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Enantioseparation of ß-carboline, tetrahydroisoquinoline and benzazepine analogues of pharmaceutical importance: Utilization of chiral stationary phases based on polysaccharides and sulfonic acid modified *Cinchona* alkaloids in high-performance liquid and subcritical fluid chromatography

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ABSTRACT

High-performance liquid chromatographic (HPLC) and subcritical fluid chromatographic (SFC) separations of the enantiomers of structurally diverse, basic ß-carboline, tetrahydroisoquinoline and benzazepine analogues of pharmacological interest were performed applying chiral stationary phases (CSPs) based on (i) neutral polysaccharides- and (ii) zwitterionic sulfonic acid derivatives of *Cinchona* alkaloids. The aim of this work was to reveal the influence of structural peculiarities on the enantiorecognition on both types of CSP through the investigation of the effects of the composition of the bulk solvent, the structures of the chiral analytes (SAs) and chiral selectors (SOs) on retention and stereoselectivity. As a general tendency, valid for all polysaccharide SOs studied, the increase of the concentration of the apolar component in the mobile phase (*n*-hexane for LC or liquid CO_2 for SFC) was found to significantly increase retention, which in most cases, was accompanied with increased selectivity and resolution. In a way, similar behaviour was registered for the zwitterionic SOs. In polar ionic mode employing eluent systems composed of methanol and acetonitrile with organic acid and base additives, moderate increases in retention factor, selectivity and resolution were observed with increasing acetonitrile content. However, under SFC conditions, an extremely high increase in retention was observed with increased CO_2 content, while selectivity and resolution changed only slightly. Thermodynamic parameters derived from temperature dependence studies revealed that separations are controlled by enthalpy.

1. Introduction

Harmane, harmine and harmaline ß-carboline alkaloids, *e.g.* (+)-harmicine, exhibit a wide range of pharmacological properties, including antimicrobial and anti-HIV activities [1–3], whereas yohimbine is an antagonist of α2-receptors located both presynaptically and postsynaptically on noradrenergic neurons [3]. Moreover, synthetic ß-carbolines display antimalarial, antiparasitic [4] and antineoplasic [5] activity. On the other hand, the ß-carboline skeleton is present in numerous naturally occurring alkaloids, such as the harman family, including eudistomines and manzamines, or canthines bearing an additional fused cycle. These compounds initially attracted interest because of their potent psychoactive and hallucinogenic abilities [1]. The 1,2,3,4-tetrahydroisoquinoline skeleton is found in a variety of alkaloids [6], such as laudanosine and salsolinol (6,7-dihydroxy-1-methyl-1,2,3,4-tetrahydroisoquinoline). It is also a useful key structure in synthetic heterocyclic chemistry. Salsolinol, being able to release prolactin selectively, is produced by the hypothalamus and the neuro-intermediate lobe of the pituitary gland; it can selectively release prolactin [7]. Benzazepine derivatives also have important biological properties such as anti-depressant, anti-hypertensive, anti-ischaemic and anorectic activity. In addition, they are anti-histamine agents, AChE inhibitors, TRPV1 antagonists and they are also used in the treatment of hyponatremia [8].

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The importance of aminonaphthols prepared via modified Mannich reactions has recently increased, because of their proven biological activities. 1-((2-Hydroxynaphthalen-1-yl)arylmethyl)piperidin-4-ol derivatives were earlier designed and synthesized as novel selective estrogen receptor modulators [9]. 1-[(6-Halo- or 4-methylbenzo[*d*]thiazol-2-ylamino)phenylmethyl] naphthalen-2-ol and 5-[(6-halo- or 4-methylbenzo[*d*]thiazol-2-ylamino)phenylmethyl]quinolin-6-ol derivatives, in turn, were found to exert repellent, insecticidal and larvicidal activity against the mosquito *Anopheles arabiensis* [10].

As a result of the very likely pharmacological differences of the individual enantiomers of the chiral analytes (SAs) described above, it is necessary to develop effective methods for their efficient separations and analyses. Enantioseparation of some *ß*-carboline analogues was previously carried out by direct methods applying chiral stationary phases (CSPs) based on macrocyclic glycopeptides [11] and polysaccharides [12]. Enantiomers of 1,2,3,4-tetrahydroisoquinoline analogues were separated utilizing *ß*-cyclodextrin and its derivatives as chiral mobile phase additives [13] and with the use of CSPs based on *ß*-cyclodextrin analogues [14]. Recently, CSPs based on polysaccharides [15,16], chiral crown ethers [17] and *Cinchona* alkaloids [18] were applied for the enantioseparation of some related tetrahydroisoquinoline derivatives.

Among numerous commercially available CSPs, nowadays the most popular phases are based on polysaccharides. The main reason is their wide application spectrum for the resolution of neutral, basic and acidic analytes [19,20]. In contrast to neutral and non-ionizable but moderately polar polysaccharide-based CSPs, chiral zwitterionic ion-exchangers based on *Cinchona* alkaloids and their sulfonic acid derivatives are characterized as charged selectors (SOs), which may provide different stereoselectivities for ionizable chiral analytes ranging from acidic to basic and zwitterionic compounds [21–24].

The main objective of the present paper is to reveal some general tendencies of structural peculiarities of the enantiomers of pharmacologically interesting analytes such as *ß*-carboline, tetrahydroisoquinoline and benzazepine analogues with respect to their enantioseparation on the above-mentioned SOs used under LC and SFC conditions. It should be underlined that these CSPs based on polysaccharides and *Cinchona* alkaloids modified by sulfonic acids are chemically highly different.

In our study we have focused on the effects of the variation of mobile phase composition in LC and SFC on the retention, selectivity and resolution of the enantiomeric basic SAs in context of the structurally entirely divergent types of SOs. A thermodynamic characterization is also an integral part of the study.

