Journal of Chromatography A 1670 (2022) 462974

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Enantioselective high-performance liquid chromatographic separation of fluorinated ß- phenylalanine derivatives utilizing *Cinchona* alkaloid-based ion-exchanger chiral stationary phases Enantioselective separation of fluorinated ß-phenylalanine derivatives

Gábor Németi[a], Róbert Berkecz[a], Sayeh Shahmohammadi[b], Enikő Forró[b], Wolfgang Lindner[c], Antal Péter[a], István Ilisz[a,*]

[a] Institute of Pharmaceutical Analysis, Interdisciplinary Excellence Centre, University of Szeged, H-6720 Szeged, Somogyi u. 4, Hungary
[b] Institute of Pharmaceutical Chemistry, University of Szeged, Eötvös u. 6, H-6720 Szeged, Hungary
[c] Department of Analytical Chemistry, University of Vienna, Währinger Strasse 38, 1090 Vienna, Austria

ARTICLE INFO

Article history:
Received 10 February 2022
Revised 11 March 2022
Accepted 13 March 2022
Available online 15 March 2022

Keywords:
Cinchona alkaloid-based chiral stationary phases
Fluorinated ß-phenylalanine derivatives
Liquid chromatography
Thermodynamic characterization

ABSTRACT

The enantioselective separation of newly synthesized fluorine-substituted β-phenylalanines has been performed utilizing *Cinchona* alkaloid-based ion-exchanger chiral stationary phases. Experiments were designed to study the effect of eluent composition, counterion content, and temperature on the chromatographic properties in a systematic manner. Mobile phase systems containing methanol or mixtures of methanol and acetonitrile together with acid and base additives ensured highly efficient enantioseparations. Zwitterionic phases [Chiralpak ZWIX (+) and ZWIX(−)] were found to provide superior performance compared to that by the anion-exchangers (Chiralpak QN-AX and QD-AX). A detailed thermodynamic characterization was also performed by employing van't Hoff analysis. Using typical liquid chromatographic experimental conditions, no marked effect of the flow rate could be observed on the calculated thermodynamic parameters. In contrast, a clear tendency has been revealed about the effect of the eluent composition on the thermodynamics for the zwitterionic phases.

1. Introduction

Enantiomerically pure β-aryl-substituted β-amino acids have attracted much attention due to their pharmaceutical importance and their utility in drug research. For example, (2*R*,3*S*)-3-amino-3-phenyl-2-hydroxypropionic acid is a key intermediate for the preparation of the taxol side-chain [1] used in the semi-synthesis of Taxol®, approved by the FDA for treatment of ovarian cancer and metastatic breast cancer [2]. (*S*)-3-Amino-3-(*o*-tolyl)propanoic acid [3] was identified as the preferred enantiomeric form for the construction of Cathepsin (CatHA) inhibitors with potential beneficial effects in cardiovascular diseases [4]. The development of fluorinated amino acids has gained increasing attention resulting from their recognition as an important class of compounds in the design and synthesis of potential pharmaceutical drugs [5,6]. As an example, Januvia[TE] (sitagliptin phosphate), a drug approved for the treatment of type 2 diabetes containing (*R*)-3-amino-4-(2,4,5-trifluorophenyl)butanoic acid as a subunit, and acts via inhibition of dipeptidyl peptidase IV [7]. To control the steps of preparation and to determine the enantiomeric impurities suitable analytical techniques and methods are needed.

Enantioselective liquid chromatography separations are the most frequently applied techniques either at analytical or preparative scale for the discrimination of chiral compounds nowadays. Due to their relevance, they are frequently discussed in review articles [8–12]. To achieve higher efficiencies using superficially or fully porous particles is a challenging area in "chiral chromatography" [13–15], however, most of the enantioselective separations are being carried out on traditional HPLC systems. Wide range of chiral compounds have been studied so far, but there is only sparse information on the liquid-phase enantioseparation of fluorinated amino acids in the literature. Utilizing ligand-exchange micellar capillary chromatography, *o*-, *m*-, and *p*-fluoro-D,L-phenylalanines were separated [16], while a Chiralcel OD-H column was applied for the enantiomeric separation of nonproteogenic polyfluoro amino acids and peptides [17]. Our group has reported a study using

* Corresponding author: Institute of Pharmaceutical Analysis, University of Szeged, Somogyi B. u. 4, H-6720 Szeged, Hungary
E-mail address: ilisz.istvan@szte.hu (I. Ilisz).

https://doi.org/10.1016/j.chroma.2022.462974

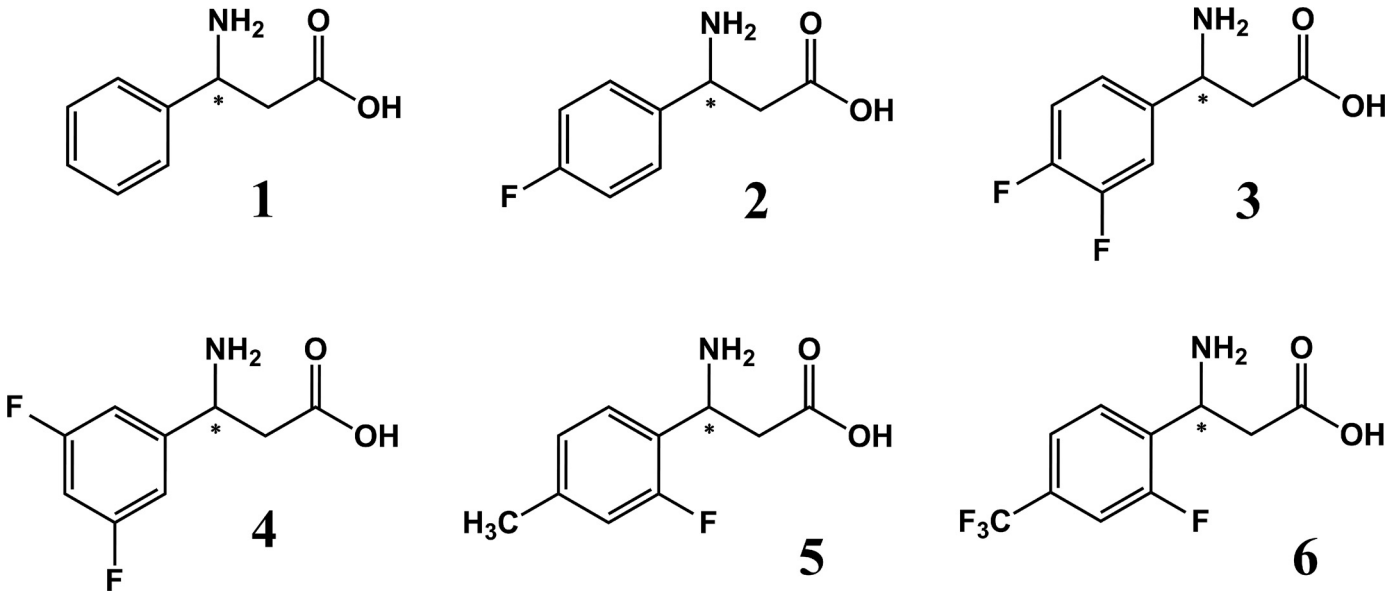

Fig. 1. Structures of analytes.

five fluorinated cyclic β^3-amino acid derivatives and their non-fluorinated counterparts on polysaccharide-based chiral stationary phases (CSPs) [18].

Of the liquid-phase "chiral chromatographic" techniques, *Cinchona* alkaloid-based ion-exchangers have found their niche for the enantioseparations of diverse chiral analytes, *e.g.*, anionic, cationic, or ampholytic compounds [19–22]. Since these CSPs have pronounced relevance in amino acid analysis [23–26], we have decided to study their applicability for the enantioselective separation of newly synthesized fluorinated ß-phenylalanines. The effects of the experimental variables have been investigated in a systematic study to acquire information on enantiorecognition. The nature and concentration of the mobile phase components and counterions as additives were varied to characterize the utilized CSPs. Based on the structural features of the applied analytes (selectands, SAs) and selectors (SOs), structure–retention (selectivity) relationships were evaluated. Analysis of the temperature dependence allowed a detailed thermodynamic characterization.

2. Experimental

2.1. Chemicals and materials

Five enantiomeric pairs of fluorine-containing ß-amino acids together with the enantiomers of non-fluorinated ß-phenylalanine (Fig. 1) were studied. Racemic amino acid **1** was prepared through ring cleavage of racemic 4-phenylazetidin-2-one with 18% HCl [27], while **2**-**6** were synthesized via a modified Rodionov synthesis, through condensation of the corresponding aldehydes with malonic acid in the presence of ammonium acetate in ethanol [28]. Phenyl-substituted β-amino acid (*S*)-**1** (*ee* $\geq$ 99%) was prepared through CAL-B (*Candida antarctica* lipase B)-catalyzed ring cleavage of 4-phenylazetidin-2-one [27]. Enantiomeric fluorophenyl-substituted β-amino acids (*S*)-**2**–(*S*)-**6** (*ee* $\geq$ 99%) were synthesized through lipase PSIM (*Burkholderia cepacia*)-catalyzed hydrolysis of racemic β-amino carboxylic ester hydrochloride salts in the presence of triethylamine (TEA) and water [28].

