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# High-performance liquid chromatographic evaluation of strong cation exchanger-based chiral stationary phases focusing on stationary phase characteristics and mobile phase effects employing enantiomers of tetrahydro-ß-carboline and 1,2,3,4-tetrahydroisoquinoline analogs

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ABSTRACT

In this study, we present results obtained on the enantioseparation of some cationic compounds of pharmaceutical relevance, namely tetrahydro-ß-carboline and 1,2,3,4-tetrahydroisoquinoline analogs. In high-performance liquid chromatography, chiral stationary phases (CSPs) based on strong cation exchanger were employed using mixtures of methanol and acetonitrile or tetrahydrofuran as mobile phase systems with organic salt additives.

Through the variation of the applied chromatographic conditions, the focus has been placed on the study of retention and enantioselectivity characteristics as well as elution order. Retention behavior of the studied analytes could be described by the stoichiometric displacement model related to the counter-ion effect of ammonium salts as mobile phase additives. For the thermodynamic characterization parameters, such as changes in standard enthalpy $\Delta(\Delta H^{\circ})$, entropy $\Delta(\Delta S^{\circ})$, and free energy $\Delta(\Delta G^{\circ})$, were calculated on the basis of van't Hoff plots derived from the ln $\alpha$ *vs.* 1/T curves. In all cases, enthalpy-driven enantioseparations were observed with a slight, but consistent dependence of the calculated thermodynamic parameters on the eluent composition. Elution sequences of the studied compounds were determined in all cases. They were found to be opposite on the enantiomeric stationary phases and they were not affected by either the temperature or the eluent composition.



## 1. Introduction

Numerous alkaloids, containing tetrahydroisoquinoline (THIQ) and tetrahydro-$\beta$-carboline (TH$\beta$C) core including their individual enantiomers, have important pharmacological activity. For example, expectorant emetine (*Ipecacuanhe*) [1], antitussive noscapine (*Papaver somniferum*) [2], and Trabectidine marketed as Yondelis® (*Ecteinascidia turbinate*) [3], show anticancer effect. Liensinine (*Nelumbo nucifera*) [4], saframycine A (*Myxococcus xanthus*) [5], and other synthetic THIQ analogs such as Zalypsis® [6], have promising pharmaceutical activities toward HIV or cancer. TH$\beta$C alkaloids, originated from both natural and synthetic sources, have also been investigated intensively in drug research. For instance, vincristine, vinblastine [7], and reserpine [8] are used in the therapies of cancer or hypertension. Callophycine A (*Callophycus oppositifolius*) [9] has cytotoxic, harmicine (*Kopsia Griffithii*) [10] exhibits antinociceptive, and (+)-7-bromotypargine (*Ancorina sp.*) shows antimalarial activity [11], whereas Tadalafil (Cialis®) was successfully applied in the treatment of erectile dysfunction [12]. In the course of the synthesis and stereochemical characterization of these compounds, enantioselective chromatographic protocols have to be integrated as well.

Accordingly, for such direct chromatographic enantiomer separation techniques appropriate chiral stationary phases (CSPs) and chiral columns need to be applied. In several review articles [13-17] the most popular methods applied for enantiomeric resolutions in both analytical and preparative scales have been discussed. In addition to the highly popular polysaccharide-based selectors (SOs)

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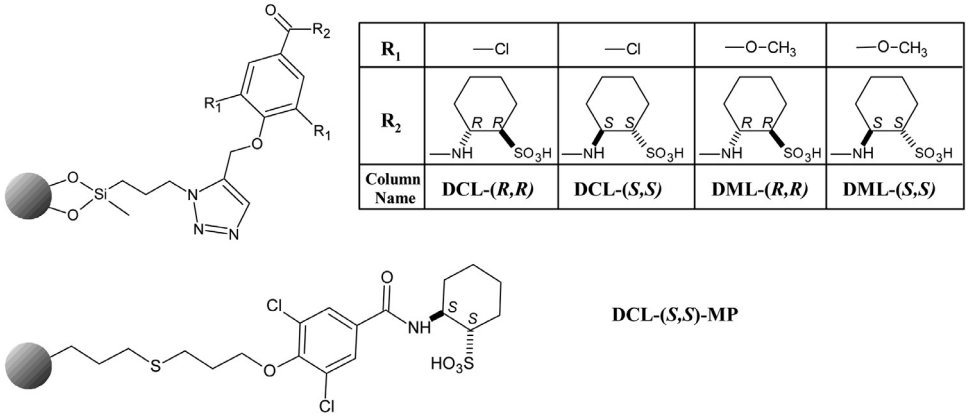


**Fig. 1.** Structure of chiral strong cation exchanger-type stationary phases.

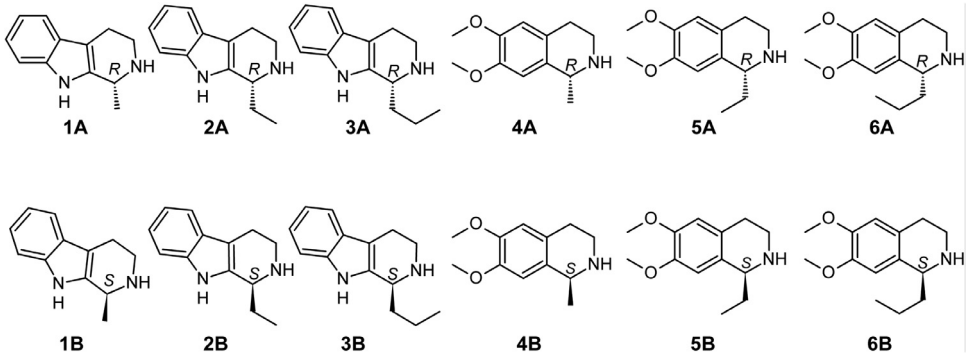


**Fig. 2.** Structure of analytes, tetrahydro-$\beta$-carboline (TH$\beta$C, **1**–**3**) and tetrahydroisoquinoline (THIQ, **4**–**6**) analogs.