2. Materials and methods

2.1. Chemicals and reagents

α-Arylated *ß*-carboline analogue **1** (the structures of analytes are depicted in Fig. 1) was synthesized by the catalyst-free direct coupling of 4,9-dihydro-3*H*-*ß*-carboline and 2-naphthol [25]. For the synthesis of analytes **2**–**5**, 2-naphthol and 1,2,3,4-tetrahydroisoquinolines were reacted with benzaldehyde, 4–chloro- or 4-methoxybenzaldehyde under neat conditions under microwave irradiation. When 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline was applied as substrate, *N*-α-hydroxynaphthylbenzyl-substituted isoquinolines (**6** and **7**) were isolated in good yields. In the synthesis of analytes **8** and **9**, 2-naphthol was reacted with secondary cyclic amines 2,3,4,5-tetrahydro-1*H*-benz[*d*]azepine or 2,3,4,5-tetrahydro-1*H*-benz[*c*]azepine in the presence of benzaldehyde [26]. Analyte **1** posesses two secondary amino groups (pK_a = 9.57 and 14.97), while each analyte of **2**–**9** has an ionizable tertiary amino group

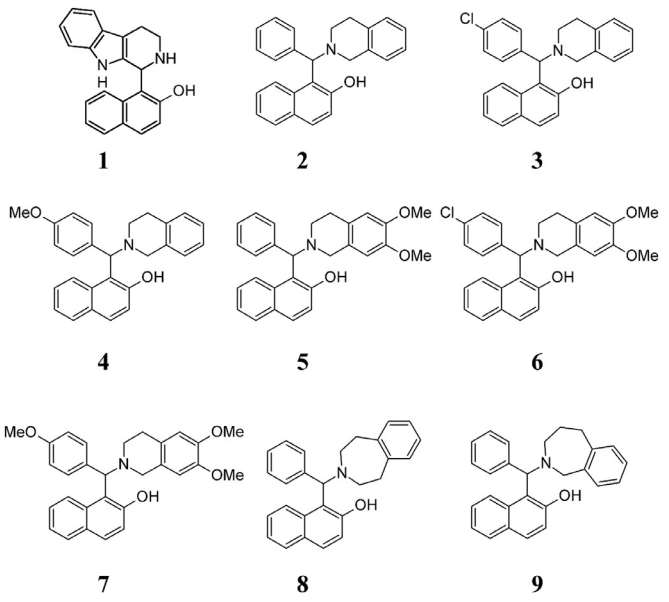

Fig. 1. Structure of analytes.

(pK_a values for **2**–**9** are 10.04, 9.22, 9.69, 9.39, 8.81, 9.13, 11.41 and 10.69, respectively). All pK_a values were calculated with MarvinSketch v. 17.28 software (ChemAxon Ltd., Budapest). It should be kept in mind that pK_a values are defined for aqueous conditions; however, in organic media, they may shift considerably to different values [27].

n-Hexane, acetonitrile (MeCN), methanol (MeOH), ethanol (EtOH) of HPLC grade as well as 1-propanol (1-PrOH), 2-propanol (2-PrOH), formic acid (FA) and diethylamine (DEA) were provided by VWR International (Radnor, PA, USA). CO_2 for the SFC experiments was from Messer (Budapest, Hungary).

2.2. Apparatus and chromatography

Liquid chromatographic (LC) measurements were performed applying a Waters Breeze system consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler and Empower 2 data manager software (Waters Corporation, Milford, MA, USA). A Lauda Alpha RA8 thermostat (Lauda Dr. R. Wobser Gmbh, Lauda-Königshofen, Germany) was used to maintain constant column temperature.

SFC measurements were carried out using a Waters Acquity Ultra Performance Convergence Chromatography™ system (UPC^2, Waters Corporation, Milford, MA, USA) containing a binary solvent delivery pump, an autosampler, a column oven, a PDA detector and Empower 2 software. Chromatographic conditions applied in LC or SFC techniques are listed in Figure legends and in footnotes to Tables. All analytes were dissolved in 2-PrOH or MeOH in the concentration range 0.5–1.0 mg mL^{-1} and injected as 20-μL and 7-μL samples for HPLC and SFC, respectively.

The commercially available polysaccharide-based CSPs applied in this study were amylose *tris*(3,5-dimethylphenylcarbamate) (Chiralpak **IA**), amylose *tris*(3-chlorophenylcarbamate) (Chiralpak **ID**), amylose *tris*(3,5-dichlorophenylcarbamate) (Chiralpak **IE**), amylose *tris*(3–chloro-4-methylphenylcarbamate) (Chiralpak **IF**) and amylose *tris*(3–chloro-5-methylphenylcarbamate) (Chiralpak **IG**). In addition, cellulose *tris*(3,5-dimethylphenylcarbamate) (Chiralpak **IB**) and cellulose *tris*(3,5-dichlorophenylcarbamate) (Chiralpak **IC**) were also used. All of these CSPs (250 mm × 4.6 mm I.D.) had the same particle size of 5 μm. The sulfonic acid modified *Cinchona* alkaloid-based Chiralpak ZWIX(+)™ and ZWIX(-)™