Methanol (MeOH) of LC-MS grade and acetonitrile (MeCN) of HPLC gradient grade were from Molar Chemicals Ltd. (Halásztelek, Hungary). Ethylamine (EA) of HPLC grade was from Sigma-Aldrich (St. Louis, MO, USA). H_2O of LC-MS grade, formic acid (FA), diethylamine (DEA), and TEA of HPLC grade were obtained from VWR International (Leuven, Belgium).

2.2. Instrumentation and chromatography

Chromatographic measurements were carried out on 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 Chromatography, Milford, MA, USA). The chromatographic system was equipped with Rheodyne Model 7125 injector (Cotati, CA, USA) with a 20-µl loop. The columns were thermostated in a Lauda Alpha RA-8 thermostat (Lauda Dr. R. Wobser GmbH & Co. KG., Lauda-Königshofen, Germany). The precision of temperature adjustment was ±0.1°C.

Chiralpak ZWIX(+) and ZWIX(−) columns (150 × 3.0 mm I.D., 3 μm particle size for both columns) and QN-AX, QD-AX columns (150 × 4.6 mm I.D., 5 μm particle size for both columns) were from Chiral Technologies Europe (Illkirch, France). Their structures are depicted in Figure S1.

Stock solutions of amino acids (1 mg ml^{-1}) were prepared by dissolution in MeOH and further dilution with the mobile phase. The dead times (t_0) of the columns were determined by injecting acetone mixed with MeOH at each investigated temperature and eluent composition. The flow rate was set at 0.6 ml min^{-1} and the column temperature at 25°C, if not otherwise stated.

2.3. Evaluation of thermodynamic data and determination of the confidence intervals

To decrease sensitivity to outliers the ln α (and ln k) *vs.* T^{-1} curves were evaluated based on weighted linear regression (weighted least squares, WLR or WLS). The weighing variable of the seeming outlier data points was reduced to obtain more accurate mean values and confidence intervals. The WLR and confidence intervals (at a confidence level of 95%) were calculated with Microsoft Excel 2016 using the Real Statistics Resource Pack Add-In. Since the free energies were calculated from enthalpy and entropy parameters confidence intervals of them were calculated by taking the propagation of error into account.

3. Results and discussion

3.1. Column selection and effects of bulk solvent composition

The *Cinchona* alkaloid-based CSPs can be applied in different chromatographic modes. However, the best performances are usually achieved in polar-ionic mode (PIM), when a mixture of MeOH (possessing polar and protic properties) and MeCN (as a polar but aprotic solvent) is applied. To achieve better peak shapes and promote ionic interactions, acid and base additives are needed in the mobile phase. The excess of acid is generally preferred. In this way, the quinuclidine group of the SO is mainly protonated promoting the enantioselective ion-pairing process.

Initially, the anion-exchanger-based QN-AX and QD-AX columns were studied applying MeOH/MeCN mobile phases of different ratios (100/0, 50/50, 25/75 v/v) with acid (FA) and base (DEA) additives. As the results summarized in Table S1 show, the *Cinchona* alkaloid-based anion-exchangers practically did not show enantiorecognition capability in the case of the studied compounds. Either enhancing the MeCN or reducing the salt (formed from the added acid and base) content of the mobile phase, higher retentions were obtained for all studied *ß*-phenylalanines without achieving any enantioresolution.

Due to the presence of the amino group in the SAs, stronger interactions and higher enantioselectivities were expected when employing zwitterionic CSPs. Therefore the ZWIX (+) and ZWIX(−) columns were studied with varying mobile phase compositions. At first, reversed-phase (RP) conditions were tested applying MeOH/H_2O mobile phase systems with different compositions using constant concentrations of acid (FA, 50 mM) and base (DEA, 25 mM) additives. Unfortunately, under all studied RP conditions poor peak shapes and no or only small enantioselectivities were obtained (data not shown).

As expected, much better performance was achieved using PI mode. In these experiments, the MeOH/MeCN ratio was varied from 100/0 to 10/90 (*v/v*), while the base (DEA) and acid (FA) modifiers were added at constant concentrations (25 and 50 mM, respectively). The chromatographic parameters (k_1, α, R_s) showing the most important results of these experiments are depicted in Fig. 2. As a result of the increase in MeCN content in the mobile phase, increased retention factors were obtained for all analytes, similar to the case of anion-exchangers discussed above. In most cases selectivity increased up to a MeOH/MeCN composition of 25/75 (*v/v*), then it decreased slightly or leveled off. Resolution values developed similarly in terms of the trend. Namely, they changed according to a maximum curve on both columns, usually reaching a maximum at a composition of MeOH/MeCN 25/75 (*v/v*) on the ZWIX(−), and 50/50 (*v/v*) on the ZWIX(+) column.

These results indicate both the similarities and differences between the separation mechanisms of the applied zwitterionic and single ion-exchanger CSPs. The increased retentions observed with higher MeCN ratios can be explained by the increased electrostatic interactions due to the decreased solvation shell of the ionized SAs and SO. In contrast, MeOH a better solvent of SAs, can decrease the accessibility of SAs to the *Cinchona* alkaloid-based CSPs resulting in lower retentions. Besides solvation-related issues, it is worth mentioning that further solvent effects might be expected since MeOH may suppress hydrogen bonding, while MeCN may interfere with aromatic $\pi-\pi$ interactions. In the case of zwitterionic CSPs, the increase in selectivity (Fig. 2) with decreasing MeOH content suggests that hydrogen bonding interactions play a notable role in enantioselective interactions. Based on these results, most further experiments were carried out using an eluent composition of MeOH/MeCN 100/0 or 50/50 (*v/v*) containing acid and base additives in a ratio of two. Earlier results have shown that the acid-to-base ratio of 2:1 provides generally optimal ionization conditions and retention characteristics for the zwitterionic CSPs [29,30].

3.2. Effects of the nature of base additive and counterion concentration

In addition to the eluent composition discussed above, both the quality and the amount of acid and base added to the mobile phase may significantly influence chromatographic properties, since the acid and the base affect both the solvation conditions and the ionization of SAs and SO. In the case of ion-exchangers dissolving acid and base in the mobile phase, counterions are formed *in situ*, and they act as competitors for the SA and SO ionic functional groups. In the case of zwitterionic SOs, both the cations and the anions can be considered as counterions. In this way, counterions interfere with ionic interactions between SO and SA, and retention can be controlled [31]. Therefore, the effects of the quality and quantity of the counterions are worth exploring.

Our previous experience has shown that the quality of the acid has no marked effect on the chromatographic parameters when using the same base [23,32]. As a consequence, for these experiments, FA was applied as the acid component (50 mM), and organic amines EA, DEA, and TEA (25 mM) were applied as bases. Under these conditions, the acid excess used in the mobile phase ensured that the amines were present in their protonated forms. The results obtained with the ZWIX(−) column with two different eluent systems [100/0 and 50/50 (*v/v*) MeOH/MeCN] are shown in Fig. 3 and Table S2. It can be established that k_1 values differ very slightly in pure MeOH, but to a greater extent when MeOH/MeCN 50/50 (*v/v*) was used. It is important to point out, that the trend of elution strength in all cases was TEA < DEA< EA. Since the basicity of these amines is rather similar (EA, DEA, TEA has pK_a values of 10.70, 10.84, 10.75, respectively [33]), it can be stated that the number of ethyl substituents of the amine can significantly affect the retentive properties through the size and shape of the alkylamine ions. Note, however, that this property depends strongly on the eluent composition, too. The changes in α and *Rs*, in turn, were much less marked. Again, they were slightly higher in MeOH/MeCN 50/50 (*v/v*) than in pure MeOH. In MeOH/MeCN 50/50 (*v/v*) both enantioselectivity and resolution decreased slightly with the more alkylated base.

