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Comparison of separation performances of novel β-cyclodextrin-based chiral stationary phases in high-performance liquid chromatographic enantioseparation

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ABSTRACT

Three β-cyclodextrin-based chiral stationary phases were developed applying novel bonding chemistry. The separation performances of β-cyclodextrin, (*R*,*S*)-2-hydroxypropyl-β-cyclodextrin, and permethyl-β-cyclodextrin-based CSPs were compared in the resolution of structurally divergent analytes, such as coumarins, dansyl amino acids, and propionic acid derivatives. Separations were carried out in reversed phase mode applying 0.1% triethylammonium phosphate (pH 3.5)/MeOH mobile phase systems in different compositions. Of the three novel CSPs the permethyl-β-cyclodextrin bonded phase proved to be the most effective one for the enantioseparation of investigated analytes.

1. Introduction

Chiral compounds may differ significantly in their biological, pharmacological and toxicological effects. Due to different effects of enantiomers there is a great need for the availability of chirally pure compounds. Liquid chromatography (LC) with chiral stationary phases (CSPs) has been widely used for enantioseparations [1–5]. Cyclodextrin (CD) technology offers various solutions for the separation of drug enantiomers in chemical, pharmaceutical and biological research. To obtain chiral selectors having desired properties a number of modified cyclodextrin derivatives have been synthesized [6–8] by functionalizing the hydroxyl groups at the two sides of the CD cavity with hydrophobic (e.g. methyl, propyl) or hydrophilic (sulphate, phosphate, quaternary amine) groups. These substituents enable to further enhance the chiral recognition towards the analytes, owing to additional interactions such as dipolar and dispersive forces, hydrogen-bonding, steric repulsion, π–π complexation. Although many CD-CSPs are commercially available, there is a need to develop new packing materials offering higher enantioselectivity in short analysis time.

Most of the CD derivatives used in chiral separations are mixtures of high variety of randomly substituted homologues and regioisomers. Therefore, reproducibility of chiral analyses depends on the batch-to-batch reproducibility of the chemical synthesis of the applied CD-derivatives. Even small differences in the degree of substitution or isomer distribution can influence the result of the separations [9–13]. Currently CD-CSPs are either chemically bonded via spacers [14,15] or adsorbed to silica gel as a cyclodextrin polymer [16]. Recently monolithic columns functionalized with cyclodextrins have also been developed for chiral separations [17].

β-Cyclodextrins and its derivatives have been used for enantiomeric separation, employing different chromatographic modes [18,19]. The enantioselectivity of CDs is influenced by several parameters such as analytes' size, CD's cavity dimensions, organic solvent and nature of polar functional groups, especially those capable of hydrogen bonding. The extent of analyte inclusion generally depends on the size of the CD cavity. Under reversed-phase conditions according to this size-fit concept of inclusion complexation, higher affinity and greater enantioselectivity for the CSP-analyte pairs generally occur for the CD that gives the best match in terms of the size of hydrophobic portions of the solute with the CD cavity. Substituted phenyl, naphthyl, and heteroaromatic rings can conveniently be accommodated in a β-CD cavity, while larger analytes such as steroids fit preferentially into γ-CD, and smaller analytes prefer α-CD.

Present paper describes evaluation of liquid chromatographic methods for the enantioseparation of 14 analytes with structural diversity on newly developed native β-CD (BCD), on the substituted

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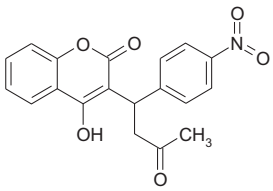
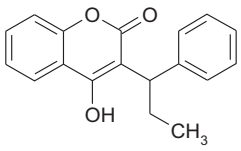
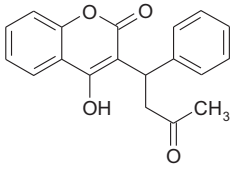
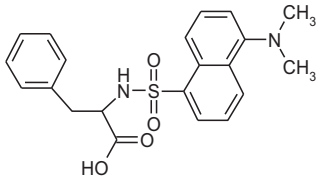
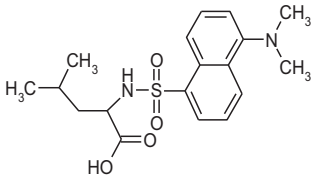
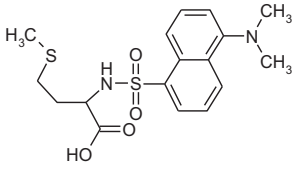
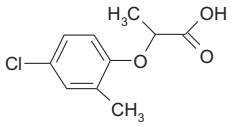
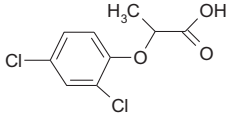
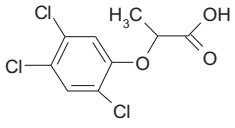
Table 1
Retention factors (k), separation factors (α) and resolutions (R_S) of stereoisomers of analytes (**1–14**).

Compound	Stationary phase	Eluent (v/v)	k_1	α	R_S
1 	BCD	66/34	5.10	1.05	0.75
	HPBCD	66/34	5.95	1.06	0.83
	PMBCD	66/34	6.12	1.14	2.15
2 	BCD	66/34	12.52	1.07	1.13
	HPBCD	66/34	18.38	1.07	1.04
	PMBCD	66/34	4.52	1.05	0.78
3 	BCD	66/34	5.10	1.02	0.46
	HPBCD	66/34	6.10	1.08	1.47
	PMBCD	66/34	2.29	1.22	2.94
4 	BCD	74/26	8.33	1.08	1.21
	HPBCD	74/26	7.45	1.00	0.00
	PMBCD	74/26	3.80	1.42	3.23
5 	BCD	74/26	7.19	1.16	2.59
	HPBCD	74/26	8.10	1.06	0.83
	PMBCD	74/26	2.57	1.03	0.47
6 	BCD	74/26	4.28	1.04	0.65
	HPBCD	74/26	4.20	1.00	0.00
	PMBCD	74/26	2.07	1.05	0.62
7 	BCD	74/26	4.76	1.08	1.09
	HPBCD	74/26	6.33	1.03	0.48
	PMBCD	77/23	2.41	1.10	1.40
8 	BCD	74/26	4.19	1.06	0.87
	HPBCD	82/18	5.52	1.04	0.63
	PMBCD	77/23	2.03	1.02	0.50
9 	BCD	66/34	6.33	1.00	0.00
	HPBCD	66/34	4.38	1.00	0.00
	PMBCD	77/23	3.17	1.17	2.37