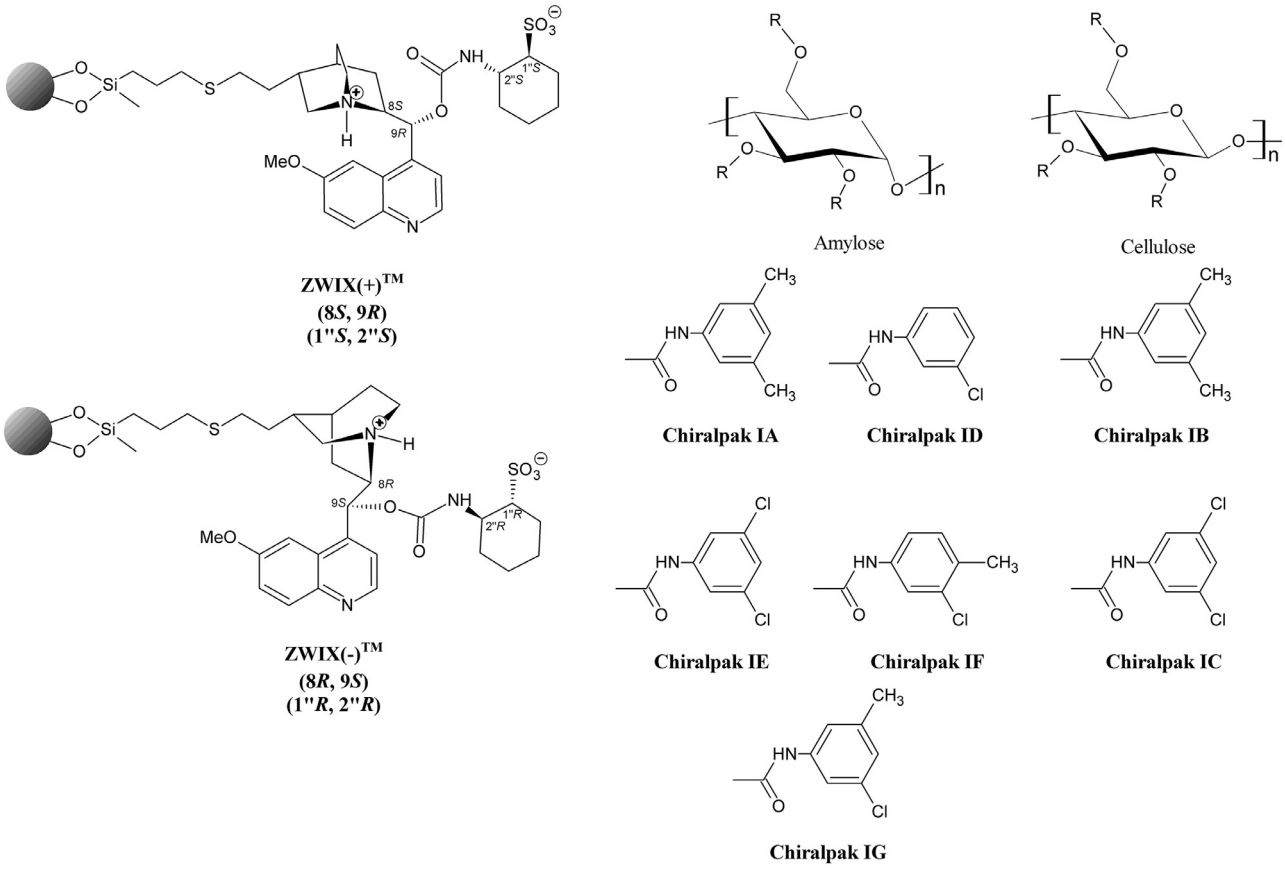

Fig. 2. Structure of selectors based on polysaccharides and *Cinchona* alkaloids.

columns (150 × 3.0 mm I.D.), however, had a different particle size of 3 μm. The void volume of the columns employed under SFC conditions was determined at the first negative peak of the CO_2/MeOH solvent. Under HPLC conditions the dead times of the ion-exchanger and polysaccharide-based columns were determined by injecting acetone dissolved in MeOH and tri-*t*-butylbenzene, respectively. All columns were gifts from Chiral Technologies Europe (Illkirch, France). The structures of the various chiral SOs investigated in this study are presented in Fig. 2.

3. Results and discussions

The enantiomeric separations of the racemic target SAs, namely, those of the α-arylated ß-carboline (**1**), *N*-α-(2–hydroxy–napht-2-yl)-benzyl isoquinolines (**2**–**7**) and *N*-α-(2–hydroxy–napht-2-yl)-benzyl benzazepine analogues (**8** and **9**), were carried out in a systematic fashion in LC and SFC modalities.

The mobile phase conditions selected in this study are either based on methods published previously [21,22,28,29] or on optimization studies discussed below.

3.1. Results obtained on polysaccharide-based CSPs

3.1.1. Effects of mobile phase composition applying polysaccharide-based CSPs in LC and SFC

Chromatographic parameters such as retention factor (k), selectivity (α) and resolution (R_S) are frequently optimized by variation of the nature of the alcohol component and its content in both normal-phase (NP-LC) measurement [30–32] and SFC separation [33–36]. To explore NP-LC conditions analyte **1** as model compound was employed with mixtures of *n*-hexane/alcohol/DEA (70/30/0.1 *v/v/v*) as mobile phase with different alcohol modifiers (EtOH, 1-PrOH or 2-PrOH). The best separation performances could generally be achieved with EtOH and 2-PrOH. (Fig. S1; Supplementary Materials). The observed differences in retention and selectivity might be explained by the alteration of the steric environment of the chiral cavities [37] within the chiral polymer-type SOs related to solvation effects of the protic solvents. Under NP-LC conditions, a decrease in the polarity of the alcohol usually results in enhanced analyte retention; however, an opposite behaviour was also reported [32]. In our case, the same trend was observed. Interestingly, 2-PrOH offered quite similar retentions compared to the linear chain counterpart. It is important to emphasize here that methanol cannot be used in NP-LC due to its limited miscibility with hexane.

Under SFC conditions on the same amylose-based CSPs, the alcohols studied were MeOH, EtOH, 1-PrOH and 2-PrOH using liquid CO_2/alcohol (50/50 *v/v*) mobile phase mixtures containing 20 mM DEA (Fig. S1). Upon varying the nature of the alcohol for analyte **1**, the largest k_1 values were obtained in the MeOH-containing mobile phase. Regarding the effect of the nature of alcohol on retention, the effect observed was quite similar to those reported earlier for NP-LC. Namely, alcohol modifiers with lower polarity resulted in reduced retentions (Fig. S1). Due to the most pronounced effectiveness of 2-PrOH in NP-LC and of MeOH in SFC reported in this and our earlier study [29], all further experiments were carried out with these two alcohols as co-solvents in the eluent systems. It is important to note that different results for the effect of the above-mentioned solvents can also be found in the literature [30–36]; that is, any generalization is hardly possible.

In a comparative study using NP-LC conditions for analyte **1** with Chiralpak **IA, IE** and **IG** columns, the composition of the

n-hexane/2-PrOH/DEA mobile phase mixture was varied between 50/50/0.1 and 90/10/0.1 *v/v/v*. As typical for a NP behaviour, an increase in the alcohol content resulted in a decreased k_1 (Fig. 3A). It is noteworthy that with the increase of the mobile phase polarity, the strength of the possible hydrogen bonds between the SA and the SO will decrease, while the solubility of the analytes in the mobile phase will increase [38]. For the given analyte, the Chiralpak **IE** column exhibited superior separation efficiency.

Employing the same Chiralpak **IA, IE** and **IG** columns under SFC conditions using MeOH as co-solvent in the range of 20 to 60 v% (all eluents contained 20 mM DEA) similar tendencies were observed as in NP-LC, although the increase in k_1 values was markedly higher with increasing CO_2 content (Fig. 3B). However, the change in α values were just as moderate as in NP-LC. That is, α, in general, increased slightly, except for Chiralpak **IA**. Without experimental verification we can only assume that the opposite behaviour of Chiralpak **IA** column might be related to the exclusive presence of electron donating (methyl) groups on the phenyl carbamate moiety. The best separation efficiency was registered for the Chiralpak **IG** column under the applied mobile phase conditions.