For the quantitative description of the chromatographic ion-exchange process, the simple stoichiometric displacement model is applied in most cases [34,35]. The model assumes a linear relationship between the logarithm of the retention factor and the logarithm of counterion concentration, where the plot of log *k vs.* log *c* provides the slope. This is related to the effective charge (ratio of the charge number of the SA and the counterion), whereas the intercept is related to the ion-exchange equilibrium constant. To gain a deeper insight into the details of the retention mechanism, the effects of counterion concentration on the chromatographic properties were examined with both zwitterionic CSPs, applying 100% MeOH with FA and DEA. In these experiments, the acid-to-base molar ratio was kept constant of two, with varying concentrations of both the acid (12.5–200 mM) and the base (6.25–100 mM). As Fig. 4 shows, linear fittings could be achieved with $R^2>0.97$ in all cases, supporting the validity of the model in the studied systems. The slopes of the log *k vs.* log *c* plots varied in a narrow range, between 0.21 and 0.25 for the ZWIX(−), and between 0.31 and 0.34 for the ZWIX(+) column. These are in accordance with earlier results obtained with zwitterionic CSPs [24,36]. As data summarized in Table S3 show, reduced retentions are obtained with increasing counterion concentration. At the same time, however, enantioselectivity is nearly unchanged, highlighting an advantageous property of the studied zwitterionic CSPs, *i.e.* the retention can be tuned by

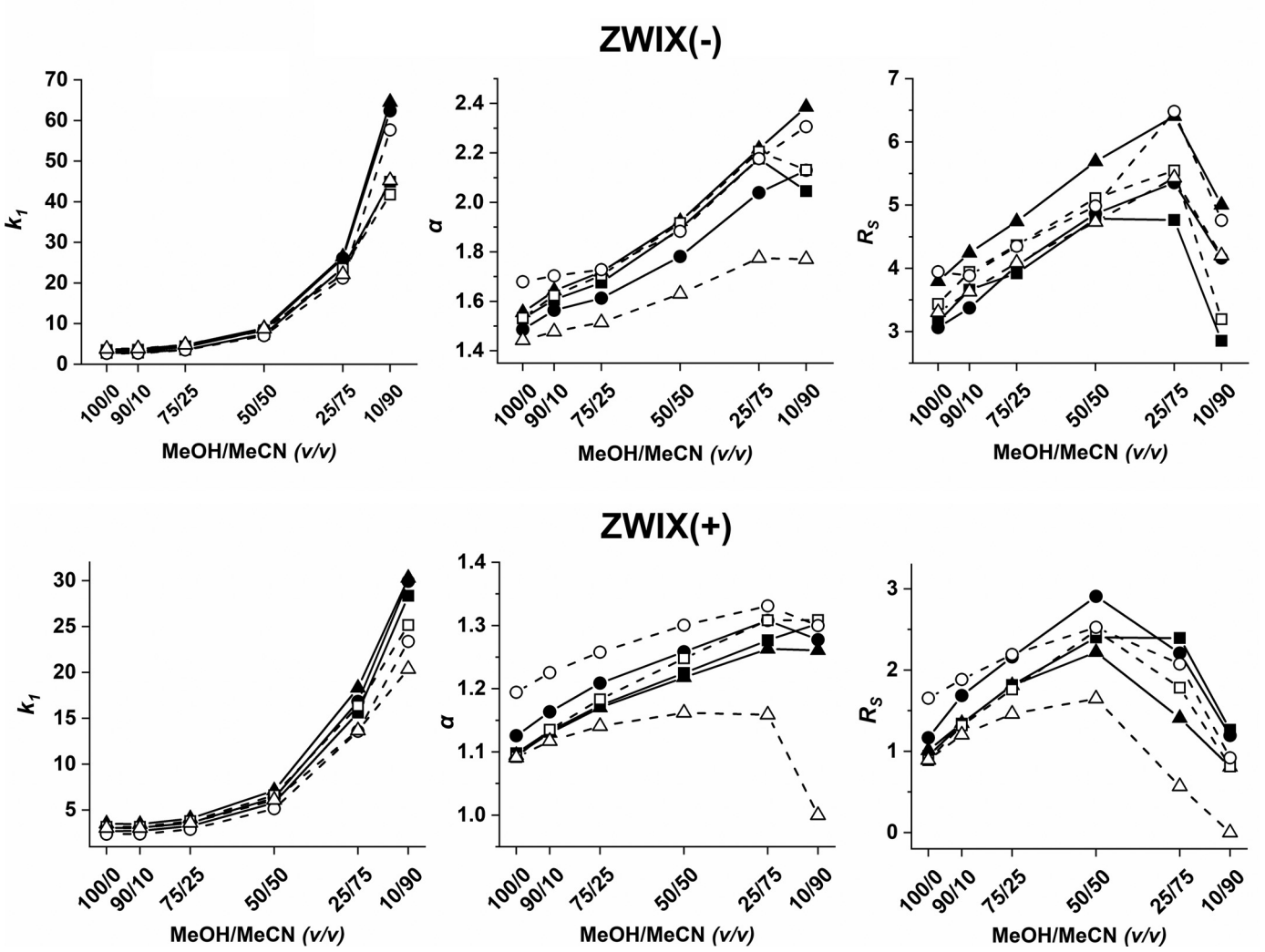

Fig. 2. Effects of the mobile phase composition on the chromatographic parameters in the separation of fluorinated *ß*-phenylalanine derivatives on zwitterionic CSPs. Chromatographic conditions: columns, ZWIX(−) and ZWIX(+); mobile phase, MeOH/MeCN (100/0 – 10/90 *v/v*) all containing 25 mM DEA and 50 mM FA; flow rate, 0.6 ml min^{-1}; detection, 262 nm; temperature, 25°C; symbols, analyte **1**, ■, **2**, ●, **3**, ▲, **4**, □, **5**, ○ and **6**, △.

varying the concentration of the counterions without having a significant loss of enantioselectivity.

3.3. Structure-retention (enantioselectivity) relationships and elution order

Fluoro substitution can lead to modified chemical and biological properties, where the substitution may significantly affect the interactions formed between the SA and the SO. Generally, it can be stated that all SAs, both the fluorinated and the non-fluorinated studied here, behaved in a rather uniform way, *i.e.*, no vital differences in the chromatographic properties could be observed (see, *e.g.*, Fig. 2). This observation suggests that the main interactions responsible for retention and enantiorecognition were not radically modified by the structural changes related to the fluoro substitution of the SAs. However, some important distinctions still can be made.

Comparing the chromatographic properties of analyte **2** *vs.* **1**, it can be noted, that retentions were lower for the non-fluorinated **1**, while no significant differences in enantioselectivities could be detected. That is, the fluorination on the aromatic ring in *para* position resulted in considerable changes only in non-selective interactions, leading to enhanced retention. Further increase in retention can be observed with an additional fluorine substitution of the aromatic ring (**3** *vs.* **2**), without significantly perturbing the enantiorecognition ability of the zwitterionic CSPs. Examining the chromatographic properties of analyte **3** *vs.* **4**, shorter retentions can be seen without noticeable changes in enantioselectivities. It means that the relative position of the fluorine atoms in the case of the double fluorine substituted SAs had a noticeable effect only on the retentive properties of the zwitterionic CSPs.

Under all applied conditions (except mobile phases containing 90 *v%* of MeCN) analyte **5** eluted with the lowest retention. Interestingly, these lowest retentions were accompanied by the highest enantioselectivities in most of the cases, suggesting that methyl substitution together with the fluorination of the aromatic ring results in such a favorable structure, where the non-selective interactions formed between the SA and SO can markedly be reduced. In the case of analyte **6**, exchanging all H atoms of the methyl group for F atoms resulted in higher k, but lower α values compared to those of **5**. No matter how different is the structure of analytes **3** and **6**, they showed a quite similar retention behaviour. In most cases, one of these SAs possessed the longest retention times independently from the applied conditions. Some marked differences between the enantioselectivities were also observed. Namely, the lowest α values were obtained in the case of **6**, suggesting that