[16-21], unique chiral cation- and zwitterion-type ion exchanger-based SOs and CSPs have also been developed in the last decade [22-26] to provide solutions for the resolution of charged analytes.

Recently, enantioseparation of some related THIQ derivatives was carried out on new CSPs based on chiral crown ethers [27], polysaccharides [28-32], and *Cinchona* alkaloids [32,33]. Compared to the THIQ analogs, there are relatively few literature data on the HPLC enantioseparations of chiral TH$\beta$C derivatives. Direct methods were based on the application of macrocyclic glycopeptides [34,35], polysaccharides [31,32,36,37], *Cinchona* alkaloids [32], and strong cation exchanger-based SOs [37].

In this study five novel, chiral strong cation exchangers (cSCXs), based on varied 3,5-disubstituted benzoic acids functionalized with *trans*-(*R,R*)- and *trans*-(*S,S*)-2-aminocyclohexanesulfonic acid (Fig. 1), have been evaluated for the enantiodiscrimination of six pairs of chiral amine-type analytes (Fig. 2) in order to gather information about the underlying cation exchange process [22,24]. This type of SOs can be operated under mild, often MS-compatible polar organic mobile phase conditions consisting of MeOH, MeCN and/or THF as organic solvents together with acidic and basic additives.

In consideration of previous results with respect to efficient separation of some ß-carboline derivatives [37], the focus of the present study is on a systematic study of the enantioseparation of the newly synthetized three THIQ and three THßC derivatives (Fig. 2) and a comparison of separation performances obtained with the cSCX-type CSPs (Fig. 1). Detailed investigations have been carried out to evaluate the effects of the composition of the polar organic mobile phase, the nature of additives, the amount and nature of the counter-ion, the specific structural features of the analytes (SAs) and SOs, as well as the temperature on retention, selectivity, and resolution of the stereoisomers. Since the configurations of all chiral analytes are known, the elution sequences were determined in all cases.

## 2. Materials and methods

### 2.1. Chemicals and reagents

On the basis of recent results on the enantioselective acylation of 1-alkyl-substituted THIQ [38] and TH$\beta$C [39], asymmetric *N*-alkoxycarbonylations of racemic 1-substituted THIQ and TH$\beta$C with phenyl allyl carbonate were carried out utilizing *Candida antarctica* lipase B in di-2-propylether ($i$Pr$_2$O) at 60°C (E > 200). The alkoxycarbonylation process provided enantiomers of 1-methyl- (**1A** and **1B**), 1-ethyl- (**2A** and **2B**), 1-propyl- (**3A** and **3B**) TH$\beta$C and 1-methyl- (**4A** and **4B**), 1-ethyl- (**5A** and **5B**), 1-propyl- (**6A** and **6B**) THIQ. The unreacted (*S*) enantiomers (**1B**–**6B**) as well as their antipods (**1A**–**6A**) were prepared through the enzymatic hydrolysis of the (*R*)-carbamates resulting in products with high enantiomeric excess (> 97%).

Acetonitrile (MeCN), methanol (MeOH), tetrahydrofuran (THF) of HPLC grade, and ammonium formate ($HCOONH_4$), ammonium acetate ($NH_4OAc$), triethylamine (TEA), formic acid (FA), acetic acid (AcOH) of analytical reagent grade were purchased from VWR International (Radnor, PA, USA). Ultrapure water was obtained from Ultrapure Water System, Puranity TU UV/UF (VWR International).

### 2.2. Apparatus and chromatography

To perform liquid chromatographic measurements, a Waters Breeze system consisting of a 1525 binary pump, a 2996 photodiode array detector, a 717 plus autosampler, and Empower 2 data manager software (Waters Corporation, Milford, MA, USA) was applied. A Lauda Alpha RA8 thermostat (Lauda Dr. R. Wobser Gmbh,

Lauda-Königshofen, Germany) was employed to maintain constant column temperature.

All analytes were dissolved in MeOH in the concentration range 0.5–1.0 mg $mL^{-1}$ and 20-µL samples were injected. The dead-times of the columns were determined by injecting acetone dissolved in MeOH. Experiments, unless otherwise stated, were carried out in isocratic mode at a flow rate of 0.6 mL $min^{-1}$ and column temperature of 25°C. The synthesis of the cSCX-type CSPs based on different 3,5-disubstituted benzoic acids functionalized with *trans*-(*R,R*)- and *trans*-(*S,S*)-2-aminocyclohexanesulfonic acids as chiral SOs and ion exchange units has already been described [24]. The structures of DCL-(*R,R*), DCL-(*S,S*), DML-(*R,R*), DML-(*S,S*), and DCL-(*S,S*)-MP CSPs, including the bonding chemistry onto silica is depicted in Fig. 1. All columns employed have the same physical size (150 × 4.0 mm I.D., 5-$\mu$m particle size).

## 3. Results and discussions

The compounds employed in this study are analogs of tetrahydro-ß-carboline and 1,2,3,4-tetrahydroisoquinoline. The three-ring THßC and two-ring THIQ parent compounds have different structural features, while the alkyl (methyl, ethyl, propyl) substitution in both types of analytes and the presence of methoxy group on THIQ afford additional structural differences. The secondary amino group in protonated (ionic) form renders electrostatic interaction with SOs of opposite charge. The calculated $pK_a$ values of secondary amino groups of analytes **1–6** are 9.16, 9.29, 9.30, 8.89, 9.04, and 9.06, respectively. (Calculations were performed with the Marvin Sketch v. 17.28 software, ChemAxon Ltd., Budapest.) The calculated $pK_a$ values of the amino group in the pyrrole moiety for analyte **1–3** were above 16, *i.e.*, no protonation can be expected under the applied conditions. All these structural features may contribute to the different noncovalent SO–SA interactions and chiral recognition characteristics.

