2.013	1.02	0.46
	MeOH:H_2O(40/60)	k_1=2.523, k_2=2.607	1.03	0.59
	MeOH:H_2O(30/70)	k_1=3.114, k_2=3.242	1.04	0.68
	MeOH:H_2O(20/80)	k_1=3.433, k_2=3.583	1.04	0.75
	MeOH:H_2O(10/90)	k_1=4.024, k_2=4.200	1.04	0.61
Ondansetron	UV=308 nm			
	ACN:MeOH(50/50)	-	-	-
	MeOH:H_2O(80/20)	k_1=2.529, k_2=2.635	1.04	0.86
	MeOH:H_2O(60/40)	k_1=6.104, k_2=6.493	1.06	1.13
Citalopram	UV=254 nm			
	ACN:MeOH(50/50)	-	-	-
	MeOH:H_2O(50/50)	k_1=5.567, k_2=5.748	1.03	0.40
	MeOH:H_2O(80/20)	k_1=1.596, k_2=2.234	1.40	4.19
Timolol	UV=303 nm			
	ACN:MeOH(99/1)	k_1=5.232, k_2=6.843	1.31	0.87
	ACN:MeOH(90/10)	k_1=1.556, k_2=1.688	1.08	0.71
	ACN:MeOH(95/5)	k_1=1.760, k_2=2.170	1.23	1.30
Atenolol	UV=275 nm			
	MeOH:H_2O(40/60)	-	-	-
	MeOH:H_2O(25/75)	k_1=1.778, k_2=1.898	1.07	0.51
	MeOH:H_2O(10/90)	k_1=3.551, k_2=4.027	1.13	0.88
	MeOH:H_2O(5/95)	k_1=4.708, k_2=5.388	1.14	0.85
	ACN:H_2O(5/95)	k_1=3.587, k_2=3.973	1.11	0.85
	ACN:MeOH:H_2O (2/2/96)	k_1=5.144, k_2=5.868	1.14	1.08

Conclusions

In this paper, the enantioseparations of 19 racemic drugs in HPLC was explored with CD clicked CSP. By adjusting the conditions, optimized resolutions of model racemic drugs were achieved with chiral resolution as high as 4.19 and 4.42 for citalopram and tropicamide, respectively. The results indicated the versatility of the as-developed CD clicked CSP in chiral separation. The use of organic modifiers such as methanol and acetonitrile may be alternative approach for the optimization of chiral analysis in HPLC.

Experimental

The HPLC grade solvents were purchased from Sigma-Aldrich (Shanghai, China). All racemic compounds including ondansetron, citalopram, galanthamine base, pregabalin, timolol, atenolol, tropicamide and ephedrine hydrochloride were obtained from China Pharmaceutical University. All racemate were prepared into 50 μg/mL concentration using methanol. The silica gel for CSP was procured from Kromasil (mean pore size 100 Å, particle size 5μm and surface area of 300 m^2/g).

Evaluation of the CD clicked CSP column was performed on Agilent HPLC system, comprised of an Agilent 1200 series, G1322A degasser, G1311A quart pump, G1313A autosampler, G1316A temperature column compartment and G1314B VWD.

All chromatographic experiments were carried out at 25°C. The UV absorbance detection was performed at the suitable wavelength, which range from 210 nm to 310 nm. The flow rate of mobile phase was 0.7 mL/min.

The resolution parameters were determined: k_n were calculated using $k_n = (t_n - t_0)/t_0$, where t_0 is the time at which the first baseline disturbance by the solvent peak occurred. The selectivity factor (α) was calculated using k_2/k_1, where R_s as evaluated using the equation $R_s = 1.18\times(t_2-t_1)/(w_2+w_1)$, where w_1 and w_2 are the half-peak widths (based on USP standards).

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