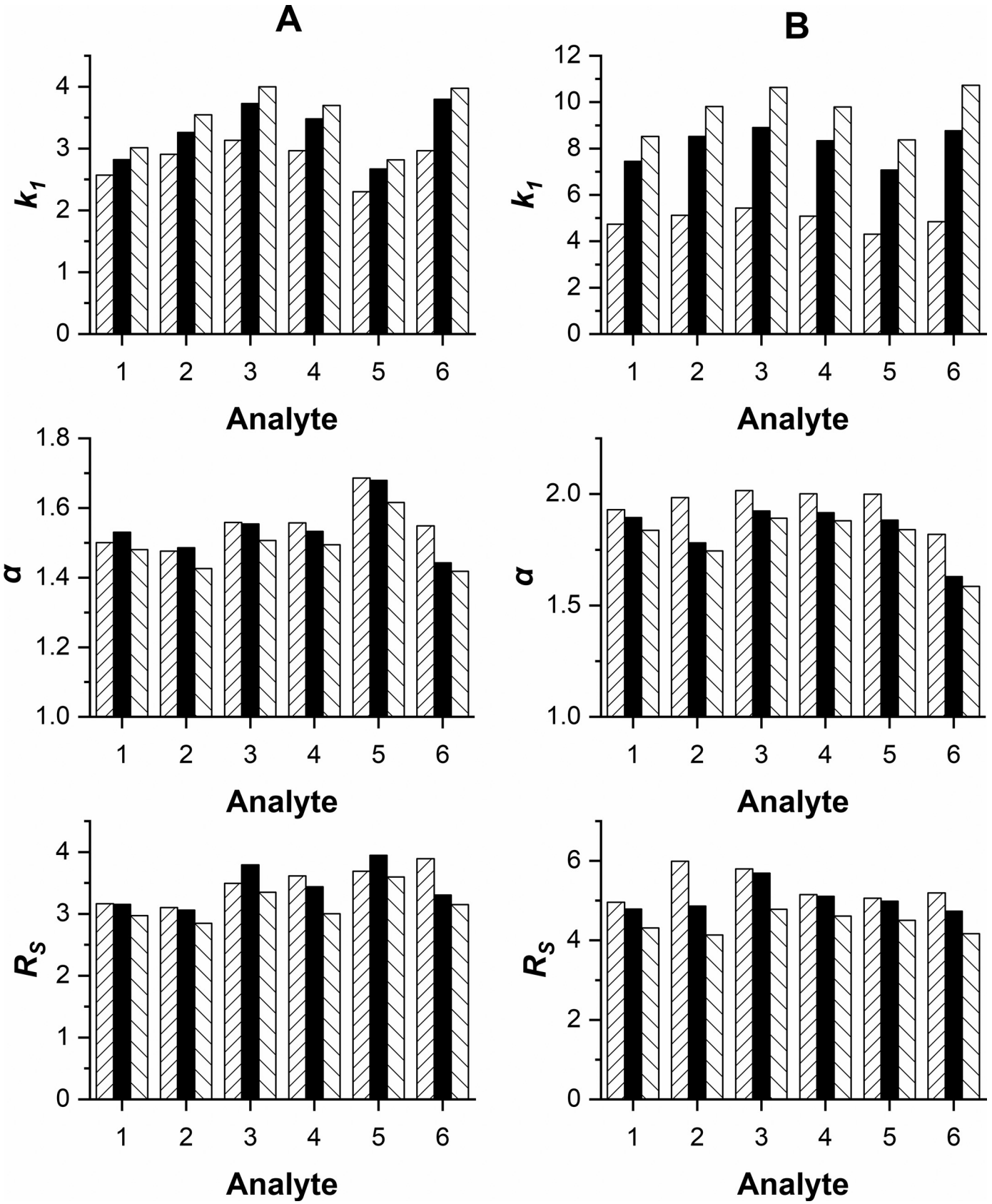

Fig. 3. Effects of base additives on the chromatographic parameters in the separation of fluorinated ß-phenylalanine derivatives on zwitterionic CSPs. Chromatographic conditions: column, Chiralpak ZWIX(−); mobile phase, **A**) MeOH and **B**) MeOH/MeCN (50/50,*v/v*), both containing 25 mM base additive and 50 mM FA; flow rate, 0.6 ml min^{-1}; detection, 262 nm; temperature, 25°C; symbols EA, ▨, DEA, ■, and TEA, ▧.

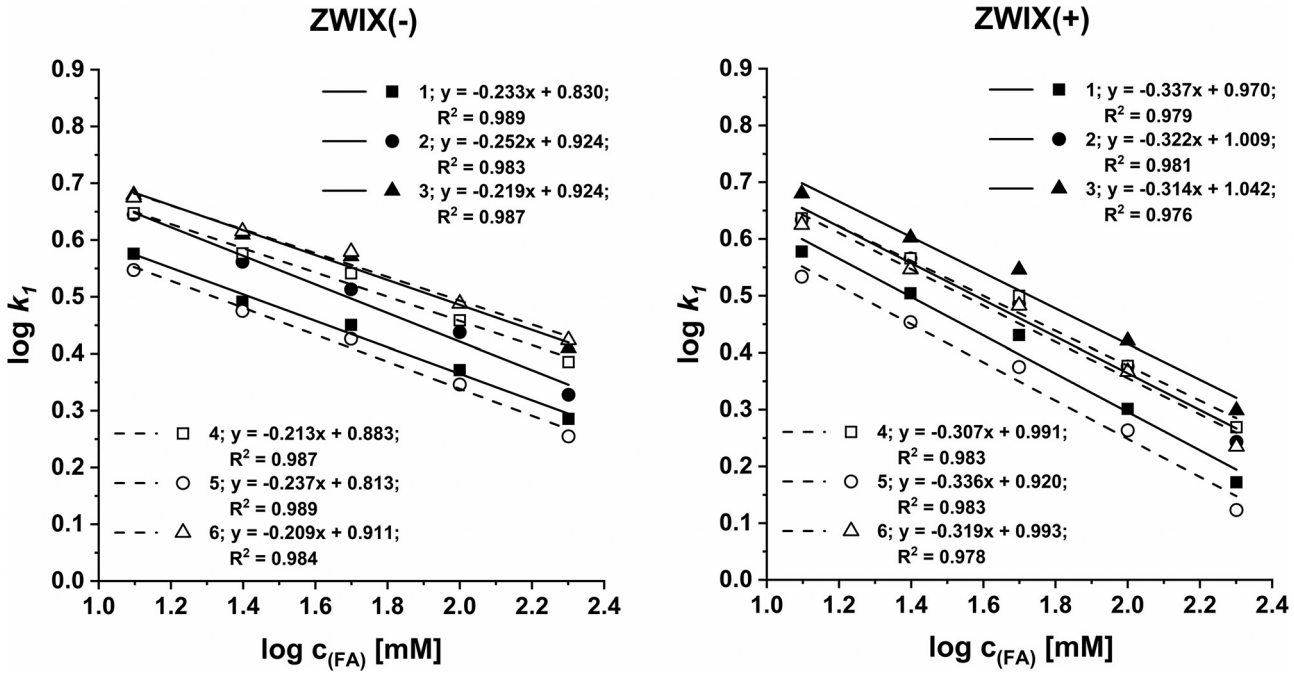

Fig. 4. Influence of the counterion concentration on the retention factor of the first-eluting enantiomer (k_1). Chromatographic conditions: columns, ZWIX(+) and ZWIX(−); mobile phase, MeOH containing DEA/FA (mM/mM), 6.25/12.5, 12.5/25, 25/50, 50/100 and 100/200 (in all cases the acid-to-base ratio being kept at 2:1); flow rate, 0.6 ml min^{-1}; detection, 262 nm; temperature, 25°C; symbols, analyte **1**, ■, **2**, ●, **3**, ▲, **4**, □, **5**, ○ and **6**, △.

the structural changes can affect the enantiorecognition markedly without strongly affecting retention.

As a summary, concerning the structural variations generated by the fluorination of *ß*-phenylalanine derivatives, it can be concluded that relatively moderate changes were observed. The fluoro substitution may have effects on both the retention and the enantiorecognition depending on the position and degree of substitution.

ZWIX(+) and ZWIX(−) are based respectively on quinine (QN) and quinidine (QD) alkaloids modified with (*R,R*)- or (*S,S*)-*trans*-2-aminocyclohexanesulfonic acid group (Figure S1). These SOs are often referred to as pseudoenantiomers because they behave as quasi-enantiomers; in fact, however, they are diastereomers. Elution orders were determined in all cases and they were found to be opposite on the studied zwitterionic CSPs without any exception (Table S4). That is, the elution order can easily be reversed by switching from ZWIX(−) to ZWIX(+) or *vice versa*. Selected chromatograms for the enantioseparation of the studied SAs are depicted in Fig. 5.

3.4. Thermodynamic characterization

In the field of liquid chromatographic enantioselective separations based on the application of different types of CSPs, despite the huge amount of experimental data generated in the last two decades, there still exists a few possibilities for the quantitative or at least semi-quantitative description of the processes affording chiral recognition. Whereas there are computer-based calculations utilizing different models in this area, their applicability is rather limited [37–39].

For the thermodynamic characterization of chiral recognition, the most frequently applied approach is the van't Hoff analysis. Its popularity originates from its simplicity, as it derives from Eq. (1),

$$ln\,\alpha = -\Delta(\Delta H^{\circ})/RT + \Delta(\Delta S^{\circ})/R \quad (1)$$

where R is the universal gas constant, T is the temperature in Kelvin, and α is the selectivity factor. The difference in the change in standard enthalpy $\Delta(\Delta H^{\circ})$ and entropy $\Delta(\Delta S^{\circ})$ for enantiomers can be obtained by plotting ln α against T^{-1}. In an outstanding review article Asnin and Stepanova enlightened all the pitfalls of this simplified approach [40]. Here, let us draw attention to only one important fact. In linear chromatography, it is impossible to separate selective and non-selective interactions; consequently, only apparent thermodynamic values can be calculated.

