Enantioseparation of β -carboline derivatives on polysaccharide- and strong cation exchanger-based chiral stationary phases. A comparative study

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ABSTRACT

In this study we attempted to describe in a comparative manner the enantioselectivity performance of six different polysaccharide- and two strong cation exchanger-type chiral stationary phases (CSPs) for the resolution of free and *N*-protected β -carboline derivatives. On commercially available cellulose- or amylose-based CSPs, the enantioseparations were carried out in normal-phase mode by variation of the nature and the concentration of the alcohol modifier in *n*-hexane as mobile phase. With the application of strong cation exchanger-type CSPs, the enantioseparations were optimized by the variation of methanol-acetonitrile bulk solvent compositions in the presence of various amounts of acid and base additives acting as counter-ions. Detailed thermodynamic investigations revealed that in all cases the enantioseparations observed were enthalpically driven, *i.e.* the retention and selectivity decreased with increasing temperature. Elution sequences were determined routinely; no general rule was found on polysaccharide-based CSPs, while on the two enantiomeric strong cation exchanger-type CSPs the predicted reversal of the elution sequence could be confirmed on switching from one enantiomeric CSP to the other form.

Keywords: enantiomer separation; β -carboline derivatives; polysaccharide-based chiral stationary phases; strong cation exchanger-type chiral stationary phases; temperature effect

1. Introduction

Thanks to their pharmacological and therapeutic activity the importance of compounds with a tetrahydro- β -carboline skeleton, such as *e.g.* vincristine and vinblastine [1] or reserpine, is well-known [2]. More and more new alkaloids isolated nowadays from natural sources have a tetrahydro- β -carboline skeleton and potential biological activities. (+)-7-Bromotrypargine, isolated from an Australian marine sponge, has been found to have an antimalarial effect [3]. Stereoisomers of a pyridoxal tetrahydro- β -carboline derivative have been synthesized and the effects of the absolute and relative configurations on the inhibitory effect on the proliferation of *Plasmodium falciparum* have been investigated [4]. Antinociceptive activity has been described for racemic harmicine [5] and the optically active form has been isolated from an extract of the leaves of *Kopsia griffithii* [6]. Some *cis* and *trans* isomers of hydantoin hybrids have been prepared and their *in vitro* cytotoxicity has been evaluated against lung, cervical, prostate and breast carcinoma [7]. A large number of stereoselective syntheses have been developed for such pharmacologically important alkaloids and some of these were reviewed by Czarnocki *et al.* [8].

The β -carboline derivatives are of great pharmaceutical potential, and attempts to synthesize novel compounds might possibly result in the discovery of effective new drugs. These new chiral compounds demand the development of effective methods offering enantioselectivity for efficient separations. In the past decade, high-performance liquid chromatography (HPLC) has become the most powerful technique for the separation and identification of both achiral and chiral compounds. There are a number of examples for the achiral HPLC analysis of β -carboline derivatives, but attempts to achieve the enantioseparations of β -carboline analogs are rare. Achiral HPLC analyses of β -carboline analogs such as harmine, harmoline and their metabolites have been performed on C18 stationary phases with the application of aqueous formic acid–

acetonitrile mobile phases with UV [9] and mass-spectrometric (MS) detection [10,11] or through the application of aqueous phosphate buffer–methanol mobile phases with MS detection [12]. Leon *et al.* applied chemically modified β -cyclodextrins as mobile phase additives for the achiral separation of norharmane, harmane and harmine on methyl- and octadecyl-silica stationary phases [13]. Pulapati *et al.* [14] applied a thin-layer chromatographic method for the determination of β -carboline derivatives.

However, there are very few literature data on the HPLC enantioseparations of chiral β -carboline derivatives. Enantioseparations of amino acid type β -carboline, 1,2,3,4-tetrahydronorharmane-1- and -3-carboxylic acid were performed by an indirect method with the application of (*S*)-*N*-(4-nitrophenoxycarbonyl)phenylalanine methoxyethyl ester as chiral derivatizing agent [15], or by direct methods applying macrocyclic glycopeptides as chiral selectors (SOs) [16,17]. In another paper, the chiral separations of enantiomers of 16 β -carboline derivatives were described by using cellulose *tris*-(3,5-dimethylphenyl carbamate)-based Chiralcel OD-H column and by capillary electrophoresis applying highly sulfated β -cyclodextrins as chiral selectors SOs [18].

~~As the column temperature is regarded as an intrinsic but sensitive parameter in chromatographic enantioseparations, the study of the temperature dependence of the retention and enantioselectivity is of interest as it offers valuable information on the chiral recognition process. The differences in the change in standard enthalpy $\Delta(\Delta H^\circ)$ and entropy $\Delta(\Delta S^\circ)$ can be expressed by the modified van't Hoff equation, which may be interpreted in terms of mechanistic aspects of the chiral recognition process [19–23]:~~

where R is the gas constant, T is temperature in Kelvin and α is the selectivity factor. In this study, the classical van't Hoff approach was applied, where quantification of the thermodynamic coefficients separately for the chiral and nonchiral sites is not possible, *i.e.* the van't Hoff plots yield apparent changes in standard enthalpy and entropy values, in which the respective contributions of the chiral and achiral interactions are combined. For a more realistic approach, the contributions of the enantioselective and nonselective sites should be distinguished [24].