The above-mentioned results allow to conclude that alcohols may affect enantioseparations in several ways. Specifically, the polar solvent may be incorporated into the polysaccharide structure, either into the cavities or between the polymer chains, affecting crystallinity and/or side chain mobility. Applying SFC conditions, the effects of the alcohol are more difficult to predict. The alcohol will affect not only the polarity, but also the viscosity and density of the mobile phase. Besides affecting the physical properties of the eluent, the debated *in situ* formation of alkylcarbonic acid may have further effects on the overall polarity and acid-base properties of the mobile phase. When applying a relatively low amount of modifier (<15%), its adsorption was found to be significant, while above 15–20% saturation of the stationary phase can be expected [34]. An experimental difficulty, as recently addressed [39], is the calculation of the operational conditions, characteristic for the SFC measurements. It is important to note that in this study we employed at least 20 v% of alcohol modifier, where no dramatic changes can be expected between the actual and set operational SFC conditions. Consequently, the set values are very reasonable, similar to those found under NP-LC conditions. It should be noted that any MeOH content will be easily dissolved in liquid CO_2 under the given SFC conditions, whereas this would not be possible when using *n*-hexane under NP conditions.

3.1.2. Structure–retention relationships of the given basic analytes on polysaccharide-based selectors

The structural characteristics of analytes **1**–**9** (Fig. 1), such as steric arrangement around the stereogenic centers, different substituents capable of forming H-bond, π–π and other interactions, as well as the structure of SOs affected retention and selectivity. The peculiarities of the nine analytes observed on the seven polysaccharide columns possessing amylose or cellulose backbone and dimethyl-, chloro–, dichloro- or methylchloro-phenylcarbamate moieties in NP-LC and in SFC were investigated. Table 1 reports the k_1, α and R_S values measured on all seven polysaccharide columns. Based on the results of preliminary experiments, we selected *n*-hexane/2-PrOH/DEA (80/20/0.1 *v/v/v*) for NP-LC measurements and CO_2/MeOH (50/50 *v/v*) mobile phase containing 20 mM DEA for SFC separations to study the structural effects ensuring similar retention factors under both NP-LC and SFC conditions.

3.1.2.1. Polysaccharide CSPs applied under NP-LC conditions. Analyte **1** has somewhat different chromatographic behaviour than the other tested amines. It is mainly due to the secondary versus tertiary amino functionality close to the chiral carbon atom and the presence of a second amino function. It seems that analyte **1** fits to both the amylose and the cellulose chain exhibiting usually good enantioselectivity: under NP-LC conditions, α ranged between 1.09–1.86 and R_S between 0.65–5.78. Note that analyte **1** was not separable on amylose-based Chiralpak **ID**. Analytes **2, 3, 8** and **9** and, in particular, analyte **4**, exhibited lower retention than analyte **1**. Values of α changed in a relatively broad range of 1.10–2.59 while R_S changed between 0.73–9.07 and, in most cases, baseline separation was achieved. On Chiralpak **IB**, stereoisomers of analyte **8** exhibited no separation.

The rigidity/flexibility of the 1,2,3,4-tetrahydroisoqunoline ring was found to influence the chromatographic behaviour. A comparison of the chromatographic properties of analytes **8** and **9** possessing a more flexible seven-numbered ring *vs.* **2** bearing a less flexible six-numbered ring shows that retention factors do not differ considerably on the seven polysaccharide-based CSPs. Namely, k_1 varied between 0.46–1.0 on Chiralpak **IA**, between 0.39–0.58 on **IB**, between 0.35–0.37 on **IC**, between 0.52–0.77 on **ID**, between 0.60–0.68 on **IE**, between 0.63–0.73 on **IF** and between 0.70–1.48 on **IG** (Table 1). In contrast, however, a significant difference was registered for α (and R_S). In all cases, higher α and R_S values were obtained for the 1,2,3,4-tetrahydroisoqunoline analogue (**2**) than for the two benzazepine analogues (**8** and **9**). This suggests that enantioselective interactions are much more dependent on the flexibility of the skeleton of the molecule than nonselective interactions.

For dimethoxy-substituted analytes **5, 6** and **7**, a definite increase can be observed in both retention and α as well as R_S values. The polar carbamate groups of these polysaccharide-type CSPs are located more inside, while the hydrophobic aromatic groups are more outside the polymer chain. Analytes can interact relatively easily with the carbamate groups via H-bonding and dipole–dipole interactions; however, π–π interactions between the aryl groups of the CSP and an aromatic group of the solute may play a role in the chiral recognition event [40,41]. Methoxy groups may behave as additional H-bonding sites. Moreover, due to the electron withdrawing characteristics of their aryl ring, they may facilitate stronger π–π interactions resulting in higher retention for **5, 6** and **7**.

A comparison of analytes **2** *vs.* **5, 3** *vs.* **6** and **4** *vs.* **7** revealed that in all cases higher k_1 values were observed for the dimethoxy-substituted analogues and the enhanced interactions formed between SOs and SAs in most cases were stereoselective resulting in higher α and R_S values. It is noteworthy that the presence of a Cl atom or an additional methoxy group (in **6** and **7**) capable of H-bond interactions usually resulted in the highest α and R_S values.

On the basis of the obtained chromatographic parameters (k, α and R_S), several conclusions can be drawn for the performance of the applied columns (**IA** *vs.* **IB, IE** *vs.* **IC** and **IF** *vs.* **IG,** Table 1). Amylose-based Chiralpak **IA** exhibited better separation efficiency than cellulose-based Chiralpak **IB** with the exception of analytes **1** and **3**. Furthermore, particularly high differences in α and R_S were observed for analytes **4**–**7** containing methoxy or dimethoxy groups as substituents.

A comparison of the performances of Chiralpak **IE** *vs.* Chiralpak **IC** shows that, with the exception of analyte **7**, the amylose-based **IE** column offered enhanced interactions resulting in higher retention. Moreover, these enhanced retentive forces offered better enantiodiscrimination for most compounds, except for analytes **5, 8** and **9**.

Of the two chloromethyl-substituted amylose-based CSPs (Chiralpak **IG** and **IF**), the 3–chloro-5-methyl derivative ensures better fit of analytes to the selector providing higher retentions in all cases. With the exception of analyte **5, 7** and **9**, the stronger retentive interactions also resulted in higher α and R_S values (Table 1).