### 3.1. Effect of mobile phase composition on chromatographic performances

On cSCX 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 SAs and the deprotonated aminocyclohexanesulfonic acid moiety of the SO. These work in cooperation with additional short-range noncovalent interactions such as H-bonding, dipole–dipole, $\pi$–$\pi$, and steric interactions [22,24,37]. As reported previously, cSCX columns afforded the best results when mixtures of MeOH (as polar protic solvent) and MeCN (as polar, but aprotic solvent) are applied in the presence of a weak organic base and a weak organic acid providing an overall slight acidity to the mobile phase [22,24]. On the basis of our preliminary experiments, the enantioseparation of THßC and THIQ analogs on the studied cSCX CSPs was first carried out with the application of MeOH and MeCN or THF as bulk solvents in different ratios containing base and acid additives.

First, the effects of the bulk solvent composition were investigated for analytes **1–6** by varying the MeOH/MeCN ratio between 100/0 and 25/75 (*v/v*), in the presence of 25 mM TEA and 50 mM FA. As illustrated in Fig. 3, for the $k_1$ values of all studied analytes significant increases were registered with increasing MeCN contents. The observed changes in the retention of THßC analogs were especially high compared to those of the THIQ analogs. These mobile phase systems were highly effective in the enantioseparation of THßC analogs (especially with DCL type CSPs). Regarding $\alpha$ and $R_S$ values, they increased markedly for the THßC analogs, but THIQ analogs were not separable under these conditions. As found earlier [26], the change of the polar but aprotic MeCN to THF may substantially affects the chiral discrimination of basic analytes due to the different hydrogen-bonding properties of the solvents. In the present study, the change of MeCN to THF revealed a significant effect on the retention behavior of the basic target analytes as visualized in Fig. 4. Namely, starting from a mobile phase containing 100% MeOH (in addition to 25 mM TEA and 50 mM FA) with increasing THF content $k_1$ first decreased and then after about 50% THF content it increased considerably (Fig. 4). A similar behavior was observed earlier with basic and acidic analytes on zwitterionic CSPs [40].

A comparison of Fig. 4 and Fig. 3 reveals that applying THF instead of MeCN as a co-solvent in MeOH, the retention profiles of analytes have a different shape. The observed retention factors are based on concerted multiple interactions between the SAs and the SO, which depend on the size of the solvation shells of all charged interaction sites of SO and SAs. The solvation shells of the charged compounds, in addition to their physical and chemical properties, will also be affected by both the acid and basic additives and the solvent mixture applied as mobile phase. Consequently, the observed retention behavior represents a rather complex situation. Based on data discussed above, an exact and validated explanation cannot be provided here. Therefore, it can only be hypothesized that the larger sizes of the solvation shells of the charged sites with a solvent component of higher acidity present in the eluent will influence the strength of the SO–SA electrostatic interactions resulting in lower retention factors. Simultaneously, the elution strength of the counter-ion is also affected by the mobile phase composition; *i.e.*, the larger the size of the solvation shell of the counter-ion, the lower its eluent strength will be, affording higher retention times. Since the retention will be the result of these two opposite effects, the measured retention times might increase or decrease with higher protic solvent ratios in the eluent, thus leading to a U-shape retention curve. Naturally, additional stereoselective SO–SA interactions will also be affected by the solvent composition, thus the observed $\alpha$ values may change, as it can also be deduced from Fig. 3 and Fig. 4. As expected, all these effects depend on the analyte and may somewhat be different for the TH$\beta$C and THIQ analogs. To validate this hypothesis, further experiments are planned to be performed.

These cSCX columns, in principle, can be operated with diverse amines in their protonated forms as counter-ions leading to conditions more compatible with MS [24]. As a consequence, further experiments with MeOH/MeCN and MeOH/THF bulk solvents containing $NH_4OAc$ as salt additive instead of TEA–FA mixtures were carried out. The effects of the bulk solvent composition were investigated for analytes **1–6** varying the MeOH/MeCN or MeOH/THF ratio between 100/0 and 20/80 (*v/v*) in the presence of 60 mM $NH_4OAc$. Results are visualized in Fig. S1 and Fig. S2. The retention behavior was similar to that of the MeOH/THF system applying TEA/FA additives with $k_1$ exhibiting a minimum curve upon changing MeOH/MeCN or MeOH/THF ratios. Interestingly, chiral discrimination for analytes **1–3** was independent of the MeOH/MeCN ratio, $\alpha$ remained practically constant, while in resolution a slight increase was observed with increasing MeCN content (Fig. S1). THIQ analogs could not be resolved under these conditions.

A comparison of the four cSCX columns linked with “triazole” revealed that under all studied conditions, at least partial separation could be achieved on all columns for the THßC analogs. The two 3,5-dichloro-substituted DCL-(*R,R*) and DCL-(*S,S*) type SOs and related columns exhibit particularly high separation performances for analytes **1**–**3** with resolutions ranging between 2.2–6.1. The two 3,5-dimethoxy-substituted SOs leading to a $\pi$-basic aryl moiety were less effective in the separation of THßC analogs; namely, $k_1$, $\alpha$, and $R_S$ were markedly smaller with the DML columns under identical conditions. For a set of experiments applying DCL-(*S,S*)-MP with MeOH/MeCN containing $NH_4OAc$ eluents the linkage type of the DCL SOs was also probed. The obtained results (data not