After the first report by Okamoto *et al.* on the application of CSPs based on cellulose and amylose derivatives [1925], the polysaccharide-based CSPs went through a very dynamic period of development becoming the most popular and most successful chiral columns [2026]. Chankvetadze *et al.* further extended the large pool of “Okamoto” phases with the synthesis of additional polysaccharide-based CSPs by incorporating the halo-methyl-phenylcarbamate moieties to the cellulose and amylose chains [21-2527-31].

The portfolio of chiral columns was recently extended by the introduction of novel strong cation exchangers (SCXs) based on different 3,5-disubstituted benzoic acids functionalized with *trans*-(*R,R*)- and *trans*-(*S,S*)-2-aminocyclohexanesulfonic acid as chiral selectors (SOs) and ion exchange unit [DCL-RR (CSP-7) and DCL-SS (CSP-8)] were developed (Fig. 1). This type of SOs can operate under slightly acidic polar-ionic mobile phase conditions (PIM) and can be applied efficiently for the separation of chiral amine-type analytes [26-2933-35].

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expressed by the modified van't Hoff equation, which may be interpreted in terms of mechanistic aspects of the chiral recognition process [30-34]:

$$\ln \alpha = -\frac{\Delta(\Delta H^\circ)}{RT} + \frac{\Delta(\Delta S^\circ)}{R}$$

where R is the gas constant, T is temperature in Kelvin and α is the selectivity factor. In this study, the classical van't Hoff approach was applied, where quantification of the thermodynamic coefficients separately for the chiral and nonchiral sites is not possible, *i.e.* the van't Hoff plots yield apparent changes in standard enthalpy and entropy values, in which the respective contributions of the chiral and achiral interactions are combined. For a more realistic approach, the contributions of the enantioselective and nonselective sites should be distinguished [35].

The focus of the present paper is placed on the study of the enantioseparation of free and *N*-Boc-protected β -carboline derivatives and a comparison of separation performances obtained with the quite different polysaccharide- and SCX-type CSPs and columns composed of these (see Experimental section). A detailed study was carried out to investigate the effects of the mobile phase composition (normal phase or ~~polar ionic mode~~ (PIM)), the nature and concentrations of different mobile phase additives, the specific structural features of the analytes and SOs and the temperature on the chromatographic behavior. The elution sequence was determined in all cases, by spiking the racemic samples with one of the fully characterized enantiomers.

2. Experimental

2.1. Chemicals and reagents

The racemic 1-hydroxymethyl β -carboline derivatives were synthesized with Pictet-Spengler ring closure [36], from the corresponding tryptamine hydrochloride derivatives. Next, the nitrogen at position 2 was protected with *tert*-butoxycarbonyl (Boc) group to ensure the asymmetric acylation at the primary hydroxy group. Enzymatic resolutions were performed with acetic anhydride in the presence of immobilized *Candida antarctica* lipase B, in toluene at 60 °C. The resulting *N*-protected amino alcohols **1A**, **3A** and **5A** and the respective *O*-acyl enantiomers were separated and purified by column chromatography, then the enantiomers were hydrolysed and deprotected to result in the tetrahydro- β -carboline enantiomers **2A**, **4A**, **6A**, **4B** and **6B** and **1B**, **3B** and **5B** [37] (Fig. 2).

n-Hexane, methanol (MeOH), acetonitrile (MeCN) of HPLC grade were obtained from VWR International (Radnor, PA, USA). The alcohol additives were ethanol (EtOH), 1-propanol (PrOH), 2-propanol (2-PrOH), 1-butanol (BuOH) and *t*-butanol (*t*-BuOH), the base additives were diethylamine (DEA) and triethylamine (TEA), while the acid additive was acetic acid (AcOH). All these additives were of analytical grade and were obtained from VWR International.

2.2. Apparatus and chromatography

A 1100 Series HPLC system from Agilent Technologies (Waldbronn, Germany), consisting of a solvent degasser, a pump, an autosampler, a column thermostat, a

multiwavelength UV-Vis detector and a corona-charged aerosol detector from ESA Biosciences, Inc. (Chelmsford, MA, USA), was applied for the chromatographic measurements. Data acquisition and analysis were carried out with ChemStation chromatographic data software from Agilent Technologies.

The polysaccharide-based HPLC columns used were cellulose *tris*-(3,5-dimethylphenyl carbamate) (Lux Cellulose-1, CSP-1), cellulose *tris*-(3-chloro-4-methylphenyl carbamate) (Lux Cellulose-2, CSP-2), cellulose *tris*-(4-methylbenzoate) (Lux Cellulose-3, CSP-3), cellulose *tris*-(4-chloro-3-methylphenyl carbamate) (Lux Cellulose-4, CSP-4), amylose *tris*-(3,5-dimethylphenyl carbamate) (Lux Amylose-1, CSP-5) and amylose *tris*-(5-chloro-2-methylphenyl carbamate) (Lux Amylose-2, CSP-6) (250 x 4.6-mm I.D., 5- μ m particle size for all columns) (Phenomenex, Torrance, CA, USA) (Fig. 1). The dead-times (t_0) of the polysaccharide-based columns were determined via the injection of tri-*t*-butylbenzene. The SCX-type columns DCL-RR (CSP-6) and DCL-SS (CSP-7) (150 x 4.0-mm I.D., 5- μ m particle size for both) were prepared according to [33]. The t_0 data of the SCX-type columns were determined by the injection of methanolic acetone.