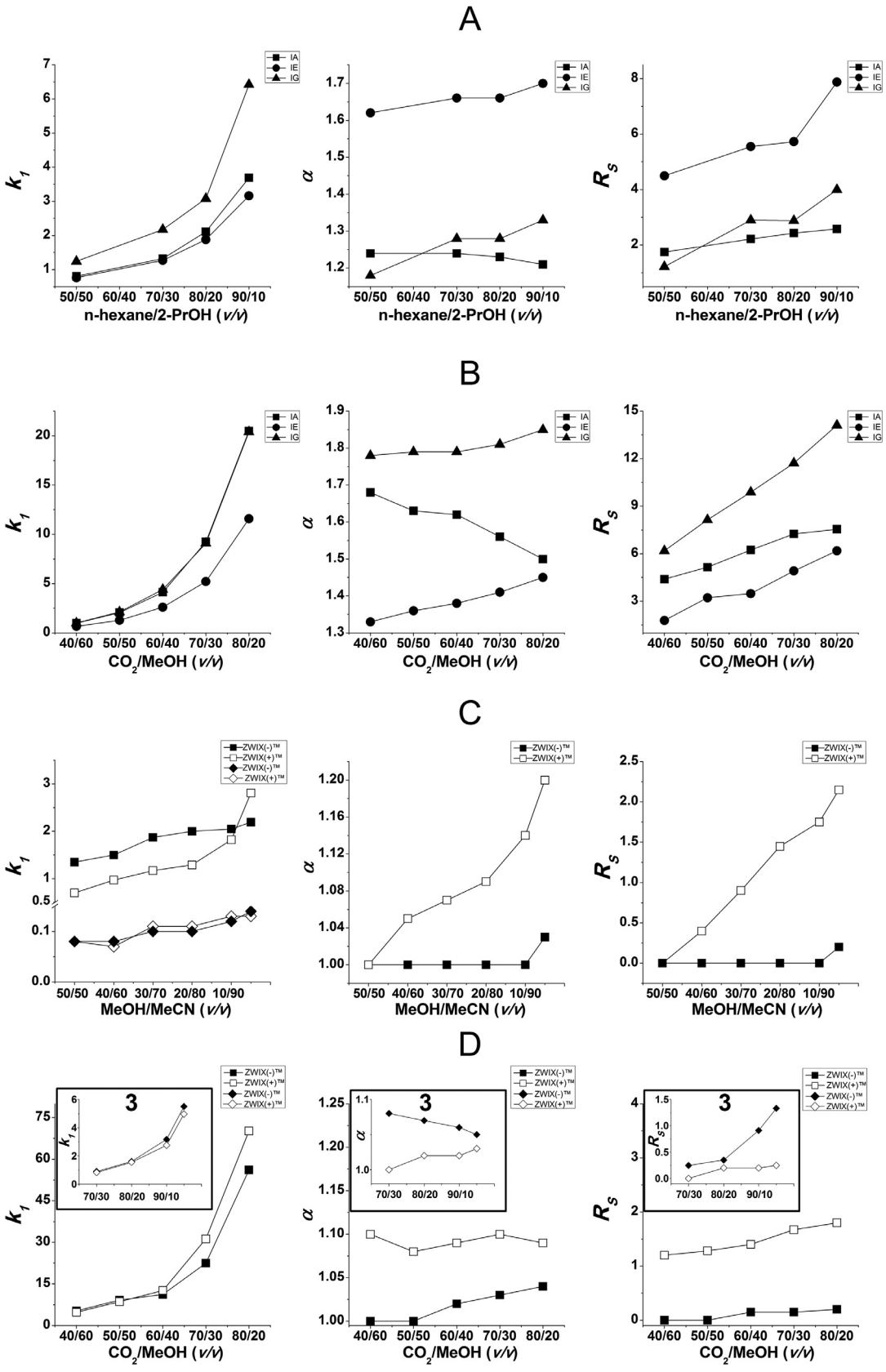

Fig. 3. Effect of mobile phase composition on k_1, α, and R_S for analyte **1** on polysaccharide phases in NP-LC (**A**), in SFC (**B**), and for analyte **1** and **3** on zwitterionic phases in PI mode (**C**) and in SFC (**D**) Chromatographic conditions: columns, A and B, Chiralpak IA, IE, and IG, C and D, ZWIX(+)™ and ZWIX(-)™; mobile phase, **A**, *n*-hexane/2-PrOH/DEA (50/50/0.1– 90/10/0.1 *v/v/v*), **B,** CO_2/MeOH (40/60–80/20 *v/v*) containing 20 mM DEA, **C**, MeOH/MeCN (50/50–5/95 *v/v*) containing 30 mM DEA and 60 mM FA and **D**, for analyte **1** CO_2/MeOH (40/60–80/20 *v/v*) and CO_2/MeOH for analyte **3** (70/30–95/5 *v/v*) all containing 30 mM DEA and 60 mM FA; flow rate, **A** and **C**, 1.0 mL min^{-1}, **B** and **D**, 2.0 mL min^{-1}; detection, 215–250 nm; temperature, **A** and **B**, ambient, **C** and **D**, 40 °C; back pressure, **B** and **D**, 150 bar; symbols, for analyte **1,** Fig. 3**A** and **B**, ■, Chiralpak **IA**, ●, Chiralpak **IE,** ▲**,** Chiralpak **IG,** for analyte **1,** Fig. 3**C** and **D,** ■, ZWIX(-)™, □, ZWIX(+)™ and for analyte **3,** Fig. 3**C** and **D,** ◆, ZWIX(-)™, ◇, ZWIX(+)™.

Table 1
Chromatographic data, k_1, α and R_S for the separation of stereoisomers of ß-carboline, 1,2,3,4-tetrahydroisoquinoline and benzazepine analogues on polysaccharide-based chiral stationary phases in normal phase and SFC modalities.