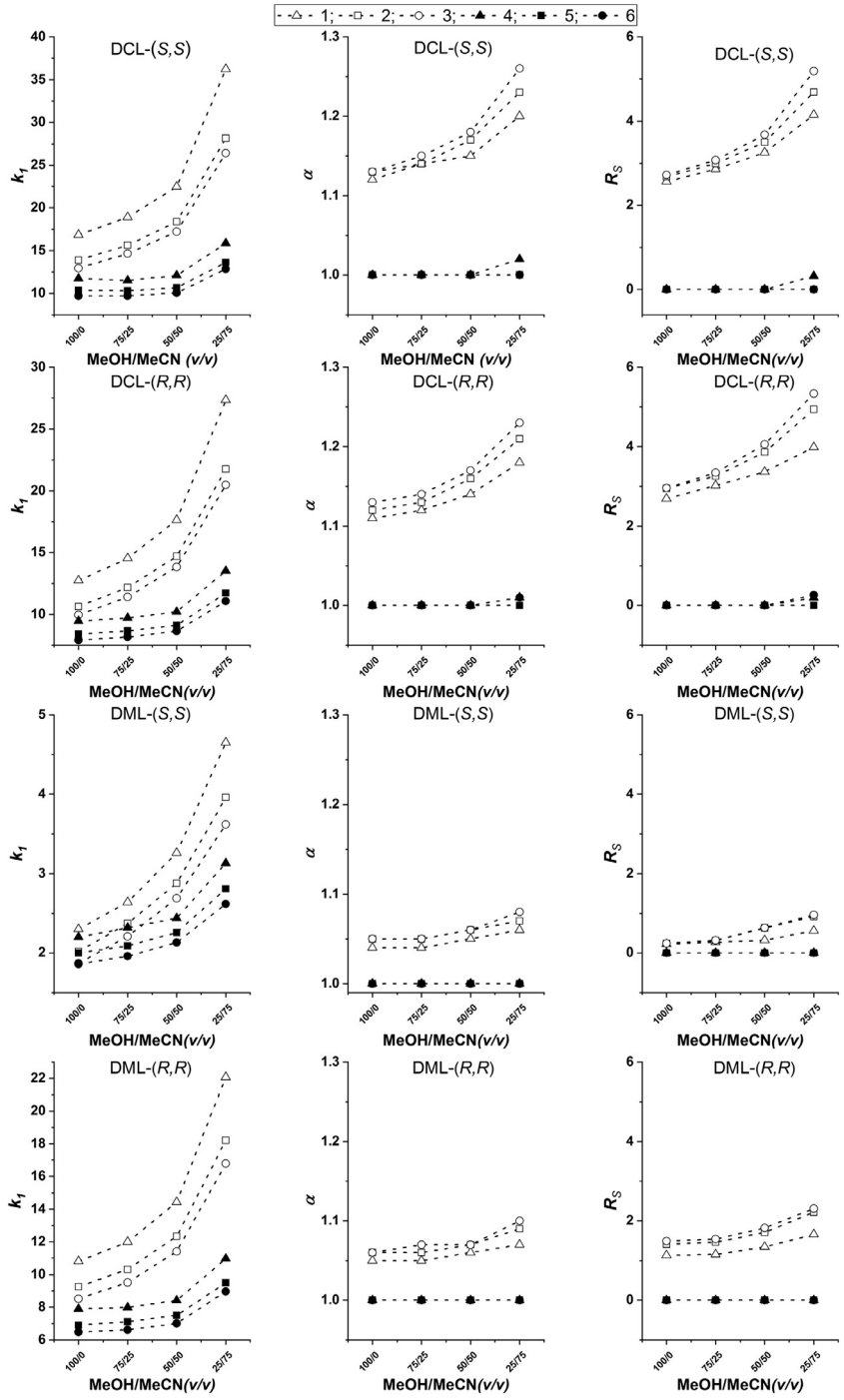


**Fig. 3.** Effects of mobile phase composition on the retention factor of the first-eluting enantiomer ($k_1$), the separation factor, ($\alpha$) and resolution ($R_S$).
Chromatographic conditions: columns, DCL-(*S,S*), DCL-(*R,R*), DML-(*S,S*), and DML-(*R,R*); mobile phase, MeOH/MeCN (100/0, 75/25, 50/50, and 25/75 *v/v*) all containing 25 mM TEA and 50 mM FA; flow rate, 0.6 ml min$^{-1}$; detection, 220–250 nm, temperature, 25 °C; symbols, for analyte **1**, △, for **2**, □, for **3**, ○, for **4**, ▲, for **5**, ■, for **6**, •.

shown in detail) provided evidence for an additional SO–SA interaction effect of the "triazole" linkage over the mercaptopropyl-bonding chemistry in the case of TH*ß*C analogs. The "triazole" moiety probably takes part in chiral discrimination through H-bonding interaction and its application results in higher retention and improved enantioselectivity.

### 3.2. Effect of the counter-ion concentration

The stoichiometric displacement model [41] is most often used to describe the retention behavior based on ion-pairing and ion-exchange mechanisms. As Eq. (1) shows, the model predicts that the logarithm of the retention factor is linearly related to the logarithm of the counter-ion concentration,

$$\log k = \log K_Z - -Z \log c_{counter-ion} \quad (1)$$

where $Z=m/n$, the ratio of the number of charges of the cation and the counter-ion and $K_Z$ is related to the ion-exchange equilibrium constant. That is, the log *k vs.* log $c_{\text{counter-ion}}$ function shows a linear relationship, where the slope of the line is proportional to the effective charge during ion exchange, while the intercept carries information about the equilibrium constant of ion exchange.