Chromatography was performed in isocratic mode at a flow rate of 0.6 or 0.8 mL min⁻¹ and a column temperature of 25 °C (if not otherwise stated). UV detection was accomplished at the selected wavelength of 230 nm. All analytes (selectands, SAs) were dissolved in the starting mobile phase or MeOH in the concentration range 0.5-1.0 mg mL⁻¹.

3. Results and Discussion

3.1. Enantioseparation on polysaccharide-based CSPs

The separations of the stereoisomers (Fig. 2) were evaluated on six different cellulose and amylose-based CSPs with mobile phases of *n*-hexane containing different alcohol additives (EtOH, PrOH, 2-PrOH, BuOH or *t*-BuOH). (To improve peak shapes, a base additive was also applied in the mobile phase.) The effect of the nature of alcohol was investigated for **SAs 3** and **4** on CSP-2 in the *n*-hexane/alcohol/DEA mobile phase system at constant alcohol molar concentration (0.26 mol L⁻¹), which refers to the volume ratio of alcohol in *n*-hexane for EtOH (15.2 vol.%), PrOH (20 vol.%), 2-PrOH (20 vol.%), BuOH (23.7 vol.%) or *t*-BuOH (24.6 vol.%). Figure 3 depicts the chromatographic data obtained using a constant molar alcohol concentration for **SA 3**. The retention factors for the first-eluting enantiomers did not differ considerably and were highest in the *t*-BuOH-containing mobile phase systems. The enantioselectivity changed explicitly, α varied between $1.9 < \alpha < 3.2$, *i.e.* the nature of the alcohol affects the ratio of the nonenantioselective and enantioselective interactions. The highest α values were observed when 2-PrOH or *t*-BuOH was added, but the application of *t*-BuOH was disadvantageous due to its higher viscosity. The changes in selectivity might be explained by the specific changes in the supramolecular structure of the SO ~~selector~~ and the changes in the solvation of the SAs. The change caused in the structure of the CSP by the change of the nature of the alcohol may affect the enantioselectivity of the CSP, depending on the size and structure of the SA [38]. The nature of the alcohol also slightly influenced the resolution; the highest R_S for both **3** and **4** was attained with 2-PrOH. Because of the advantageous features of 2-PrOH

(lower viscosity and higher resolutions), most of the experiments were carried out with 2-PrOH as alcohol modifier.

The amount of the alcohol in the mobile phase is expected to affect the chromatographic parameters. For **SAs 2, 4 and 6**, typical normal phase behavior was observed. On CSP-1, increase of the 2-PrOH content from 20 to 40% resulted in much smaller retention factors (data not shown). Simultaneously, α and R_S also decreased with increasing 2-PrOH content (these SAs could be resolved only partially in the *n*-heptane/2-PrOH/DEA mobile phase system).

For the studied SAs, a structure–retention relationship was investigated. Chromatographic data, including k_I , α and R_S at the same eluent composition [*n*-heptane/2-PrOH/DEA (80/20/0.1 v/v/v)] on all six columns were measured and are reported in Table 1. Comparison of the separations of *N*-protected and non-protected β -carboline derivatives (**1** vs. **2**; **3** vs. **4**; and **5** vs. **6**) revealed that **SAs 2, 4 and 6** possessing a free secondary amino group generally exhibited higher k , but significantly lower α and R_S values, indicating more effective nonchiral, probably H-bond or remaining silanol group-driven interactions between SiO_2 CSP and SA. The *N*-protected analogs usually exhibited smaller k , but higher α and R_S values (an exception was on CSP-5, where a higher k_I was observed for **1** than for **2**). Thus, it can be noted that the *N*-Boc protection of **1, 3 and 5** favors for the chiral interactions between the SiO_2 CSP and SA, resulting higher α and R_S values, probably through more favorable steric interactions. Substitution of the aromatic ring with an electron-withdrawing fluoro or an electron-donating methoxy group affects the chiral discrimination of the studied SAs in different ways. In the case of *N*-Boc protection, the fluoro substitution (**1** vs. **3**) did not result in marked changes, while methoxy substitution (**1** vs. **5**) usually led to higher retention and lower selectivity and resolution. In the case of the non-protected SAs, the fluoro substitution (**2** vs. **4**) generally resulted in lower retention, without

significant changes in the selectivity and resolution. In the case of the methoxy substitution (**2** vs. **6**), for almost all the CSPs the observed higher retentions were accompanied by higher α and R_S values. In Table 1, the CSP resulting the best resolution in case of a certain analyte is highlighted with bold.

On comparison of the chromatographic characteristics of the polysaccharide-based CSPs, no general rules could be set up. As presented in Table 1, the largest k_I was obtained on CSP-5 for **1**, **3** and **5**, on CSP-3 for **2** and **4**, and on CSP-4 for **6**. Especially high k values were observed on CSP-4 for analytes **2**, **4** and **6** containing a free secondary $-\text{NH}-$ group, but the higher retentions were not accompanied by the highest selectivities.