Column	Analyte	*NP-LC*			*SFC*		
		k_1	α	R_S	k_1	α	R_S
IA	1	2.21	1.43	2.83	2.06	1.63	5.15
	2	1.00	1.35	3.05	1.31	1.46	5.77
	3	0.89	1.11	3.19	1.66	1.37	4.93
	4	1.27	1.55	5.83	1.79	1.50	2.33
	5	1.76	1.37	4.50	0.82	1.10	1.37
	6	1.46	1.73	7.37	1.95	1.37	4.77
	7	1.85	2.19	10.86	1.80	1.36	4.48
	8	0.46	1.33	2.23	0.90	1.17	2.08
	9	1.00	1.35	2.75	1.76	1.24	3.23
IB	1	2.70	1.86	5.78	1.42	1.33	2.37
	2	0.54	1.33	2.40	0.90	1.17	2.23
	3	0.65	1.44	3.71	1.12	1.18	2.55
	4	0.74	1.48	3.90	1.15	1.21	2.19
	5	1.33	1.57	1.95	0.95	1.20	1.84
	6	1.53	1.31	2.38	1.35	1.24	3.30
	7	1.77	1.24	1.67	1.30	1.26	3.49
	8	0.39	1.00	0.00	0.71	1.00	0.00
	9	0.58	1.24	1.50	1.07	1.15	1.98
IC	1	1.35	1.09	0.65	0.69	1.00	0.00
	2	0.34	1.52	2.21	0.66	1.00	0.00
	3	0.30	1.39	1.62	0.74	1.00	0.00
	4	0.35	1.55	1.80	0.70	1.29	1.41
	5	1.48	1.85	6.29	1.56	1.23	1.12
	6	2.09	1.95	7.00	1.99	1.11	1.80
	7	3.69	1.53	5.48	2.39	1.00	0.00
	8	0.37	1.30	1.61	0.66	1.00	0.00
	9	0.35	1.26	1.38	0.75	1.00	0.00
ID	1	3.22	1.00	0.00	0.73	1.52	3.19
	2	0.62	1.78	5.50	0.82	1.28	3.62
	3	0.69	1.38	2.00	0.94	1.20	2.83
	4	0.76	1.95	2.35	0.98	1.29	2.19
	5	1.58	1.30	4.09	1.65	1.23	1.78
	6	2.00	1.78	7.47	1.67	1.24	3.80
	7	3.60	1.72	7.31	1.92	1.25	3.98
	8	0.52	1.33	2.67	0.75	1.13	1.74
	9	0.77	1.43	3.40	1.10	1.14	2.01
IE	1	1.98	1.86	5.73	1.29	1.36	3.22
	2	0.60	1.52	3.71	1.00	1.15	2.08
	3	0.57	1.29	2.55	1.07	1.12	1.75
	4	0.61	1.21	2.08	1.10	1.27	2.22
	5	1.98	1.33	4.84	1.71	1.25	2.22
	6	2.20	1.60	7.57	2.53	1.20	3.07
	7	3.63	1.57	5.68	3.16	1.18	2.85
	8	0.60	1.13	1.00	1.08	1.09	1.21
	9	0.68	1.10	0.73	1.32	1.08	1.18
IF	1	2.24	1.23	2.46	1.28	1.26	2.15
	2	0.72	1.67	5.18	0.67	1.30	3.39
	3	0.75	1.42	3.17	0.79	1.43	5.18
	4	0.81	2.28	3.90	0.83	1.39	4.07
	5	2.04	1.58	4.34	2.36	1.30	3.43
	6	1.78	1.74	7.58	2.56	1.25	3.62
	7	2.74	1.73	5.03	2.66	1.28	4.00
	8	0.63	1.25	1.78	1.25	1.10	1.61
	9	0.73	1.46	2.92	2.02	1.61	6.39
IG	1	3.58	1.48	5.88	2.12	1.79	5.38
	2	0.98	2.59	9.07	1.91	2.40	14.52
	3	1.15	1.66	5.33	2.34	1.92	8.00
	4	1.12	2.49	8.29	2.63	1.46	6.20
	5	3.30	1.53	4.84	1.08	1.36	4.95
	6	3.31	1.87	7.86	3.53	1.36	5.15
	7	5.06	1.44	5.50	3.96	1.45	6.04
	8	0.70	1.43	3.27	1.44	1.21	2.95
	9	1.48	1.30	2.93	2.55	1.50	7.23

Chromatographic conditions: column, Chiralpak **IA, IB, IC, ID, IE, IF** and **IG**; mobile phase, in NP-LC *n*-hexane/2-PrOH/DEA (80/20/0.1 *v/v/v*); in SFC CO_2/MeOH (50/50 *v/v*) containing 20 mM DEA; flow rate, in NP-LC, 1.0 mL $min^{-1,}$ in SFC, 2.0 mL min^{-1}; detection, 220–230 nm; temperature, in NP-LC, ambient, in SFC, 40 °C; back pressure, in SFC, 150 bar.

3.1.2.2. Polysaccharide CSPs applied under SFC conditions. Under SFC conditions, the behaviour of the compounds was somewhat similar to that in NP-LC (Table 1). Analyte **1** fits nicely to both amylose and cellulose chains resulting in rather high stereoselectivity and resolution. It should be noted that among the seven CSPs, cellulose-based Chiralpak **IC** exhibited unexpectedly poor stereoselectivity, since it was effective only in the separation of stereoisomers of **4**–**6**. A comparison of the chromatographic behaviour of analytes **3** and **4** *vs.* **2** offers the possibility to visualize the effect of the substitution pattern of analytes on enantioseparation. The inserted chlorine (at compound **3**) enhanced the retentive interactions, but, in general reduced the enantioselectivity. The introduction of a methoxy group (**4**), in turn, afforded higher retention in most cases on polysaccharide-based CSPs with moderate effects on enantioselectivity depending on the nature of selector (Table 1). The substitution of the benzene ring influences the capability of both H-bond and π–π interactions. In summary, it is highly probable, that the H-bond and π–π interactions will jointly regulate the effects of substitution on the SO-SA interactions.

A comparison of the chromatographic characteristics of analytes **2** as well as **8** and **9** revealed a behaviour similar to that observed in the case of NP-LC. The k_1 values do not differ considerably (Table 1), while for α and R_S a slight or moderate increase was registered in the case of analyte **2** (the only exception was analyte **9** on Chiralpak **IF**). This behaviour draws attention to the importance of the rigidity/flexibility of the molecule for chiral recognition both in LC and SFC.

The effect of the methoxy group on the chromatographic properties of analytes **4**–**7** is evident just as the marked differences between NP-LC and SFC observed not only in retention but also in enantioselectivity.

In all cases, a comparison of amylose- and cellulose-based CSPs for the separations of the investigated stereoisomers shows higher retention and an improved enantioselectivity on the amylose-based CSPs (**IA** *vs.* **IB** and **IE** *vs.* **IC**).

Interestingly, under SFC conditions, similar to NP-LC separations, practically in all cases the two chloromethylphenylcarbamate Chiralpak **IF** and **IG** columns afforded the highest α and R_S values indicating the role of both π–π-type and H-bonding SO-SA interaction increments for the given series of analytes.

In order to be able to characterize the chromatographic performances of the optimized methods, the limit of detection (LOD) and limit of quantitation (LOQ) were determined and reported in Table S1. These values allow comparison with those found in the literature for compounds with similar structures.