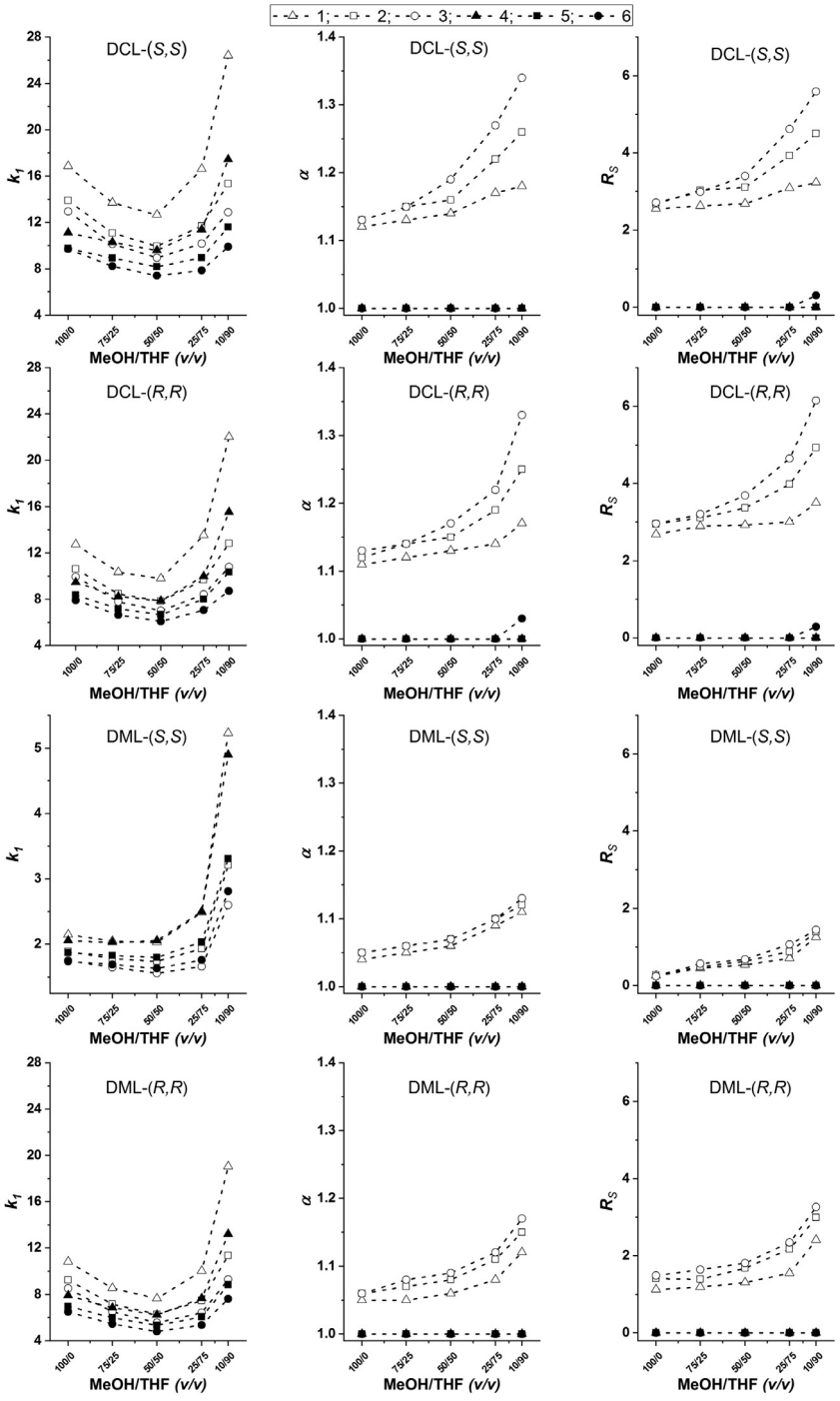


**Fig. 4.** Effects of mobile phase composition on the retention factor of the first-eluting enantiomer ($k_1$), for the separation factor ($\alpha$), and resolution ($R_S$). Chromatographic conditions: columns, DCL-(*S,S*), DCL-(*R,R*), DML-(*S,S*), and DML-(*R,R*); mobile phase, MeOH/THF (100/0, 75/25, 50/50, 25/75, and 10/90 *v/v*) all containing 25 mM TEA and 50 mM FA; flow rate, 0.6 ml $min^{-1}$; detection, 220–250 nm, temperature, 25 °C; symbols, for analyte **1**, △, for **2**, □, for **3**, ○, for **4**, ▲, for **5**, ■, for **6**, •.

Applying a mobile phase of MeOH/MeCN (50/50 *v/v*) in the presence of $NH_4OAc$ in the ion-pairing process, the protonated ammonium ion acts as a competitor. The effects of variation of the concentration of the counter-ion on retention for analytes 1–3 on three cSCX CSPs [DCL-(*S,S*), DCL-(*S,S*)-MP, and DCL-(*R,R*)] are depicted in Fig. S3. Under the studied conditions, linear relationships were found between log $k_1$ *vs.* log $c_{\text{counter-ion}}$ with slopes varying between (−0.86)–(−0.97). The observed slopes around −1.0 were not significantly affected by the linkage chemistry of the applied CSPs and they correspond well to the values found for different amines examined on cation-exchanger-type CSPs [22].

Varying the type of the counter-ion using mixtures of TEA and AcOH (*i.e.*, triethylammonium ion served as a counter-ion), slopes (Fig. S4) and enantioselectivities rather similar to those with $NH_4OAc$ were obtained. What becomes evident, however, is the effect of the type of the counter-ion (ammonium ion *vs* triethylammonium ion) on the retention behavior. At similar eluent compositions (MeOH/MeCN 50/50 *v/v*), the ammonium ion leads to much smaller retention factors (data not shown). This might be explained by the effect of the size of the solvated counter-ion. The smaller the size of the solvated counter-ion, the closer it can get to the ion-exchanger site and its elution ability will be the stronger. It is

**Table 1**
Effects of eluent composition on chromatographic data $k_1$, $\alpha$, $R_S$ of tetrahydro-ß-carboline and 1,2,3,4-tetrahydroisoquinoline analogs.

