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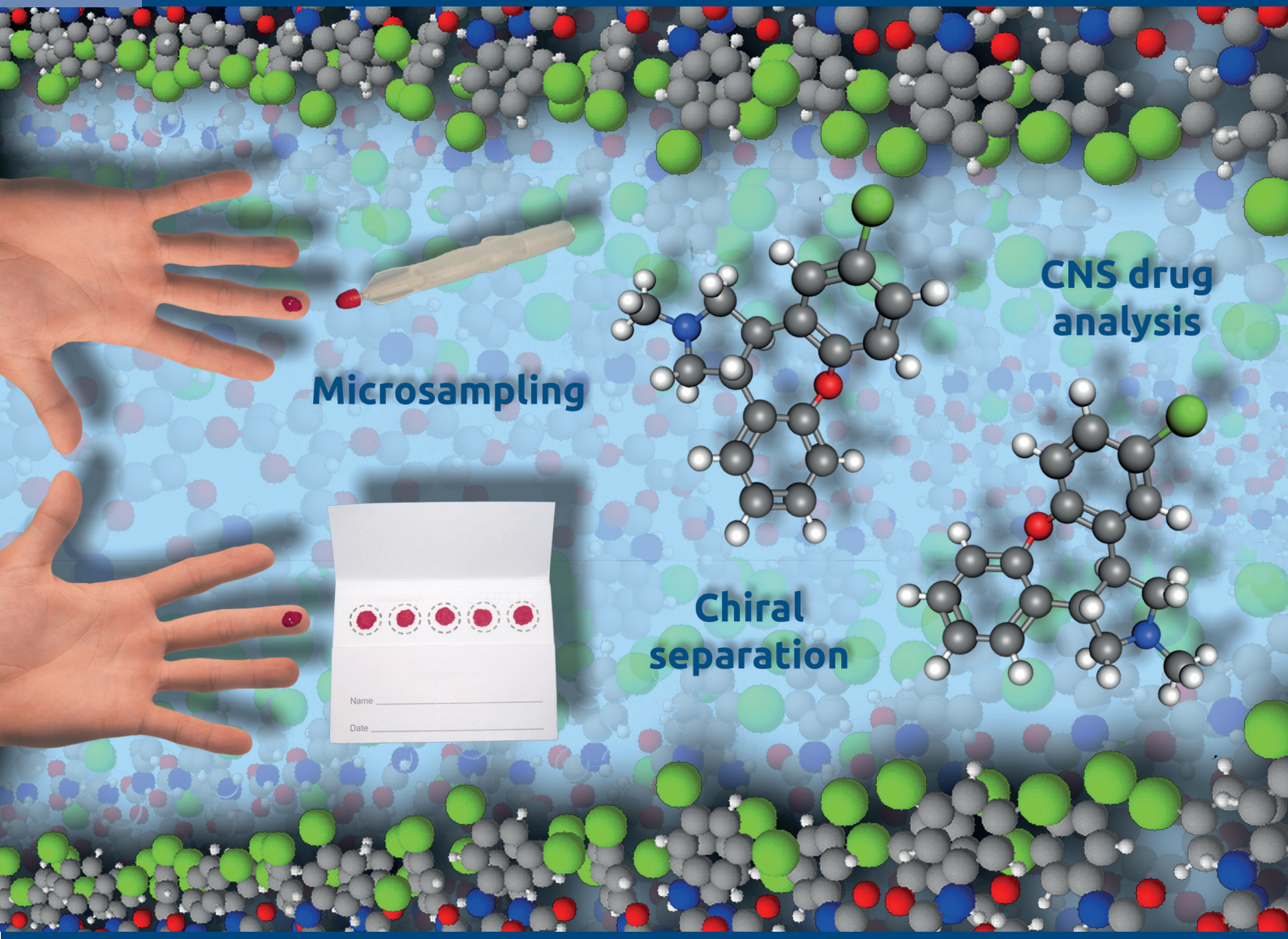

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RESEARCH ARTICLE

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Comparative study on the liquid chromatographic enantioseparation of cyclic β-amino acids and the related cyclic β-aminohydroxamic acids on *Cinchona* alkaloid-based zwitterionic chiral stationary phases