3.2. Results obtained on zwitterionic CSPs

3.2.1. Effects of mobile phase composition applying sulfonic acid modified Cinchona alkaloid-based CSPs in LC and SFC

Zwitterionic CSPs as chiral cation-exchangers can be employed for the enantioseparations of the basic analytes studied. In these cases retention follows the ion-exchange mechanism although working in non-aqueous conditions with polar protic mobile phases. Apparent pK_a values of the analytes will have a different effect on the retention, whether or not the analyte is mono- or bibasic. Due to this reason analyte **1** and analyte **3** were chosen as model compounds for method evaluation.

For a comparison to the neutral polysaccharide-type CSPs discussed above, the effects of the composition of the polar protic bulk solvent on chromatographic parameters measured on ZWIX(+)^{TM} and ZWIX(-)^{TM} columns are treated here. Chromatographic data obtained with MeOH/MeCN (50/50–10/90 *v/v*) as the mobile phase containing 60 mM FA and 30 mM DEA are depicted in Fig. 3C. Because of the acid and base additives these conditions are called polar ionic (PI) mode. Analyte **1** was moderately retained and retention increased with increasing MeCN content, due to enhanced ionic interactions and reduced solvation. This observation is in accordance with results obtained earlier for α-amino and β-amino acids [21,22] as well as 1,2,3,4-tetrahydroisoquinoline and indole analogues [18,28]. In contrast, analyte **3** was very weakly retained and a mild increase in retention was registered with increasing MeCN content (k increased from 0.08 to 0.14; Fig. 3C). Regarding α and R_S values for analyte **1** on ZWIX(+)^{TM}, α increased from 1.0 to 1.20 and R_S from 0.0 to 2.15. On ZWIX(-)^{TM}, in turn, analyte **1** exhibited separation only at the highest MeCN content, whereas stereoisomers of analyte **3** were not separable (Fig. 3C). It is important to emphasize that the retention behaviour of analyte **3** significantly differs from that of analyte **1**, although the pK_a values of analytes **1** and **3** are quite close (9.57 and 9.22, respectively). This behaviour is presumably be explained by the difference in steric effects. Note that the secondary amino group of analyte **1** is most probably sterically somewhat better accessible for the interaction with the solvated aminocyclohexanesulfonic acid moiety of the selector and this may markedly contribute to its retention. In contrast, the interaction of the tertiary amino group of analyte **3** with the aminocyclohexanesulfonic acid moiety may be more hindered. In addition, the second amino group of analyte **1** can weakly interact with the deprotonated sulfonic acid site, although its binding strength will be lower.

Under SFC conditions using a slightly acidic polar ionic mobile phase of liquid CO_2/MeOH containing 30 mM DEA and 60 mM FA, a higher increase in k_1 was registered compared to that in the PI mode (Fig. 3C *vs.* Fig. 3D). For analyte **1**, the increase in k_1 was extremely high; by increasing the CO_2 content from 40 to 80 *v%*, k_1 enhanced from 5.3 to 56.0 and 70.0 on ZWIX(+)^{TM} and ZWIX(-)^{TM}, respectively. For analyte **3**, k_1 increased from 0.9 to *ca.* 5.5 by increasing the CO_2 content from 70 to 95 v%. In contrast with k_1 values, α and R_S changed only moderately with increasing liquid CO_2 content (Fig. 3D). Baseline separation was achieved for analyte **1** on ZWIX(+)^{TM} and for analyte **3** on ZWIX(-)^{TM}. An important conclusion here is that the zwitterionic ion-exchangers perfectly compatible also with SFC conditions.

3.2.2. Structure–retention relationships and structural effects of Zwitterionic sulfonic acid modified Cinchona alkaloid-based selectors

3.2.2.1. Zwitterionic CSPs applied under polar ionic (PI) conditions. According to data presented in Table S2 (and Fig. 3C), the interaction of analytes containing a tertiary amino group (**2**–**9**) with zwitterionic SOs is rather weak and, obviously, enantiodiscrimination is not supported. However, analyte **1** possessing two secondary amino groups can interact more strongly with the zwitterionic selectors resulting in higher retention, which might also be associated with more dominant ion pairing supported by hydrogen bonding. The high MeCN content in the mobile phase promoted H-bond interactions and resulted in partial or baseline separation of the stereoisomers of analyte **1**. The lack of the retardation of compounds possessing tertiary amino group indicates the importance of steric effects.

3.2.2.2. Zwitterionic CSPs applied under SFC conditions. When comparing the retention behaviour observed under SFC conditions using liquid CO_2/MeOH (80/20 *v/v*) containing 30 mM DEA and 60 mM FA (Table S1), it becomes clear that ionic interactions are particularly important. For analytes **2**–**9** containing a tertiary amino group, k_1 values were usually 3–5 times higher in comparison with those obtained on polysaccharide CSPs in SFC. Values of α and R_S were lower than those obtained on polysaccharide-based CSPs, while for other analytes, at least partial or baseline separation could be achieved.

Selected chromatograms for the nine analogues obtained with different chromatographic techniques are depicted in Fig. 4.