Comparison of the retentive characteristics of CSP-2 and CSP-4 (which differ in the positions of the methyl and chloro groups on the phenyl ring) shows that k_I was higher for all the studied SAs on CSP-4. Interestingly, despite the higher k , better selectivities were obtained with CSP-2 (**4** exhibited no separation either on CSP-2 or on CSP-4), suggesting that the steric arrangement of the methyl and chloro groups on CSP-2 is more advantageous for the chiral recognition of β -carboline derivatives. The four cellulose-based columns effectively differentiated the *N*-protected β -carboline enantiomers (**1**, **3** and **5**), while for the efficient separation of the enantiomers of the nonprotected derivatives the amylose-based CSPs were needed.

The chromatographic performances of the cellulose- and amylose-based CSPs containing the same *tris*-(3,5-dimethylphenyl carbamate) moiety can be compared based on the basis of the data obtained with CSP-1 and CSP-5. When the same mobile phases were used, it can be stated that much higher retentions were obtained with the amylose-based CSP for the *N*-Boc-protected SAs (**1**, **3** and **5**) than with the cellulose based CSP. Interestingly, this situation was reversed in

the case of the nonprotected SAs (**2**, **4** and **6**), *i.e.* significantly stronger retention was observed with the cellulose-based CSP. This fact shed light on the importance of the polysaccharide chain in the formation of nonselective interactions. The enantioselectivity of the cellulose-based CSP was higher for the *N*-Boc-protected SAs, while in the case of the nonprotected SAs, in contrast, the amylose-based CSP offered higher selectivity. It is interesting to note that in most cases the resolution observed was better with the amylose-based CSP due to the better peak shapes.

In summary, it can be concluded that the six recently commercialized polysaccharide-type CSPs displayed a complementary character. The *tris*-(3,5-dimethylphenylcarbamate) substitution and helical structure of amylose (CSP-5) leads to effective chiral interactions for both non protected and *N*-protected β -carboline derivatives. All six analytes can be well resolved with resolution values between 2.86 and 5.33. None of the other five polysaccharide-based CSPs showed a similar performance; in fact some of them failed in one or more cases.

The elution sequences were determined in all cases. No consistent elution sequence was observed, which shed light on the importance of the identification of the chromatographic peaks (Table 1). The directly comparable cellulose- and amylose-based CSP-1 and CSP-5 offered the same elution sequence for the investigated SAs.

3.2. Enantioseparation on SCX-type CSPs

As described above, the polysaccharide-based CSPs were quite effective in the enantioseparation of neutral *N*-protected β -carboline derivatives, while except for CSP-5 their efficiency was significantly lower for the enantioseparation of the basic compounds **2**, **4** and **6** containing a free secondary amine (-NH-) group. One aim of this study was to evaluate chiral cation exchangers as alternative CSPs for the resolution of the free and *N*-protected β -carbolines.

It is known that for SCX columns the primary driving force for retention is the formation of ion pairs via long-range electrostatic interactions between the protonated amino group of the SA (cationic species) and the deprotonated sulfonic acid group of the SO (anionic species) in PIM [27,2833,34]. For chiral recognition, however, not only ionic interactions, but also additional short-range, such as π - π , H-bond, dipole-dipole and steric interactions need to become active in order to fulfill the multiple intermolecular SO^- CSP-SA interaction model for chiral discrimination concepts. For the ionization state of SO and SA, the apparent pH of the mobile phase is critical; generally slightly acidic mobile phases are applied.

As reported previously, SCX columns afforded the best results in PIM, when a mixture of MeOH (as polar protic solvent) and MeCN (as non-polar aprotic solvent) was applied in the presence of organic acid and base, keeping the acid-to-base ratio at 2:1 [27,2833,34]. The protic MeOH can competitively suppress intermolecular H-bonding, while the aprotic MeCN may support ionic and H-bonding events. On the other hand, MeCN may weaken intermolecular π - π interactions and the chromatographic parameters (*i.e.* k and α values) can be regulated through the variation of the ratio of MeOH/MeCN. Accordingly, the effects of the bulk solvent compositions were investigated for SAs **2**, **4** and **6** containing a free secondary amino group in different ratios of MeOH/MeCN, while the acid (AcOH, 200 mM) and base (TEA, 100 mM) were kept at constant concentrations. As depicted in Fig. 4, with increasing MeCN content, the retention increased when CSP-7 was applied, while on CSP-8 a minimum curve was observed α and R_s increased slightly with increasing MeCN concentration. The enhanced retention observed for SAs **2**, **4** and **6** at increased MeCN content can probably be explained by the decreased solvation of the ionizable/ionized species, *i.e.* solvation of the polar compounds in a mobile phase with a higher MeCN content was less effective, and thus the electrostatic interactions

between the SO and the SA become stronger, resulting in higher retention. With increasing MeCN content, α and R_S increased slightly.

It should be noted that under normal phase conditions the *N*-protected β -carboline derivatives (despite the retardation) exhibited no separations on SCX columns on variation of the mobile phase composition of the *n*-hexane/2-propanol/DEA system between 98/2/0.1-50/50/0.1 (v/v/v) (data not shown).