Besides theoretical limitations discussed comprehensively by Asnin and Stepanova [40], the correctness of van't Hoff plots was examined focusing on instrumental and experimental conditions by Felinger *et al.* [41]. In their study, the heterogeneous surface of a CSP was simulated by the serial connection of two reversed-phase achiral columns, and both interaction sites were evaluated individually by using van't Hoff analysis. Flow rate (pressure drop across the column) was found to affect the calculated thermodynamic parameters. However, it is important to see, that in this study achiral conditions were applied, and ΔH° and ΔS° values were calculated. Inspired by the work of Felinger *et al.*, we designed a systematic study to reveal further details of the applicability of the van't Hoff approach in enantioselective chromatography, where the effect of temperature was investigated between 5 and 50°C (5°C, 10°C, then with 10°C increments up to 50°C) on the ZWIX(−) and ZWIX(+) CSPs.

3.4.1. Effect of the flow rate on the thermodynamic parameters

Evaluation of the effects of flow rate on the thermodynamic parameters was performed setting 0.3, 0.6, or 0.9 ml min^{-1} flow rate and employing constant mobile phase composition [MeOH/MeCN 50/50 (*v/v*) with FA (50 mM) and DEA (25 mM)] with the ZWIX(−) column. Experimental data obtained for the six studied SAs using van't Hoff analysis are summarized in Table 1.

Most frequently, the least negative $\Delta(\Delta H^{\circ})$ and $\Delta(\Delta S^{\circ})$ values were obtained at the highest flow rate, but changes were rather small, and no monotonous change could be discovered in the thermodynamic parameters with increasing flow rate. It can clearly be

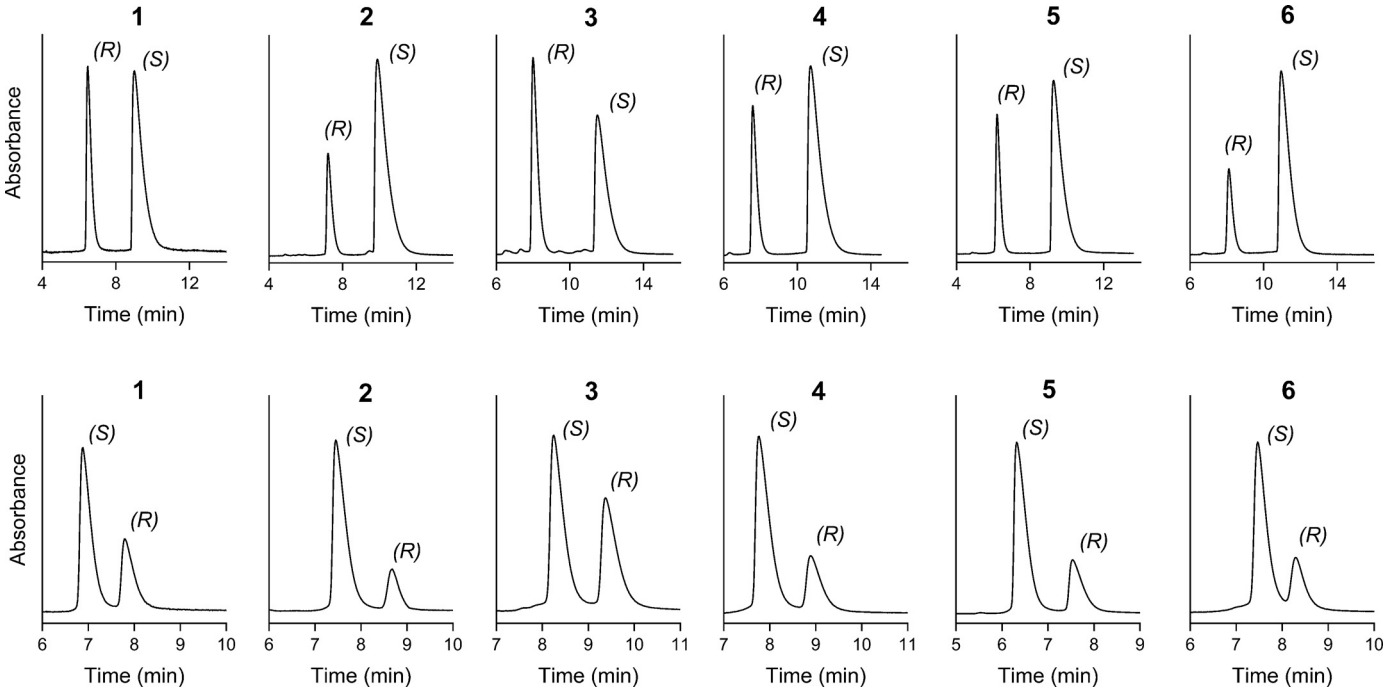

Fig. 5. Selected chromatograms of analytes **1-6.** Chromatographic conditions: columns, Chiralpak ZWIX(−) and ZWIX(+); mobile phase, for ZWIX(−)100 *v%* MeOH and for ZWIX(+) MeOH/MeCN (75/25, *v/v*) all containing 25 mM DEA and 50 mM FA; flow rate, 0.6 ml min^{-1}; detection, 262 nm; temperature, 25°C.

Table 1
Effects of flow rate on the thermodynamic parameters of fluorinated *ß*-phenylalanine derivatives on ZWIX(−) column.

Analyte	$-\Delta(\Delta H^0)$ (kJ mol^{-1})			$-\Delta(\Delta S^0)$ (J mol^{-1} K^{-1})			$-\Delta(\Delta G^0)_{298K}$ (kJ mol^{-1})		
	a	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**
1	5.43 ± 0.13	5.00 ± 0.13	4.84 ± 0.11	12.35 ± 0.44	10.87 ± 0.42	10.34 ± 0.37	1.75 ± 0.19	1.76 ± 0.18	1.76 ± 0.16
2	5.85 ± 0.14	5.14 ± 0.16	5.18 ± 0.10	13.72 ± 0.48	11.29 ± 0.54	11.46 ± 0.34	1.76 ± 0.20	1.77 ± 0.23	1.76 ± 0.14
3	5.46 ± 0.16	5.21 ± 0.16	5.09 ± 0.10	12.42 ± 0.54	11.60 ± 0.52	11.16 ± 0.35	1.75 ± 0.23	1.75 ± 0.22	1.76 ± 0.15
4	5.36 ± 0.17	5.60 ± 0.14	5.28 ± 0.16	11.84 ± 0.55	12.62 ± 0.47	11.54 ± 0.54	1.83 ± 0.23	1.83 ± 0.20	1.84 ± 0.23
5	4.37 ± 0.13	4.40 ± 0.16	3.92 ± 0.08	8.59 ± 0.44	8.68 ± 0.53	7.17 ± 0.25	1.81 ± 0.16	1.81 ± 0.22	1.79 ± 0.11
6	3.77 ± 0.15	3.56 ± 0.11	2.98 ± 0.13	7.85 ± 0.49	7.18 ± 0.37	5.27 ± 0.43	1.43 ± 0.21	1.42 ± 0.16	1.40 ± 0.18

Chromatographic conditions: column, ZWIX(−); mobile phase, MeOH/MeCN (50/50 *v/v*) containing 25 mM DEA and 50 mM FA, flow rate, **a)** 0.3 ml min^{-1}, **b)** 0.6 ml min^{-1}, **c)** 0.9 ml min^{-1}; detection, 262 nm. Confidence intervals were calculated as described in Section 2.3.

stated that the thermodynamic parameters of the studied SAs are affected in different ways by the flow rate, but these slight changes do not follow a trend. In a limited set of experiments, the effect of flow rate on the thermodynamic parameters was also studied with the ZWIX(+) column. In this case, no significant changes in $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ values were observed applying a flow rate of 0.6 or 0.9 ml min^{-1} (Table S5). Consequently, the only reliable conclusion that can be drawn is that using typical operational conditions (*i.e.*, flow rate is around the optimal value corresponding to the dimensions of the column) the $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ values are influenced more significantly by the structural peculiarities of the SAs than by the flow rate, even if the analytes are structurally closely related. With respect to the thermodynamic parameters calculated for the zwitterionic CSPs, it is interesting to note that each thermodynamic parameter varied in a fairly narrow range. Furthermore, markedly more negative $\Delta(\Delta H°)$, $\Delta(\Delta S°)$, and $\Delta(\Delta G°)$ values were obtained with the ZWIX(−) column, showing its superiority over the ZWIX(+) column in the enantioselective separation of fluorinated *ß*-phenylalanines.