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The enantiomeric pairs of *cis* and *trans* stereoisomers of cyclic β-aminohydroxamic acids and their related *cis* and *trans* cyclic β-amino acids containing two chiral centers were directly separated on four structurally related chiral stationary phases derived from quinine and quinidine modified with (*R*,*R*)- and (*S*,*S*)-aminocyclohexanesulfonic acids. Applying these zwitterionic ion-exchangers as chiral selectors, the effects of the composition of the bulk solvent, the acid and base additives, the structures of the analytes, and temperature on the enantioresolution were investigated. To study the effects of temperature and obtain thermodynamic parameters, experiments were carried out at constant mobile phase compositions in the temperature range 5–50°C. The differences in the changes in standard enthalpy $\Delta(\Delta H°)$, entropy $\Delta(\Delta S°)$, and free energy $\Delta(\Delta G°)$ were calculated from the linear van't Hoff plots derived from the ln α versus $1/T$ curves in the studied temperature range. Results thus obtained indicated enthalpy-driven separations in all cases. The sequence of elution of the enantiomers was determined and found to be reversed when ZWIX(–)^{TM} was changed to ZWIX(+)^{TM} or ZWIX(–A) to ZWIX(+A).

KEYWORDS
amino acids, beta-aminohydroxamic acids, enantioseparation, high-performance liquid chromatography

1 | INTRODUCTION

Hydroxamic acids have recently attracted further interest due to their valuable biological and pharmaceutical properties. Hydroxamates are well-known for their ability to scavenge metal ions. They are able to form chelate complexes with the metal ions of the active site of several metalloenzymes and inhibit their function [1]. Hydroxamates can act as antimicrobials [2–4], anticancer [5] and anti-inflammatory [6] drugs. For example, vorinostat (suberoylanilidehydroxamic acid) [7] has been approved by the Food and Drug Administration for the treatment of cutaneous T-cell lymphoma, while ciclopiroxis is a topical antifungal drug [4]. The hydroxamic acid moiety can be found in natural products of plants [8] and bacteria [9] as well. These compounds help iron uptake from soil. Desferrioxamine B, a natural hydroxamate-type siderophore produced by *Streptomyces pylosus*, is used in the therapy of iron overload [10]. Hydroxamates can also be effective tools in asymmetric synthesis as chiral ligands [11–14], the spectrophotometric determination of vanadium ions [15], and the separation and concentration of rare earth elements from seawater [16].

Abbreviations: ACHSA, *trans*-2-aminocyclohexanesulfonic acid; AcOH, acetic acid; CSP, chiral stationary phase; DEA, diethylamine; MeOH, methanol; PI, polar-ionic; QD, quinidine; QN, quinine; SA, selectand; SO, selector

Conflict of interest: The authors have declared no conflict of interest.

Aromatic and aliphatic hydroxamic acids have increasing applications in medical and organic chemistry; however, information about alicyclic derivatives is rather limited [17]. There are only a handful of studies that have reported successful separation methods for hydroxamic acid stereoisomers. CE has been utilized with 18-crown-6-tetracarboxylic acid as chiral selector (SO) [18], while chiral HPLC analysis of hydroxamic acids and their analogs are comparatively rare. The control of enantiomeric purity of hydroxamic acids and their analogs on various stationary phases (CSPs) has been described [19–22].

We have recently reported the separation of the enantiomers of bicyclic β-aminohydroxamic acids using zwitterionic CSPs [23]. *Cinchona* alkaloid and sulfonic acid-based zwitterionic CSPs are frequently used for the resolution of enantiomers of acids, amines, amino acids, and small peptides in polar-ionic (PI) and reversed-phase (RP) modes by HPLC [24–27], and there is a recent review about their applications as well [28].