As indicated earlier for the chiral discrimination of amines on chiral SCX-type CSPs, multiple SO-SA intermolecular interactions have to come into play in a concerted action whereby the long-ranged electrostatic forces are the primary ones [27,28,33,34]. In the ion-pairing process, the protonated base additives act as basic counter-ions (displacer) in the mobile phase by competing with the protonated analytes for the bonding sites of the SOs. Thus, the retention can be adjusted through variation of the concentrations of the counter-ions [39,40]. The effects of variation of the concentrations of basic counter-ion additives on the retention are depicted in Fig. 5. Under the studied conditions, linear relationships were found between $\log k'$ and $\log c_{TEA}$, with slopes around 1.0.

According to the displacement model described earlier [41], the slopes of these plots are determined by the ratio of the effective charges of the solute and the counter-ions; the observed slopes around 1.0 correspond with the values of around 0.9 found for an SCX CSP and different amines [39]. On the SCX CSPs under the applied conditions, practically identical slopes were obtained for each enantiomer, *i.e.* the enantioselectivity remained almost constant (data not shown). When the counter-ion concentration was changed on both CSPs the α values stayed practically constant for SA 2, SA 4 and 6 (1.16-1.17).

The SAs **2**, **4** and **6** in this study (Fig. 2) differed on the nature of the substitution pattern (fluoro or methoxy group) on the aromatic ring. These structural differences lead to different effects influencing the electron-withdrawing/donating character of the aromatic moiety and hence their interactions with the chiral SOs.

On both CSPs, at a given mobile phase composition, the fluoro- and methoxy-substituted analogs interacted more efficiently with the CSP selector than did the nonsubstituted one (**4** and **6** vs. **2**) (Table 2), which was manifested in slightly higher k values and more pronounced α and R_S values. On comparison of the performances of the two SCX-type CSPs, it can be noted that slightly higher retention was observed on CSP-8 than on CSP-7, but the α values on both columns were very similar.

In contrast with the polysaccharide-based columns, the elution sequence exhibited consistent behavior; as shown in Table 2, the observed elution sequence was reversed in every case on switching from CSP-7 [$S < R$] to CSP-8 [$R < S$], revealing that these two CSPs behave as enantiomers (see Fig. 1). Under the given mobile phase conditions, on the polysaccharide-based columns compounds **2**, **4** and **6** could not be separated efficiently, whereas on SCX columns under the mobile phase conditions useful for the retention and elution, the free bases **2**, **4** and **6** were very strongly retained and could be separated efficiently.

Chromatograms obtained under various chromatographic conditions are presented in Fig. 6.

(To compare the performance of two different types of CSPs (e.g. CSPs studied here) applying polar ionic mobile phase conditions would be quite reasonable and it would be a subject of a separate comprehensive study, including additional model compounds.)

3.3. Effects of temperature and thermodynamic parameters

In order to investigate the effects of temperature on the chromatographic parameters and to be able to draw mechanistic conclusions, a variable-temperature study was carried out over the temperature range 5-40 °C on the selected polysaccharide-based and SCX-type CSPs. Experimental data for **SAs 1, 3 and 5** on CSP-2 and for **SAs 1-6** on CSP-5 in the mobile phase hexane/2-PrOH/DEA (80/20/0.1 v/v/v) are presented in Table S1, and similarly chromatographic data for **SAs 2, 4 and 6** on CSP-7 and CSP-8 in mobile phase **a**, MeOH/MeCN (50/50 v/v) containing 100 mM TEA and 200 mM AcOH, and on CSP-8 for mobile phase **b**, MeOH (100%) containing 100 mM TEA and 200 mM FA, are given in Table S2.

A comparison of the retention factors (Tables S1 and S2) revealed that all of the recorded k_I values decreased with increasing temperature on all columns in all eluent systems. Similarly to k_I , α decreased with increasing temperature. The dependence of the resolution on temperature, however, was more complex. On SCX-based columns, R_S decreased with increasing temperature (Table S2), while on polysaccharide-based CSP-2 and CSP-5, R_S increased with increasing temperature (an exception was **1** on both columns) (Table S1). The increase in R_S at higher temperatures may be explained by the dominating effect of the increased column efficiency and improvement of the peak symmetry.

Since the effect of temperature on the separation was more complex, an extensive study dealing with the thermodynamics of enantiomer separation was carried out. For the calculation of thermodynamic parameters, chromatographic data were collected, from which van't Hoff plots were constructed. As a general trend, $\ln \alpha$ vs. $1/T$ gave linear van't Hoff plots, as indicated by the correlation coefficients in Tables 3 and 4.

On polysaccharide-based columns, the differences in the changes in standard enthalpy, $\Delta(\Delta H^\circ)$, reflecting the relative ease of transfer of the SAs from the mobile to the stationary phase, on both CSP-2 and CSP-5 were negative (Table 3). It is widely accepted that both enantiomers undergo the same nonspecific interactions, whereas the more strongly retained one is subjected to additional stereospecific interactions. The lower $\Delta(\Delta H^\circ)$ values point to more efficient transfer of the enantiomers between the mobile and the stationary phase and/or the stronger interaction of the more retained enantiomers with the CSP ~~SO~~. At the same mobile phase composition, *n*-hexane/2-PrOH/DEA (80/20/0.1 v/v/v) for SAs **1**, **3** and **5**, $\Delta(\Delta H^\circ)$ was lower on cellulose-based CSP-2 than on amylose-based CSP-5, indicating a more favorable exothermic process. However, the $\Delta(\Delta H^\circ)$ values on CSP-5 for *N*-Boc-protected and non-protected SAs did not differ considerably; the interactions of structurally different SAs with an amylose-based CSP ~~selector~~ were similar.