As an extension of data evaluation, we also explored the effects of flow rate on the change in standard enthalpy ($\Delta H°$), entropy ($\Delta S°$), and free energy ($\Delta G°$) by the evaluation of the ln k *vs* T^{-1} plots (data not shown). In this case $\Delta S°$ contains the product of R *x ln* φ, where φ is the reversal of the phase ratio unless the latter is determined independently [42]. Most frequently, the least negative $\Delta H°$, $\Delta S°$, and $\Delta G°$ values were obtained at 0.9 ml min^{-1}, and about the same values were obtained at flow rates of 0.3 and 0.6 ml min^{-1} in the case of the ZWIX(−) column. In the case of the ZWIX(+) column, no significant difference could be found between the thermodynamic data obtained at 0.6 and 0.9 ml min^{-1}. This shows that if the flow rate has any effect on the thermodynamic parameters, both enantiomers are affected in the same way.

3.4.2. Effect of the mobile phase composition on the thermodynamic parameters

The adsorption in chromatography (defined as the transfer of a solute from the mobile to the stationary phase) is a complex process involving five steps: 1) desolvation of the solute in the liquid phase (desolv), 2) desorption of the solvent from the surface of the stationary phase (desorp), 3) formation of a transient complex on the surface (netads), 4) resolvation of the transient complex (resolv), and, finally, 5) dilution of the liquid phase by the solvent molecules desorbed from the surface (dil), as it is described in Eq. (2),

$$\Delta X^0 = \Delta X^0_{desolv} + \Delta X^0_{desorp} + \Delta X^0_{netads} + \Delta X^0_{resolv} + \Delta X^0_{dil} \qquad (2)$$

where ΔX^0 is the change in the thermodynamic quantity (H, S, or G) [40]. Desolvation, occurring in the liquid phase is a non-

Table 2
Effects of eluent composition on the thermodynamic parameters of fluorinated ß-phenylalanine derivatives on ZWIX(−) column.

Analyte	$-\Delta(\Delta H^0)$ (kJ mol^{-1})			$-\Delta(\Delta S^0)$ (J mol^{-1} K^{-1})			$-\Delta(\Delta G^0)_{298K}$ (kJ mol^{-1})		
	a	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**
1	3.43 ± 0.14	4.27 ± 0.07	5.00 ± 0.13	8.10 ± 0.45	9.63 ± 0.24	10.87 ± 0.42	1.01 ± 0.19	1.40 ± 0.10	1.76 ± 0.18
2	3.32 ± 0.15	4.43 ± 0.10	5.14 ± 0.16	7.94 ± 0.50	10.11 ± 0.33	11.29 ± 0.54	0.95 ± 0.21	1.42 ± 0.14	1.77 ± 0.23
3	3.84 ± 0.11	4.52 ± 0.12	5.21 ± 0.16	9.36 ± 0.36	10.40 ± 0.41	11.60 ± 0.52	1.05 ± 0.15	1.42 ± 0.17	1.75 ± 0.22
4	3.93 ± 0.12	4.69 ± 0.14	5.60 ± 0.14	9.66 ± 0.40	10.77 ± 0.47	12.62 ± 0.47	1.05 ± 0.17	1.48 ± 0.20	1.83 ± 0.20
5	3.33 ± 0.11	3.87 ± 0.14	4.40 ± 0.16	7.01 ± 0.38	7.82 ± 0.47	8.68 ± 0.53	1.24 ± 0.16	1.54 ± 0.20	1.81 ± 0.22
6	2.40 ± 0.13	2.87 ± 0.10	3.56 ± 0.11	4.89 ± 0.42	5.67 ± 0.32	7.18 ± 0.37	0.94 ± 0.18	1.18 ± 0.13	1.42 ± 0.16

Chromatographic conditions: column, ZWIX(−); mobile phase, **a)** MeOH; **b)** MeOH/MeCN (75/25 *v/v*); **c)** MeOH/MeCN (50/50 *v/v*), all containing 25 mM DEA and 50 mM FA; flow rate, 0.6 ml min^{-1}; detection, 262 nm. Confidence intervals were calculated as described in Section 2.3.

enantioselective process, while all other components of equation 2 depend on chirality. Enantiomers may replace a different number of solvent molecules when linked to the CSP, and, as a consequence, both desorption and dilution may depend on stereochemical properties. Since the contribution of the dilution step is low, it can be neglected, and for a pair of enantiomers, $\Delta(\Delta X^0)$ can be calculated according to Eq. (3).

$$\Delta(\Delta X^0) = \Delta X_2^0 - \Delta X_1^0 = \Delta\left(\Delta X_{desorp}^0\right) + \Delta\left(\Delta X_{netads}^0\right) + \Delta\left(\Delta X_{resolv}^0\right) \quad (3)$$

Obviously, the measured $\Delta(\Delta X^0)$ values are still lumped values, characterizing a seemingly homogeneous surface [40].

Systematic studies on the effect of mobile phase composition on thermodynamics can hardly be found in the field of chiral separations. Asnin *et al.* studied the enantioselective separation of dipeptides on antibiotic-based CSPs and found a correlation between the mobile phase pH and $\Delta H°$ and $\Delta S°$ values, but only for Chirobiotic T, not for Chirobiotic R [43]. As an explanation, it was suggested that the acidity of the mobile phase affects the binding affinity of the teicoplanin-based CSP due to its ionic character. In a subsequent publication, the effect of MeOH content was studied on a Chirobiotic R column applying MeOH/H_2O-based eluents, where diverged correlations were found between the MeOH content and the thermodynamic parameters for the studied dipeptides [44].

A study of the possible effects of mobile phase composition on the thermodynamic parameters was performed with different eluent compositions of MeOH/MeCN with FA (50 mM) and DEA (25 mM) using 0.6 ml min^{-1} flow rate. In the case of the ZWIX(−) column MeOH/MeCN 100/0, 75/25, and 50/50 (*v/v*), while in case of the ZWIX(+) column 100/0, and 50/50 (*v/v*) eluent compositions were applied. The thermodynamic parameters calculated as discussed above, summarized in Table 2 and Table S6, show a clear tendency. Namely, the higher the MeCN content of the eluent the more negative the $\Delta(\Delta H°)$, $\Delta(\Delta S°)$, and $\Delta(\Delta G°)$ values obtained on both zwitterionic CSPs. It is important to note that all $\Delta(\Delta H°)$, $\Delta(\Delta S°)$, and $\Delta(\Delta G°)$ values were negative, indicating that enthalpy-controlled enantiorecognition takes place on the studied CSPs. All calculated thermodynamic parameters changed with similar tendencies for all studied SAs in support of the earlier finding that enantiorecognition is not seriously affected by the structural changes related to the fluoro substitution of the SAs. To reveal the contribution of the enthalpy and entropy terms to theenantioseparation, Q=$\Delta(\Delta H°)/[T^*\Delta(\Delta S°)$; T = 298 K] values were also calculated (Table 3). The changes in Q values did not exceed the experimental error, which suggests that $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ are affected to a similar extent with higher MeCN ratios.

In an earlier paper, we emphasized the importance of solvation of the SA and SO in the case of ion-exchanger-based CSPs [19]. The electrostatic forces formed between SO and SA were found to be strongly affected by the thickness of solvation spheres developed around the charged species. Since MeCN possesses lower solvation power of the chargeable sites of SA and SO, increasing its ratio in the mobile phase results in an enhanced Coulomb attraction. In the case of the zwitterionic CSPs, adsorption relates to electrostatic forces which, in turn, is affected by the solvation shells. Therefore, the solvent can influence the adsorption and trigger the overall stereorecognition, as observed in the present study.

Table 3
Effects of eluent composition on the $\Delta(\Delta H^0)/[T\text{x}\Delta(\Delta S^0)]$ ratio of fluorinated ß-phenylalanine derivatives on ZWIX(−) column.

Analyte	$Q = \Delta(\Delta H^0)/[T\text{x}\Delta(\Delta S^0)]$		
	a	**b**	**c**
1	1.42 ± 0.10	1.49 ± 0.04	1.54 ± 0.07
2	1.40 ± 0.11	1.47 ± 0.06	1.53 ± 0.09
3	1.38 ± 0.07	1.46 ± 0.07	1.51 ± 0.08
4	1.36 ± 0.07	1.46 ± 0.08	1.49 ± 0.07
5	1.59 ± 0.10	1.66 ± 0.12	1.70 ± 0.12
6	1.65 ± 0.17	1.70 ± 0.11	1.66 ± 0.10

Chromatographic conditions: column, ZWIX(−); mobile phase, **a)** MeOH; **b)** MeOH/MeCN (75/25 *v/v*); **c)** MeOH/MeCN (50/50 *v/v*), all containing 25 mM DEA and 50 mM FA; flow rate, 0.6 ml min^{-1}; detection, 262 nm. Confidence intervals were calculated as described in Section 2.3.