| Analyte | $k_1$, $\alpha$, $R_S$ | Column | MeOH/MeCN | MeOH/THF | Column | MeOH/MeCN | MeOH/THF |
|---|---|---|---|---|---|---|---|
| **1** | $k_1$ | **DCL-(*S,S*)** | 36.24(*S*) | 16.60(*S*) | **DML-(*S,S*)** | 4.65 (*S*) | 2.49 (*S*) |
| | $\alpha$ | | 1.20 | 1.17 | | 1.06 | 1.09 |
| | $R_S$ | | 4.15 | 3.09 | | 0.56 | 0.71 |
| **2** | $k_1$ | | 28.13(*S*) | 11.69(*S*) | | 3.96 (*S*) | 1.93 (*S*) |
| | $\alpha$ | | 1.23 | 1.22 | | 1.07 | 1.10 |
| | $R_S$ | | 4.69 | 3.93 | | 0.92 | 0.88 |
| **3** | $k_1$ | | 26.40(*S*) | 10.16(*S*) | | 3.62 (*S*) | 1.66 (*S*) |
| | $\alpha$ | | 1.26 | 1.27 | | 1.08 | 1.10 |
| | $R_S$ | | 5.19 | 4.62 | | 0.96 | 1.07 |
| **1** | $k_1$ | **DCL-(*S,S*)-MP** | 12.00 (*S*) | 7.81 (*S*) | **DML-(*R,R*)** | 22.07 (*R*) | 10.03 (*R*) |
| | $\alpha$ | | 1.13 | 1.10 | | 1.07 | 1.08 |
| | $R_S$ | | 3.07 | 2.56 | | 1.66 | 1.55 |
| **2** | $k_1$ | | 10.41 (*S*) | 6.01 (*S*) | | 18.22 (*R*) | 7.48 (*R*) |
| | $\alpha$ | | 1.14 | 1.14 | | 1.09 | 1.11 |
| | $R_S$ | | 3.68 | 3.32 | | 2.22 | 2.17 |
| **3** | $k_1$ | | 10.16 (*S*) | 5.46 (*S*) | | 16.80 (*R*) | 6.41 (*R*) |
| | $\alpha$ | | 1.15 | 1.16 | | 1.10 | 1.12 |
| | $R_S$ | | 3.71 | 3.68 | | 2.31 | 2.35 |
| **1** | $k_1$ | **DCL-(*R,R*)** | 27.34(*R*) | 13.54(*R*) | | | |
| | $\alpha$ | | 1.18 | 1.14 | | | |
| | $R_S$ | | 3.99 | 3.00 | | | |
| **2** | $k_1$ | | 21.76(*R*) | 9.68 (*R*) | | | |
| | $\alpha$ | | 1.21 | 1.19 | | | |
| | $R_S$ | | 4.94 | 3.98 | | | |
| **3** | $k_1$ | | 20.47(*R*) | 8.43 (*R*) | | | |
| | $\alpha$ | | 1.23 | 1.22 | | | |
| | $R_S$ | | 5.34 | 4.65 | | | |

Chromatographic conditions: columns, DCL-(*R,R*), DCL-(*S,S*), DML-(*R,R*), DML-(*R,R*), DCL-(*R,R*)-MP; mobile phase, MeOH/MeCN (25/75 *v/v*) or MeOH/THF (25/75 *v/v*) both containing 25 mM TEA and 50 mM FA; flow rate, 0.6 ml $min^{-1}$ detection at 223 or 230 nm; temperature, 25°C; (*R*) or (*S*), configuration of the first-eluting enantiomer.

important to keep in mind that the size of the solvated counter-ion depends not only on the size of the protonated amine, but also on the eluent composition (see earlier discussion). The aprotic solvent is a poor solvating agent for the cation resulting in a thinner solvation shell which, in turn, will enable stronger electrostatic interactions. Because of rather limited data, our hypothesis must not necessarily be generalized; therefore, the screening of the effect of the type and size of the amine used as counter-ion will necessary be performed.

### 3.3. Structure–retention relationships and elution sequences

In organic chemistry, the steric effect of a substituent pattern on the reaction rate of a particular reaction scenario had been characterized by Meyer with the so-called size descriptor (Meyer parameter, $V^a$) [42]. Accordingly, to gain a deeper understanding of the effect of alkyl substituents of the investigated SAs, we attempted to investigate a relationship between the Meyer parameter and the chromatographic characteristics. The effect of alkyl side-chain was studied with mobile phases of different compositions on the four cSCX columns. Data obtained in MeOH/MeCN and MeOH/THF (25/75 *v/v*) mobile phases, all containing 25 mM TEA and 50 mM FA, are depicted in Fig. S5. The corresponding results show a linear relationship for $k_1$ *vs.* $V^a$ with good correlation coefficients ($R^2 \geq 0.991$)**.** Therefore, it can be concluded that, for the present case, the retention clearly depends on the volume of the alkyl side chain. With increasing Meyer parameters (increasing volume of the substituents of analytes **1**–**3** and **4**–**6**) retention decreased correspondingly, while stereoselectivity increased on all cSCXs. Through a steric effect, a bulkier substituent, to a certain extent, can evidently inhibit the selective interactions formed between SA and SO leading to a reduced retention under the given mobile phase conditions. The application of mobile phases containing $NH_4OAc$ as additive instead of TEA and FA (see above) showed similar retention behavior: $k_1$ depended strongly on the bulkiness of the side chain; however, the separation factor remained practically constant (data not shown). According to the slight increase of the $pK_a$ values of analytes 1 to 3, the retention order based on only electrostatically driven interactions, should be 3<2<1. In the present case, in contrast, it is actually reversed, because it is outbalanced by the sterically driven size effect.

It is important to mention that the elution order was not influenced by the size of the substituent, *i.e.*, ion pair formation plays a decisive role in the chiral discrimination through multisite interactions in synergy with steric effects.