The trends in the change in $\Delta(\Delta S^\circ)$ were similar to the trends in the change in $\Delta(\Delta H^\circ)$ and showed that the lowest entropies on CSP-2 were observed for SAs **1** and **3** (-3.1 and -3.8 J mol⁻¹ K⁻¹, respectively) and on CSP-5 for SAs **2** and **6** (-1.4 and -1.2 J mol⁻¹ K⁻¹, respectively), indicating a thermodynamically unfavorable process. The $\Delta(\Delta S^\circ)$ values are governed by the difference in the number of degrees of freedom between the stereoisomers on the CSP, and mainly by the numbers of solvent molecules released from the SO and the SA when the analyte is associated with the CSP. The $\Delta(\Delta S^\circ)$ values for SAs **1** and **3** were significantly lower on CSP-2 than on CSP-5, while for SA **5** the $\Delta(\Delta S^\circ)$ values on both CSPs were very similar, suggesting a similar contribution of entropy change to chiral discrimination.

On SCX-based columns, the differences in the changes in standard enthalpy, $\Delta(\Delta H^\circ)$, were negative relating to a favorable exothermic process (Table 4). At the MeOH/MeCN (50/50 v/v) mobile phase composition containing 100 mM TEA and 200 mM AcOH, the $\Delta(\Delta H^\circ)$ values on CSP-7 ranged from -1.1 to -1.9 kJ mol⁻¹, and those on CSP-8 ranged from -1.1 to -1.8 kJ mol⁻¹; the $-\Delta(\Delta H^\circ)$ values were practically the same on the two CSPs. Transfer of the solute from the mobile phase to the stationary phase can usually be characterized as an exothermic process, and consequently the retention and selectivity decrease with increasing temperature, as observed in our case. It was also observed that the change of eluent composition (application of 100% MeOH containing 100 mM TEA and 200 mM AcOH) has no marked effect on the standard enthalpy change. On both columns, the $\Delta(\Delta H^\circ)$ values were slightly less negative for **2** than for **4** and **6**, indicating more efficient binding of fluoro- and methoxy-substituted derivatives to the SOs.

The trend in the change in $\Delta(\Delta S^\circ)$ was similar to that observed in $\Delta(\Delta H^\circ)$. Under the same mobile phase conditions on CSP-7, $\Delta(\Delta S^\circ)$ ranged from -2.7 to -4.6 J mol⁻¹ K⁻¹, and that on CSP-8 ranged from -2.6 to -4.4 J mol⁻¹ K⁻¹, *i.e.* no significant changes were observed in comparison with the two SCX-type CSPs. The negative $\Delta(\Delta S^\circ)$ indicates that the adsorbed enantiomers exhibited increased order and/or a loss in the degrees of freedom on the SO, indicating a thermodynamically unfavorable process. Similarly to $\Delta(\Delta H^\circ)$, the $\Delta(\Delta S^\circ)$ values were lower for **2** than for **4** and **6**. The mobile phase composition had slight effects on the change in entropy. Increase of the polar component of MeOH in the mobile phase resulted in only a slight change in $\Delta(\Delta S^\circ)$.

The relative contributions of the enthalpic and entropic terms to the free energy of adsorption can be visualized through the enthalpy/entropy ratio Q [$Q = \Delta(\Delta H^\circ) / [298 \times \Delta(\Delta S^\circ)]$] calculated at 298 K (Tables 3 and 4). Comparison of the Q values for the individual analytes revealed that the enantioselective discrimination was enthalpically driven ($Q > 1.0$) in all cases and the high Q values ($Q > 3$) indicate that the enthalpic contribution to the free energy was much higher on polysaccharide-based CSPs than on the brush-type CSPs of the SCX character.

4. Conclusions

In a comparative study, the enantioseparations of β -carboline derivatives were investigated on CSPs containing either polysaccharide-based chiral SOs ~~selectors~~ such as cellulose *tris*-(3,5-dimethylphenyl carbamate), cellulose *tris*-(3-chloro-4-methylphenyl carbamate), cellulose *tris*-(4-methylbenzoate), cellulose *tris*-(4-chloro-3-methylphenyl carbamate), amylose *tris*-(3,5-dimethylphenyl carbamate) and amylose *tris*-(3-chloro-4-methylphenyl carbamate) and on CSPs of the brush-type strong cation exchanger based on 3,5-disubstituted benzoic acid amides of *trans*-(*R,R*)- and *trans*-(*S,S*)-2-aminocyclohexanesulfonic acid. As mobile phase, either various normal phase (*n*-hexane/alcohol/alkylamine) or polar ionic (MeOH/MeCN containing organic acid and base) variants were employed. The chromatographic retention behavior and resolution proved to depend significantly on the nature and the concentration of the mobile phase additives, the temperature and the structures of the SOs and SAs.

The values of thermodynamic parameters such as the changes in $\Delta(\Delta H^\circ)$ and $\Delta(\Delta S^\circ)$ proved to depend on the structures of the analytes and on the CSP ~~chiral-selector~~ used. All of the

separations were enthalpically-driven, and the enthalpic contribution to the free energy was much higher on polysaccharide-based CSPs than on SCX-type CSPs.