4. Conclusions

In the current work, excellent enantioseparations were achieved for newly synthesized, fluorine-containing ß-phenylalanine derivatives applying *Cinchona* alkaloid-based zwitterionic ion-exchangers in the polar ionic mode. Effects of mobile phase compositions were investigated to gain insights into the enantiorecognition processes. Acidic and basic additives served as effective counterions resulting in easily tunable retention properties without significant loss in enantioselectivity. The nature of the base was found to affect retention properties, while it has only slight effects on the observed enantioselectivities. The main interactions responsible for retention and enantiorecognition were not radically modified by the structural changes of the analytes; however, important structure-retention and enantioselectivity relationships could be revealed.

A detailed temperature study ensured a possibility for the thermodynamic characterization of the *Cinchona* alkaloid-based CSPs, not ignoring the limitations of the employed van't Hoff analysis. Assuming that the separation of the two enantiomers takes place essentially by the same SO-SA interaction mechanism, which seems to be the case in this study, based on the change in standard enthalpy and entropy values clear evidence could be provided how the eluent composition affects the difference in the change in standard enthalpy and entropy. Increase in the eluent MeCN content favored the adsorption process without significantly affecting the enthalpy and entropy contributions. Applying typical operational conditions no strong evidence could be found for the effect of flow rate on the calculated thermodynamic parameters. That is, the $\Delta(\Delta H°)$ and $\Delta(\Delta S°)$ values were found to be influenced more

significantly by the structural peculiarities of the studied analytes than the flow rate.

CRediT authorship contribution statement

Gábor Németi: Investigation, Writing – Original Draft, Visualization, Review & Editing; **Róbert Berkecz**: Conceptualization, Writing– Original Draft, Review & Editing; **Sayeh Shahmohammadi**: Resources, Writing – Original Draft; **Enikő Forró**: Resources,Writing – Original Draft, **Wolfgang Lindner**: Conceptualization, Writing– Orgiginal Draft, Review & Editing; **Antal Péter**: Conceptualization, Writing-– Original Draft, Review & Editing; **István Ilisz**: Conceptualization, Writing– Orgiginal Draft, Review & Editing; Supervision, Project Administration, Funding Acquasition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was supported by National Research, Development and Innovation Office-NKFIA through projects K137607 and K129049. Project no. TKP2021-EGA-32 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2022.462974.

References

[1] E. Forró, F. Fülöp, New enzymatic two-step cascade reaction for the preparation of a key intermediate for the taxol side-chain, European J. Org. Chem. (2010) 3074–3079, doi:10.1002/ejoc.201000262.
[2] I. Ojima, S.D. Kuduk, S. Chakravarty, Recent advances in the medicinal chemistry of taxoid anticancer agents, 1999. doi:10.1016/S1067-5698(99)80004-2.
[3] E. Forró, G. Tasnádi, F. Fülöp, Enzymatic preparation of (S)-3-amino-3-(o-tolyl)propanoic acid, a key intermediate for the construction of Cathepsin inhibitors, J. Mol. Catal. B Enzym. 93 (2013) 8–14, doi:10.1016/j.molcatb.2013.04.001.
[4] S. Ruf, C. Buning, H. Schreuder, G. Horstick, W. Linz, T. Olpp, J. Pernerstorfer, K. Hiss, K. Kroll, A. Kannt, M. Kohlmann, D. Linz, T. Hübschle, H. Rütten, K. Wirth, T. Schmidt, T. Sadowski, Novel β-amino acid derivatives as inhibitors of cathepsin A, J. Med. Chem. 55 (2012) 7636–7649, doi:10.1021/jm300663n.
[5] G.K.S. Prakash, Modern Fluororganic Chemistry. By Peer Kirsch, 2005, doi:10.1002/anie.200485237.
[6] I. Ojima, Wiley: Fluorine in Medicinal Chemistry and Chemical Biology - Iwao Ojima, 2009th ed., Wiley-Blackwell, n.d. http://as.wiley.com/WileyCDA/WileyTitle/productCd-1405167203.html.
[7] A.E. Weber, N. Thornberry, JANUVIA™ (Sitagliptin), a Selective Dipeptidyl Peptidase IV Inhibitor for the Treatment of Type2 Diabetes, Annu. Rep. Med. Chem. 42 (2007) 95–109, doi:10.1016/S0065-7743(07)42007-3.
[8] G.K.E. Scriba, Chiral recognition in separation sciences. Part I: polysaccharide and cyclodextrin selectors, TrAC - Trends Anal. Chem. 120 (2019) 115639, doi:10.1016/j.trac.2019.115639.
[9] G.K.E. Scriba, Chiral recognition in separation sciences. Part II: macrocyclic glycopeptide, donor-acceptor, ion-exchange, ligand-exchange and micellar selectors, TrAC - Trends Anal. Chem. 119 (2019) 115628, doi:10.1016/j.trac.2019.115628.
[10] G.K.E. Scriba, Chiral separations, Springer, New York, New York, NY, 2019, doi:10.1007/978-1-4939-9438-0.
[11] B. Chankvetadze, Recent trends in preparation, investigation and application of polysaccharide-based chiral stationary phases for separation of enantiomers in high-performance liquid chromatography, TrAC - Trends Anal. Chem. 122 (2020) 115709, doi:10.1016/j.trac.2019.115709.
[12] B. Chankvetadze, Application of enantioselective separation techniques to bioanalysis of chiral drugs and their metabolites, TrAC - Trends Anal. Chem. 143 (2021) 116332, doi:10.1016/j.trac.2021.116332.
[13] G. Mazzoccanti, S. Manetto, A. Ricci, W. Cabri, A. Orlandin, M. Catani, S. Felletti, A. Cavazzini, M. Ye, H. Ritchie, C. Villani, F. Gasparrini, High–throughput enantioseparation of Nα–fluorenylmethoxycarbonyl proteinogenic amino acids through fast chiral chromatography on zwitterionic-teicoplanin stationary phases, J. Chromatogr. A. 1624 (2020) 461235, doi:10.1016/j.chroma.2020.461235.
[14] O.H. Ismail, M. Antonelli, A. Ciogli, M. De Martino, M. Catani, C. Villani, A. Cavazzini, M. Ye, D.S. Bell, F. Gasparrini, Direct analysis of chiral active pharmaceutical ingredients and their counterions by ultra high performance liquid chromatography with macrocyclic glycopeptide-based chiral stationary phases, J. Chromatogr. A. 1576 (2018) 42–50, doi:10.1016/j.chroma.2018.09.029.
[15] N. Khundadze, S. Pantsulaia, C. Fanali, T. Farkas, B. Chankvetadze, On our way to sub-second separations of enantiomers in high-performance liquid chromatography, J. Chromatogr. A. 1572 (2018) 37–43, doi:10.1016/j.chroma.2018.08.027.
[16] J.M. Lin, T. Hobo, Inspection of the reversal of enantiomer migration order in ligand exchange micellar electrokinetic capillary chromatography, Biomed. Chromatogr. 15 (2001) 207–211, doi:10.1002/bmc.63.
[17] T. Tonoi, A. Nishikawa, T. Yajima, H. Nagano, K. Mikami, Fluorous substituent-based enantiomer and diastereomer separation: Orthogonal use of HPLC columns for the synthesis of nonproteinogenic polyfluoro amino acids and peptides, European J. Org. Chem. (2008) 1331–1335, doi:10.1002/ejoc.200701052.
[18] G. Lajkó, T. Orosz, L. Kiss, E. Forró, F. Fülöp, A. Péter, I. Ilisz, High-performance liquid chromatographic enantioseparation of fluorinated cyclic β3-amino acid derivatives on polysaccharide-based chiral stationary phases. Comparison with nonfluorinated counterparts, Biomed. Chromatogr. 30 (2016) 1441–1448, doi:10.1002/bmc.3702.
[19] D. Tanács, T. Orosz, I. Ilisz, A. Péter, W. Lindner, Unexpected effects of mobile phase solvents and additives on retention and resolution of N-acyl-D,L-leucine applying Cinchonane-based chiral ion exchangers, J. Chromatogr. A. 1648 (2021), doi:10.1016/j.chroma.2021.462212.
[20] A. Bajtai, I. Ilisz, D.H.O. Howan, G.K. Tóth, G.K.E. Scriba, W. Lindner, A. Péter, Enantioselective resolution of biologically active dipeptide analogs by high-performance liquid chromatography applying Cinchona alkaloid-based ion-exchanger chiral stationary phases, J. Chromatogr. A. 1611 (2020), doi:10.1016/j.chroma.2019.460574.
[21] C. Geibel, K. Dittrich, U. Woiwode, M. Kohout, T. Zhang, W. Lindner, M. Lämmerhofer, Evaluation of superficially porous particle based zwitterionic chiral ion exchangers against fully porous particle benchmarks for enantioselective ultra-high performance liquid chromatography, J. Chromatogr. A. 1603 (2019) 130–140, doi:10.1016/j.chroma.2019.06.026.
[22] U. Woiwode, M. Ferri, N.M. Maier, W. Lindner, M. Lämmerhofer, Complementary enantioselectivity profiles of chiral cinchonan carbamate selectors with distinct carbamate residues and their implementation in enantioselective two-dimensional high-performance liquid chromatography of amino acids, J. Chromatogr. A. 1558 (2018) 29–36, doi:10.1016/j.chroma.2018.04.061.
[23] I. Ilisz, N. Grecsó, A. Aranyi, P. Suchotin, D. Tymecka, B. Wilenska, A. Misicka, F. Fülöp, W. Lindner, A. Péter, Enantioseparation of β2-amino acids on cinchona alkaloid-based zwitterionic chiral stationary phases. Structural and temperature effects, J. Chromatogr. A. 1334 (2014) 44–54, doi:10.1016/j.chroma.2014.01.075.
[24] I. Ilisz, N. Grecsó, R. Papoušek, Z. Pataj, P. Barták, L. Lázár, F. Fülöp, W. Lindner, A. Péter, High-performance liquid chromatographic separation of unusual β3-amino acid enantiomers in different chromatographic modes on Cinchona alkaloid-based zwitterionic chiral stationary phases, Amino Acids 47 (2015) 2279–2291, doi:10.1007/s00726-015-2006-1.
[25] G. Lajkó, T. Orosz, I. Ugrai, Z. Szakonyi, F. Fülöp, W. Lindner, A. Péter, I. Ilisz, Liquid chromatographic enantioseparation of limonene-based carbocyclic β-amino acids on zwitterionic Cinchona alkaloid-based chiral stationary phases, J. Sep. Sci. 40 (2017) 3196–3204, doi:10.1002/jssc.201700450.
[26] T. Orosz, E. Forró, F. Fülöp, W. Lindner, I. Ilisz, A. Péter, Effects of N-methylation and amidination of cyclic β-amino acids on enantioselectivity and retention characteristics using Cinchona alkaloid- and sulfonic acid-based chiral zwitterionic stationary phases, J. Chromatogr. A. 1535 (2018) 72–79, doi:10.1016/j.chroma.2017.12.070.
[27] E. Forró, T. Paál, G. Tasnádi, F. Fülöp, A new route to enantiopure β-aryl-substituted β-amino acids and 4-aryl-substituted β-lactams through lipase-catalyzed enantioselective ring cleavage of β-lactams, Adv. Synth. Catal. 348 (2006) 917–923, doi:10.1002/adsc.200505434.
[28] S. Shahmohammadi, F. Fülöp, E. Forró, Efficient Synthesis of New Fluorinated β-Amino Acid Enantiomers through Lipase-Catalyzed Hydrolysis, Molecules 25 (2020) 5990, doi:10.3390/molecules25245990.
[29] I. Ilisz, Z. Gecse, G. Lajkó, M. Nonn, F. Fülöp, W. Lindner, A. Péter, Investigation of the structure-selectivity relationships and van't Hoff analysis of chromatographic stereoisomer separations of unusual isoxazoline-fused 2-aminocyclopentanecarboxylic acids on Cinchona alkaloid-based chiral stationary phases, J. Chromatogr. A. 1384 (2015) 67–75, doi:10.1016/j.chroma.2015.01.041.
[30] G. Lajkó, T. Orosz, N. Grecsó, B. Fekete, M. Palkó, F. Fülöp, W. Lindner, A. Péter, I. Ilisz, High-performance liquid chromatographic enantioseparation of cyclic β-aminohydroxamic acids on zwitterionic chiral stationary phases based on Cinchona alkaloids, Anal. Chim. Acta. 921 (2016) 84–94, doi:10.1016/j.aca.2016.03.044.
[31] C.V. Hoffmann, M. Laemmerhofer, W. Lindner, Novel strong cation-exchange type chiral stationary phase for the enantiomer separation of chiral amines by