A comparison of separation performances of THßC and THIQ analogs revealed that THßC derivatives could efficiently be separated on cSCX CSPs. The THIQ analogs were less retained than THßC derivatives and were not separable on cSCX phases under the applied conditions (Table 1). The elution sequences observed on the studied cSCX phases follow the general rule determined by the configuration of the chiral moiety of the SO. That is, in all studied mobile phases on CSPs possessing (*S,S*)-configuration, the elution sequence was $S < R$, while on CSPs with (*R,R*)-configuration it was $R < S$ (Table 1). It can also be extracted from Table 1 that the two DCL(*S,S*) SO-based columns slightly differ in their retention and stereoselectivity characteristics under identical mobile phase conditions. On the one hand, this can be accounted for by their different binding chemistries. On the other hand, the other factor is the slightly higher loading of selector DCL(*S,S*) compared to that of DCL(*S,S*)-MP.

### 3.4. Temperature dependence and thermodynamic study

The investigation of the temperature dependence of chromatographic characteristics is a possible way to map the retention mechanism, since thermodynamic parameters can provide valuable information about the processes that play a key role in the retention mechanism.

**Table 2**
Thermodynamic parameters $\Delta(\Delta H^o)$, $\Delta(\Delta S^o)$, $T \times \Delta(\Delta S^o)$, $\Delta(\Delta G^o)$, correlation coefficients ($R^2$), and $Q$ values of ß-carboline analogs on DCL-(*S,S*) columns.

| Analyte | Mobile phase | $-\Delta(\Delta H^o)$ (kJ/mol) | $-\Delta(\Delta S^o)$ (J/(mol*K) | Correlation coefficients ($R^2$) | $-T \times \Delta(\Delta S^o)_{298K}$ (kJ/mol) | $-\Delta(\Delta G^o)_{298K}$ (kJ/mol) | $Q$ |
|---|---|---|---|---|---|---|---|
| **1** | | 1.41 | 3.75 | 0.995 | 1.12 | 0.29 | 1.3 |
| **2** | **a** | 1.39 | 3.60 | 0.996 | 1.07 | 0.32 | 1.3 |
| **3** | | 1.42 | 3.63 | 0.997 | 1.08 | 0.34 | 1.3 |
| **1** | | 1.48 | 3.96 | 0.994 | 1.18 | 0.30 | 1.3 |
| **2** | **b** | 1.55 | 3.98 | 0.996 | 1.19 | 0.36 | 1.3 |
| **3** | | 1.68 | 4.27 | 0.997 | 1.27 | 0.40 | 1.3 |
| **1** | | 1.56 | 4.16 | 0.994 | 1.24 | 0.32 | 1.3 |
| **2** | **c** | 1.82 | 4.66 | 0.996 | 1.39 | 0.43 | 1.3 |
| **3** | | 1.97 | 4.90 | 0.994 | 1.46 | 0.50 | 1.3 |
| **1** | | 1.66 | 4.45 | 0.994 | 1.33 | 0.33 | 1.3 |
| **2** | **d** | 1.53 | 3.99 | 0.995 | 1.19 | 0.34 | 1.3 |
| **3** | | 1.49 | 3.84 | 0.997 | 1.14 | 0.35 | 1.3 |
| **1** | | 1.83 | 4.91 | 0.995 | 1.46 | 0.37 | 1.3 |
| **2** | **e** | 1.70 | 4.39 | 0.995 | 1.31 | 0.39 | 1.3 |
| **3** | | 1.74 | 4.47 | 0.996 | 1.33 | 0.41 | 1.3 |
| **1** | | 1.94 | 5.06 | 0.976 | 1.51 | 0.43 | 1.3 |
| **2** | **f** | 1.94 | 4.89 | 0.960 | 1.46 | 0.49 | 1.3 |
| **3** | | 2.10 | 5.24 | 0.957 | 1.56 | 0.54 | 1.3 |

Chromatographic conditions: column, DCL-(*S,S*); mobile phase, **a**, MeOH/THF (75/25 *v/v*) containing 50 mM FA and 25 mM TEA, **b**, MeOH/THF (50/50 *v/v*) containing 50 mM FA and 25 mM TEA, **c**, MeOH/THF (25/75 *v/v*) containing 50 mM FA and 25 mM TEA, **d**, MeOH/MeCN (75/25 *v/v*) containing 50 mM FA and 25 mM TEA, **e**, MeOH/MeCN (50/50 *v/v*) containing 50 mM FA and 25 mM TEA, **f**, MeOH/MeCN (25/75 *v/v*) containing 50 mM FA and 25 mM TEA; flow rate, 0.6 ml $min^{-1}$; detection, 218–280 nm; $Q = \Delta(\Delta H°)/298 \times \Delta(\Delta S°)$.

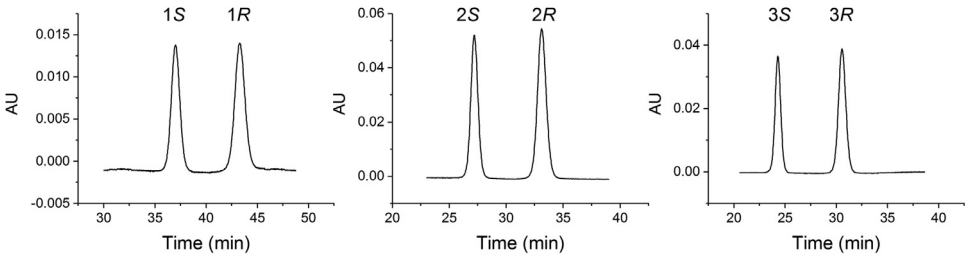


**Fig. 5.** Selected chromatograms of tetrahydro-ß-carboline analogs.
Chromatographic conditions: columns, DCL-(*S,S*); mobile phase, MeOH/THF (25/75 *v/v*) all containing 25 mM TEA and 50 mM FA; flow rate, 0.6 ml $min^{-1}$; detection, 220–250 nm, temperature, 10°C.