Of the studied CSPs, the polysaccharide-based CSPs appeared best suitable for the direct enantioseparation of the *N*-protected β -carboline derivatives, while for the enantioseparation of the nonprotected derivatives, containing a free secondary amino group only the amylose *tris*-(3,5-dimethylphenyl carbamate)-based and the SCX-type CSPs performed well, and baseline resolution was achieved for all of the analytes, ~~but not on the same CSP; the six commercial cellulose or amylose based and the strong cation-exchanger type CSPs exhibited a complementary character, which led to successful resolutions.~~

The elution sequence was determined in all cases, but on polysaccharide-based CSPs no general rule was found, which reflects the importance of the identification of the elution sequence when polysaccharide-based CSPs are used. However, the elution sequence on the two quasi-enantiomeric SCX-type CSPs could be reversed, which is an advantageous feature in the case of the determination of the minor component in the presence of the major one.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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Figure legends

Figure 1

Structures of polysaccharide- and SCX-type chiral stationary phases, CSP-1 – CSP-8

Backbone of CSP	R	Selector	Trade name	CSP
Cellulose <i>tris</i> (3,5-dimethylphenylcarbamate)	Cellulose-1	CSP-1
	Cellulose <i>tris</i> (3-chloro-4-methylphenylcarbamate)	Cellulose-2	CSP-2
	Cellulose <i>tris</i> (4-methylbenzoate)	Cellulose-3	CSP-3
	Cellulose <i>tris</i> (4-chloro-3-methylphenylcarbamate)	Cellulose-4	CSP-4
Amylose <i>tris</i> (3,5-dimethylphenylcarbamate))	Amylose-1	CSP-5
	Amylose <i>tris</i> (5-chloro-2-methylphenylcarbamate)	Amylose-2	CSP-6
3,5-dichlorobenzoic acid functionalized with <i>trans</i> -(<i>R,R</i>)-2-amino-2-cyclohexanesulfonic acid	DCL- <i>RR</i>	CSP-7
	3,5-dichlorobenzoic acid functionalized with <i>trans</i> -(<i>S,S</i>)-2-amino-2-cyclohexanesulfonic acid	DCL- <i>SS</i>	CSP-8

Figure 2

Structures of analytes

Figure 3

Effects of the nature of the alcohol on the chromatographic parameters k_I , α and R_S for analyte **3** on CSP-2

Chromatographic conditions: column, CSP-2; mobile phase, *n*-hexane/alcohol/DEA concentration of alcohol in *n*-hexane was 0.26 mol l⁻¹ (see text); flow rate, 0.6 ml min⁻¹;

detection, 230 nm; symbols, k_I , , α , , R_S ,

Figure 4

Effects of MeCN content on the chromatographic parameters of k'_t , α and R_s for analytes **2**, **4** and **6** on CSP-7 and CSP-8

Chromatographic conditions: columns, CSP-7 and CSP-8; mobile phase, MeOH/MeCN (100/0, 90/10, 70/70 and 50/50 v/v) all containing 100 mM TEA and 200 mM AcOH; flow rate, 0.6 ml min⁻¹; detection, 230 nm; symbols, analyte **2**, ■; analyte **4**, ● and analyte **6**, ▲

Figure 5

Influence of the counter-ion concentration of selected analytes **2**, **4** and **6** on the retention of the first-eluting enantiomer, k_I

Chromatographic conditions: column, CSP-7 and CSP-8; mobile phase, MeOH containing 25 mM TEA and 50 mM AcOH, 50 mM TEA and 100 mM AcOH, 100 mM TEA and 200 mM AcOH and 200 mM TEA and 400 mM AcOH, respectively; flow rate, 0.6 ml min^{-1} ; detection, 230 nm; symbols, analyte **2**, ■; analyte **4**, ○ and analyte **6**, ▲

Figure 6

Chromatograms of selected SAs

~~Chromatographic conditions: column, for 1 and 3 CSP-2, for 2 and 4 CSP-7, for 2 CSP-8, for 5 and 6 CSP-5 and for 6 CSP-1; mobile phase, on CSP-1, CSP-2 and CSP-5 *n*-hexane-2-PrOH/DEA (80/20/0.1 v/v/v), on CSP-7 and CSP-8 MeOH/MeCN (50/50 v/v) containing 100 mM TEA and 200 mM AcOH; flow rate, 0.6 and 0.8 ml min⁻¹ (For more details see Tables 1 and 2.); detection, 230 nm~~

Table 1

Comparison of separation performances on polysaccharide-based columns: separation factor (k), selectivity factor (α), resolution (R_s) and elution sequences

Compound	Column	k_I	α	R_s	<i>Elution sequence</i>
1	CSP-1	0.63	1.80	3.38	$S < R$
	CSP-2	0.73	2.30	5.30	$S < R$
	CSP-3	0.69	1.56	2.41	$S < R$
	CSP-4	0.83	1.79	4.64	$S < R$
	CSP-5	1.54	1.54	4.83	$S < R$
	CSP-6	0.80	1.19	0.87	$S < R$
2	CSP-1	4.77	1.06	0.47	$S < R$
	CSP-2	4.84	1.05	0.31	$S < R$
	CSP-3	7.79	1.00	0.00	-
	CSP-4	7.48	1.05	0.30	$S < R$
	CSP-5	1.56	1.27	2.86	$S < R$
	CSP-6	3.53	1.00	0.00	-
3	CSP-1	0.57	2.03	4.16	$S < R$
	CSP-2	0.70	2.30	5.37	$S < R$
	CSP-3	0.78	1.15	0.60	$S < R$
	CSP-4	0.78	1.68	4.13	$S < R$
	CSP-5	1.69	1.29	3.16	$S < R$
	CSP-6	0.68	1.59	2.16	$S < R$
4	CSP-1	4.03	1.13	0.86	$R < S$
	CSP-2	3.99	1.00	0.00	-
	CSP-3	7.22	1.00	0.00	-
	CSP-4	5.99	1.00	0.00	-
	CSP-5	1.55	1.25	3.19	$S < R$
	CSP-6	2.96	1.00	0.00	-