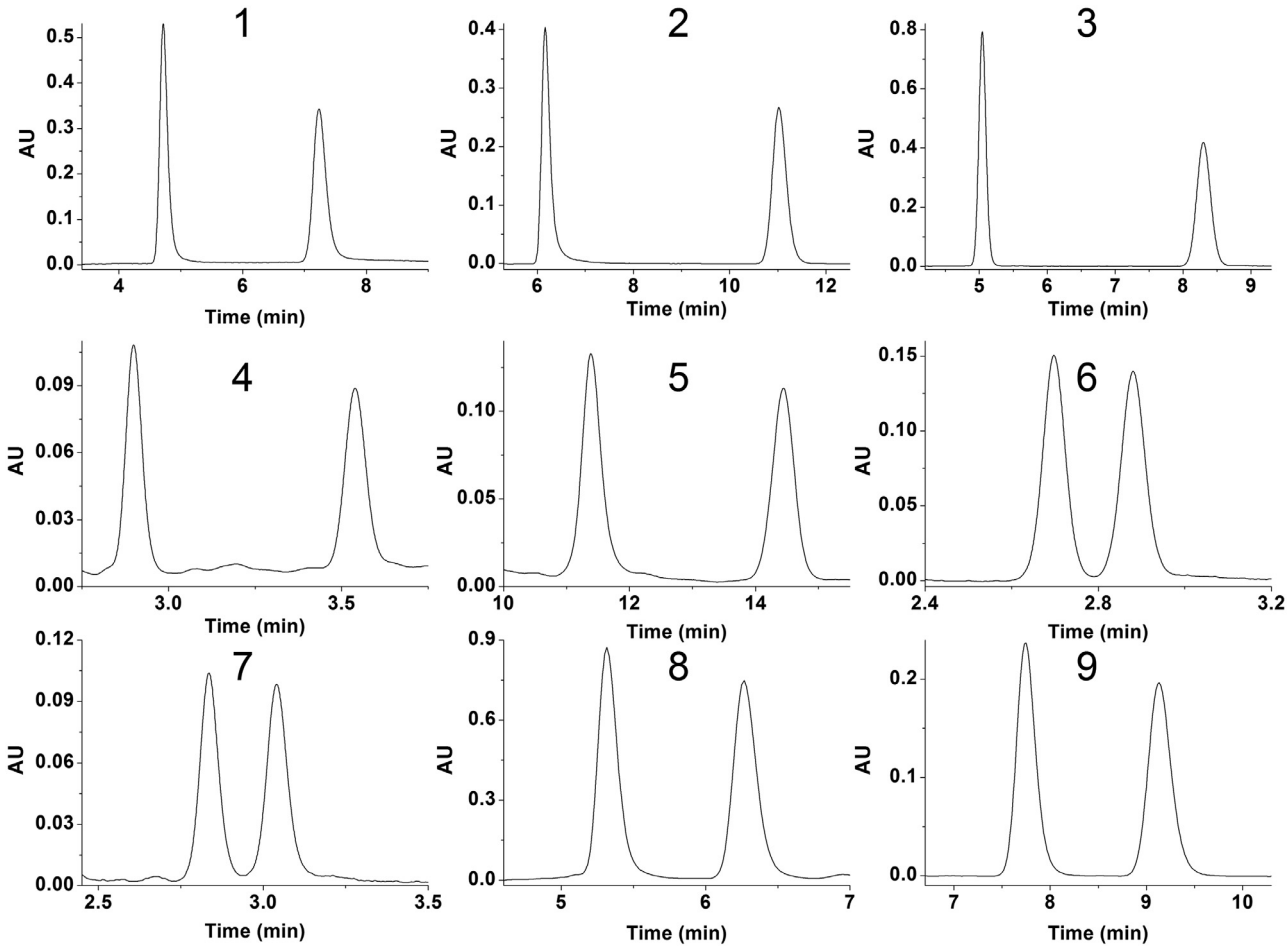

Fig. 4. Selected chromatograms for analytes **1–9** in NP-LC or SFC.Chromatographic conditions: column, ZWIX(-)^{TM} for **6**, ZWIX(+)^{TM} for **7, IG** for **1, 2, 3, 4, 8, 9**, and **IA** for **5**; mobile phase, *n*-hexane/2-PrOH/DEA 80/20/0.1 (*v/v/v*) for **2, 5, 8, 9**, CO_2/MeOH (50/50 *v/v*) containing 20 mM DEA for **1, 3** and **4,** CO_2/MeOH (90/10 *v/v*) containing 30 mM DEA and 60 mM FA for **6** and **7**; flow rate, 2.0 ml min^{-1} for **1, 3, 4, 6** and **7**; 1.0 ml min^{-1} for **2, 8, 9** and **5**; detection 215 nm to 290 nm; temperature, ambient; for **2, 8, 9** and **5,** 40 °C and back pressure, 150 bar for **1, 3, 4, 6** and **7**.

3.3. Influence of temperature and thermodynamic parameters

The study of the temperature dependence of retention and enantioselectivity may offer valuable information on the chiral recognition process. By the careful interpretation of the van't Hoff equation, the differences in the change in standard enthalpy $\Delta(\Delta H°)$ and entropy $\Delta(\Delta S°)$ can be expressed, not forgetting about the limitations of the simplified approach applied in this study, *i.e.* not differentiating between chiral and achiral contributions [42–44].

In order to see whether enantioseparations are dominated by enthalpy or entropy control, variable-temperature studies were carried out over the temperature range 10–50 °C under NP-LC and 20–50 °C under SFC conditions. The corresponding data are listed in Table S3. The collected chromatographic data were utilized to construct van't Hoff plots and thermodynamic parameters were calculated (Table S4). As a general trend, van't Hoff analysis of the separation factors (ln α *vs. 1/T*) gave linear plots.

Applying either the polysaccharide-based or the zwitterionic CSPs, retention as well as separation factor generally decreased with increasing temperature. The relative contribution of the free energy can be described by the enthalpy/entropy ratio *Q* [$Q = \Delta(\Delta H°)/[298 \times \Delta(\Delta S°)]$]. As represented in Table S4, the chiral recognition is found to be enthalpically-controlled for both types of CSPs.

4. Conclusion

The study of the effects of mobile phase composition for the resolution of the nine basic target analytes on the two chemically entirely different types of CSPs revealed that the increased ratio of the apolar component in the mobile phase (*n*-hexane for LC or liquid CO_2 for SFC) resulted in considerably higher retentions. On polysaccharide phases, in turn, the ratio of apolar/polar components in the mobile phase affected only slightly the discrimination between the enantiomers.

Regarding the effects of the nature of alcoholic modifier on α and R_S values, application of EtOH and 2-PrOH under NP-LC conditions and MeOH under SFC condition seemed to be more efficient.

The analysis of structure–retention relationships allows the conclusion that amylose-based selectors were more efficient than their cellulose-based counterpart. Of the two chloromethyl-substituted amylose-based CSPs, operated in NP-LC and SFC modalities, the 3–chloro-5-methyl substitution pattern (Chiralpak **IG**) ensures better fitting and/or H-bond and π–π interaction pattern of analytes to the solvated amylose chain, resulting in higher k_1, α and R_S values in almost all cases.

A comparison of NP-LC and SFC modalities on polysaccharide phases indicated that α-arylated ß-carboline and 1,2,3,4-tetrahydroisoquinoline analogues were separated more efficiently by SFC, while the separation efficiency for the benzazepine analogues was better in NP-LC.

In contrast to the polysaccharide-type CSPs the zwitterionic CSPs were much less efficient for the separation of stereoisomers of ß-carboline and dimethoxy-substituted 1,2,3,4-tetrahydroisoquinoline analogues. However, the retention factors were too low to arrive at a clear-cut final conclusion and further experimental work is needed to be more conclusive. The substitution pattern of the studied analytes has rather similar effects on enantioseparations both in NP-LC and SFC.

The thermodynamic study revealed that separations are controlled by enthalpy on both types of CSPs both under SFC and LC conditions.

Declaration of Competing Interest

Authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2019.460771.

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