high-performance liquid chromatography, J. Chromatogr. A. 1161 (2007) 242–251, doi:10.1016/j.chroma.2007.05.092.
[32] I. Ilisz, N. Grecsó, M. Palkó, F. Fülöp, W. Lindner, A. Péter, Structural and temperature effects on enantiomer separations of bicyclo[2.2.2]octane-based 3-amino-2-carboxylic acids on cinchona alkaloid-based zwitterionic chiral stationary phases, J. Pharm. Biomed. Anal. 98 (2014) 130–139, doi:10.1016/j.jpba.2014.05.012.
[33] David R. Lide, ed., CRC Handbook of Chemistry and Physics, Internet V, CRC Press, n.d.
[34] W. Kopaciewicz, M.A. Rounds, J. Fausnaugh, F.E. Regnier, Retention model for high-performance ion-exchange chromatography, J. Chromatogr. A. 266 (1983) 3–21, doi:10.1016/S0021-9673(01)90875-1.
[35] B. Sellergren, K.J. Shea, Chiral ion-exchange chromatography. Correlation between solute retention and a theoretical ion-exchange model using imprinted polymers, J. Chromatogr. A. 654 (1993) 17–28, doi:10.1016/0021-9673(93)83061-V.
[36] N. Grecsó, E. Forró, F. Fülöp, A. Péter, I. Ilisz, W. Lindner, Combinatorial effects of the configuration of the cationic and the anionic chiral subunits of four zwitterionic chiral stationary phases leading to reversal of elution order of cyclic β3-amino acid enantiomers as ampholytic model compounds, J. Chromatogr. A. 1467 (2016) 178–187, doi:10.1016/j.chroma.2016.05.041.
[37] N. Grecsó, M. Kohout, A. Carotti, R. Sardella, B. Natalini, F. Fülöp, W. Lindner, A. Péter, I. Ilisz, Mechanistic considerations of enantiorecognition on novel Cinchona alkaloid-based zwitterionic chiral stationary phases from the aspect of the separation of trans-paroxetine enantiomers as model compounds, J. Pharm. Biomed. Anal. 124 (2016) 164–173, doi:10.1016/j.jpba.2016.02.043.
[38] R. Sardella, E. Camaioni, A. Macchiarulo, A. Gioiello, M. Marinozzi, A. Carotti, Computational studies in enantioselective liquid chromatography: Forty years of evolution in docking- and molecular dynamics-based simulations, TrAC - Trends Anal. Chem. 122 (2020) 115703, doi:10.1016/j.trac.2019.115703.
[39] I. Varfaj, M. Protti, A. Di Michele, A. Macchioni, W. Lindner, A. Carotti, R. Sardella, L. Mercolini, Efficient enantioresolution of aromatic α-hydroxy acids with Cinchona alkaloid-based zwitterionic stationary phases and volatile polar-ionic eluents, Anal. Chim. Acta. 1180 (2021) 338928, doi:10.1016/j.aca.2021.338928.
[40] L.D. Asnin, M.V. Stepanova, Van't Hoff analysis in chiral chromatography, J. Sep. Sci. 41 (2018) 1319–1337, doi:10.1002/jssc.201701264.
[41] A. Sepsey, É. Horváth, M. Catani, A. Felinger, The correctness of van 't Hoff plots in chiral and achiral chromatography, J. Chromatogr. A. 1611 (2020) 6–8, doi:10.1016/j.chroma.2019.460594.
[42] T.L. Chester, J.W. Coym, Effect of phase ratio on van't Hoff analysis in reversed-phase liquid chromatography, and phase-ratio-independent estimation of transfer enthalpy, J. Chromatogr. A. 1003 (2003) 101–111, doi:10.1016/S0021-9673(03)00846-X.
[43] E.N. Reshetova, M.V. Kopchenova, S.E. Vozisov, A.N. Vasyanin, L.D. Asnin, Enantioselective retention mechanisms of dipeptides on antibiotic-based chiral stationary phases: Leucyl-leucine, glycyl-leucine, and leucyl-glycine as case studies, J. Chromatogr. A. 1602 (2019) 368–377, doi:10.1016/j.chroma.2019.06.025.
[44] L.D. Asnin, M.V. Kopchenova, S.E. Vozisov, M.A. Klochkova, Y.A. Klimova, Enantioselective retention mechanisms of dipeptides on antibiotic-based chiral stationary phases. II. Effect of the methanol content in the mobile phase, J. Chromatogr. A. 1626 (2020) 461371, doi:10.1016/j.chroma.2020.461371.