Table 2

Comparison of separation performances on CSP-7 and CSP-8: separation factor (k), selectivity factor (α), resolution (R_S) and elution sequences

Compound	k_I	α	R_S	<i>Elution sequence</i>
CSP-7				
2	4.29	1.13	2.50	$S < R$
4	4.26	1.20	3.64	$S < R$
6	4.46	1.19	3.38	$S < R$
CSP-8				
2	5.27	1.14	2.33	$R < S$
4	5.32	1.21	3.52	$R < S$
6	5.46	1.20	3.16	$R < S$

Chromatographic conditions: column, CSP-7 and CSP-8; mobile phase, MeOH/MeCN (50/50 v/v) containing 100 mM TEA and 200 mM AcOH; flow rate, 0.6 ml min⁻¹; detection, 230 nm

Table 3

Thermodynamic parameters, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $Tx\Delta(\Delta S^\circ)_{298K}$, $\Delta(\Delta G^\circ)$, correlation coefficients, (R^2) and Q values of β -carboline derivatives on CSP-2 and CSP-5

Analyte	$-\Delta(\Delta H^\circ)$ (kJ mol ⁻¹)	$-\Delta(\Delta S^\circ)$ (J mol ⁻¹ K ⁻¹)	Corr. coeff. (R^2)	$-Tx\Delta(\Delta S^\circ)_{298K}$ (kJ mol ⁻¹)	$-\Delta(\Delta G^\circ)_{298K}$ (kJ mol ⁻¹)	Q
CSP-2						
1	2.8	3.1	0.999	0.9	1.9	3.1
3	3.0	3.8	0.995	1.1	1.9	2.7
5	1.0	<0.1	0.996	<0.1	<1.0	>10
CSP-5						
1	1.0	0.2	0.998	0.1	0.9	10
3	0.8	0.6	0.994	0.2	0.6	4.0
5	0.8	0.1	0.991	<0.1	<0.8	>8.0
2	1.0	1.4	0.997	0.4	0.6	2.5
4	0.6	0.1	0.915	<0.1	<0.6	>6.0
6	1.2	1.2	0.991	0.4	0.8	3.0

Chromatographic conditions: column, CSP-2 and CSP-5, mobile phase, *n*-hexane/2-PrOH/DEA (80/20/0.1 v/v/v); flow rate, 0.8 ml min⁻¹; detection, 230 nm; R^2 , correlation coefficient of van't Hoff plot, $\ln \alpha - 1/T$ curves; $Q = \Delta(\Delta H^\circ) / Tx\Delta(\Delta S^\circ)_{298K}$

Table 4

Thermodynamic parameters, $\Delta(\Delta H^\circ)$, $\Delta(\Delta S^\circ)$, $Tx\Delta(\Delta S^\circ)_{298K}$, $\Delta(\Delta G^\circ)$, correlation coefficients, (R^2) and Q values of β -carboline derivatives on CSP-7 and CSP-8

Analyte	Eluent	$-\Delta(\Delta H^\circ)$ (kJ mol ⁻¹)	$-\Delta(\Delta S^\circ)$ (J mol ⁻¹ K ⁻¹)	Corr. coeff. (R^2)	$-Tx\Delta(\Delta S^\circ)_{298K}$ (kJ mol ⁻¹)	$-\Delta(\Delta G^\circ)_{298K}$ (kJ mol ⁻¹)	Q
CSP-7							
2	a	1.1	2.7	0.999	0.8	0.3	1.4
4	a	1.9	4.6	0.999	1.4	0.5	1.4
6	a	1.6	3.9	0.999	1.2	0.4	1.3
CSP-8							
2	a	1.1	2.6	0.997	0.8	0.3	1.4
	b	1.1	2.8	0.950	0.8	0.3	1.4
4	a	1.8	4.4	0.998	1.3	0.5	1.4
	b	1.6	4.0	0.997	1.2	0.4	1.3
6	a	1.6	3.7	0.998	1.1	0.5	1.5
	b	1.6	3.9	0.996	1.2	0.4	1.3

Chromatographic conditions: column, CSP-7 and CSP-8, mobile phase, **a**, MeOH/MeCN (50/50 v/v) containing 100 mM TEA and 200 mM AcOH; **b**, MeOH containing 100 mM TEA and 200 mM AcOH; flow rate, 0.6 ml min⁻¹; detection, 230 nm; R^2 , correlation coefficient of van't Hoff plot, $\ln \alpha - 1/T$ curves; $Q = \Delta(\Delta H^\circ) / Tx\Delta(\Delta S^\circ)_{298K}$