Applying the van't Hoff representation, as suggested by Chester and Coym [43] the difference in the change in standard enthalpy $\Delta(\Delta H°)$ and entropy $\Delta(\Delta S°)$ for the two enantiomers can be calculated on the basis of Eq. (2)

$$\ln \alpha = -\frac{\Delta(\Delta H°)}{RT} + \frac{\Delta(\Delta S°)}{R} \quad (2)$$

where $T$ is the absolute temperature (K), and $R$ is the universal gas constant. Since the contribution of nonselective interactions cannot be extracted only by subtracting the appropriate thermodynamic parameters (or in a "chromatographic way"), it is important to emphasize that the thermodynamic data presented here cover apparent values from a combination of enantioselective and nonselective interactions. Keeping the limitations of this approach in mind, the evaluation based on the chromatographic characteristics obtained under the same conditions (given stationary phase, mobile phase with constant composition, constant flow rate [44]) in the case of compounds showing significant structural analogy still can provide useful information for a better understanding of the molecular recognition mechanism. The pitfalls of the thermodynamic calculations were excellently summarized by Asnin and Stepanova [45].

To explore the effects of temperature on the chromatographic parameters, a variable temperature study was carried out in the temperature range 10–50°C (at 10°C increments) on the best-performing DCL-(*S,S*) CSP employing the THßC analogs. To gather information about the effects of the mobile phase composition on the thermodynamic parameters, six different eluent compositions were tested, in duplicates at each studied temperature. Experimental data are listed in Table S1, while the calculated thermodynamic parameters are summarized in Table 2. Under all applied chromatographic conditions, retentions decreased with increasing temperature for all studied THßC analogs. The transfer of the SA from the mobile phase to the stationary phase is an exothermic process and $k_1$ decreases with increasing temperature. Changes observed in $\alpha$ and $R_S$ were also consistent: both $\alpha$ and $R_S$ decreased with increasing temperature. The calculated thermodynamic parameters were all negative indicating that the adsorption is preferential from view of the enthalpy term, while it is unfavorable from view of the entropy term. Data varied in a relatively narrow range: $\Delta(\Delta H°)$ ranged from –1.41 to –2.10 kJ $mol^{-1}$, $\Delta(\Delta S°)$ varied between–3.60 to –5.24 J $mol^{-1}K^{-1}$, while $\Delta(\Delta G°)$ ranged from –0.29 to –0.54 kJ $mol^{-1}$. The relative contribution of the enthalpic and entropic terms to the free energy of adsorption is reflected in the enthalpy/entropy ratios $Q=\Delta(\Delta H°)/298 \times \Delta(\Delta S°)$ (Table 2). In all studied cases, $Q$ was higher than one, *i.e.*, the separations were enthalpically driven independently from the applied mobile phase systems. Systematic studies for exploring how the chromatographic conditions affect the thermodynamic parameters are rare to find. Very recently Asnin and co-workers investigated the enantioselective separation of some dipeptides applying macrocyclic antibiotic-based (Chirobiotic R and T) CSPs reporting correlation between $\Delta H°$, $\Delta S°$ or $\Delta G°$ and the mobile phase pH or MeOH content [46,47]. As can be seen from data given in Table 2, all calculated thermodynamic parameters changed monotonically with the eluent composition in both the MeOH/MeCN and the MeOH/THF mobile phase systems. The calculated thermodynamic parameters became increasingly negative for all three analogs with decreasing

MeOH content in both systems, suggesting that the difference between the sum of the enantioselective and non-selective processes, related to the adsorption and desorption steps of the enantiomers, became higher in eluents of lower MeOH content. A further exploration of the effect of eluent composition on the binding affinity of ionic CSPs requires additional studies with zwitterionic CSPs.

Selected chromatograms for the illustration of the best enantioseparations are depicted in Fig. 5.

## 4. Conclusions

In this comprehensive investigation the enantioseparation of tetrahydro-ß-carboline and 1,2,3,4-tetrahydroisoquinoline analogs were carried out utilizing chiral strong cation exchangers. Focusing on the retention behavior, the applicability of stoichiometric displacement model was confirmed using mixtures of methanol with acetonitrile or tetrahydrofuran as mobile phase systems with organic salt additives. The nature (size) of counter-ion was found to be an important factor markedly affecting retention, while it had much less effect on the observed enantioselectivities. A hypothesis based on the size of the solvated counter-ion is applied consistently for the description of the observed retention characteristics; however, it needs further approval.

Since elution orders in every case were found to be opposite on the enantiomeric stationary phases and they were not affected by either the temperature or the eluent composition, the developed methods can easily be employed for the effective enantioresolution of the studied tetrahydro-ß-carboline analogs. The enantiomers of 1,2,3,4-tetrahydroisoquinoline analogs could not be separated under the applied conditions. The temperature-dependence study revealed enthalpically driven recognitions in all cases, where the calculated thermodynamic parameters were slightly dependent on the eluent composition.

This study also demonstrates the consistent use of appropriate chiral cation exchangers working as CSPs for liquid chromatography of basic analytes with mobile phase conditions compatible with LC-MS applications.

## Declaration of Competing Interest

Authors declare no conflict of interest.

## CRediT authorship contribution statement

**Attila Bajtai:** Investigation, Writing - original draft, Visualization. **Dániel Tanács:** Investigation, Writing - original draft, Visualization. **Róbert Berkecz:** Writing - review & editing. **Enikő Forró:** Resources, Writing - original draft. **Ferenc Fülöp:** Writing - review & editing. **Wolfgang Lindner:** Conceptualization, Writing - review & editing. **Antal Péter:** Conceptualization, Writing - original draft. **István Ilisz:** Conceptualization, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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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.2021.462121.

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