In addition to mobile phase components, stereoselective interactions in chiral chromatography are highly sensitive to temperature. Specifically, retention determined by "achiral" and "chiral" contributions can change markedly with temperature [29–34]. On the basis of the van't Hoff equation, thermodynamic quantities of chromatographic equilibrium processes such as the difference of standard enthalpy change $\Delta(\Delta H°)$ and standard entropy change $\Delta(\Delta S°)$ for the two corresponding enantiomers can be derived from the plot of R lnα versus $1/T$ [23], where α is the selectivity factor, R is the universal gas constant and T is temperature (in K). It is important to realize that the thermodynamic quantities obtained assuming only one type of interaction sites allows a simplified approach to characterize the system from a thermodynamic point of view. To be more realistic, the enantioselective and nonselective sites should also be distinguished [34].

The present study describes the enantioseparation of two pairs of *cis* and *trans* cyclic β-aminohydroxamic acids and their carboxylic acid analogs (selectands, SAs; Fig. 1) on the ampholytic CSPs in polar-ionic mode. (The structures of the SOs are shown in Supporting Information Fig. S1 and [35]). The influence of the bulk solvent composition of the mobile phase, the impact of different counter-ion concentrations, temperature, and structural features of the SAs and SOs on the retention and enantioselectivity are discussed. The sequence of elution of the enantiomers was determined in all cases.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Direct enzymatic method was performed for the synthesis of *cis*- and *trans*-β-amino acid enantiomers **1A-D** and **3A-D** through the *Candida antarctica* lipase B-catalyzed enantioselective hydrolysis of alicyclic β-amino esters in iPr$_2$O at 65°C [36]. The absolute configurations were proved by comparing the [α] values with the literature data [36].

Racemic *cis* and *trans*-2-amino-*N*-hydroxycyclohexanecarboxamides (**2**) and *cis*- and *trans*-6-amino-*N*-hydroxycyclohex-3-enecarboxamides (**4**) were prepared from the racemic ethyl *cis*- and *trans*-2-aminocyclohexanecarboxylates or ethyl *cis*- and *trans*-6-aminocyclohex-3-enecarboxylates at room temperature with three equivalents of 50 w/w% aqueous hydroxyl amine solution.

Ethyl *cis*- and *trans*-6-aminocyclohex-3-enecarboxylates were resolved with (*S*)-mandelic acid in EtOH by diastereomeric salt formation. The enantiomeric excess values for the esters were determined after derivatization with benzoyl chloride [37,38]. Ethyl 6-aminocyclohex-3-enecarboxylate enantiomers were also transformed into ethyl 2-aminocyclohexanecarboxylate enantiomers with H_2 in the presence of Pd/C. Continuous-flow hydrogenations were carried out in a ThalesNano H-cube™ system.

The enantiopure amino acid ester bases were then reacted with hydroxyl amine solution by the method described in [39, 40], affording the hydroxamic acid enantiomers **2A**, **2D**, **4A,** and **4C**. The stereochemistry of the synthesized compounds was determined by ^{1}H NMR spectroscopy (Supporting Information Appendix I).

Methanol (MeOH) and acetonitrile (ACN) of HPLC grade were purchased from VWR International (Radnor, PA, USA). Diethylamine (DEA) and glacial acetic acid (AcOH) of analytical reagent grade were from Sigma–Aldrich (St. Louis, MO, USA). Purified water was obtained from the Ultrapure Water System, Puranity TU UV/UF (VWR International).

2.2 | Apparatus and chromatography

Analytical HPLC measurements were carried out on a 1100 Series HPLC system from Agilent Technologies (Waldbronn, Germany), equipped with a solvent degasser, a pump, an autosampler, a column thermostat, a multiwavelength UV-V is and a corona-charged aerosol detector (ESA Biosciences, Chelmsford, MA, USA). ChemStation chromatographic data software from Agilent Technologies allowed performing the acquisition and processing of the chromatographic data. Optical rotations of the amino ester enantiomers were measured with a Perkin–Elmer 341 polarimeter (PerkinElmer, Waltham Massachusetts, USA).

The Chiralpak ZWIX(+)™, ZWIX(–)™ and ZWIX(+A) and ZWIX(–A) columns (150 × 3.0 mm id, 3-μm particle size for all columns) were provided by Chiral Technologies Europe (Illkirch, France) and by Michal Kohout (Department of Organic Chemistry, University of Chemistry and Technology, Prague, Czech Republic).

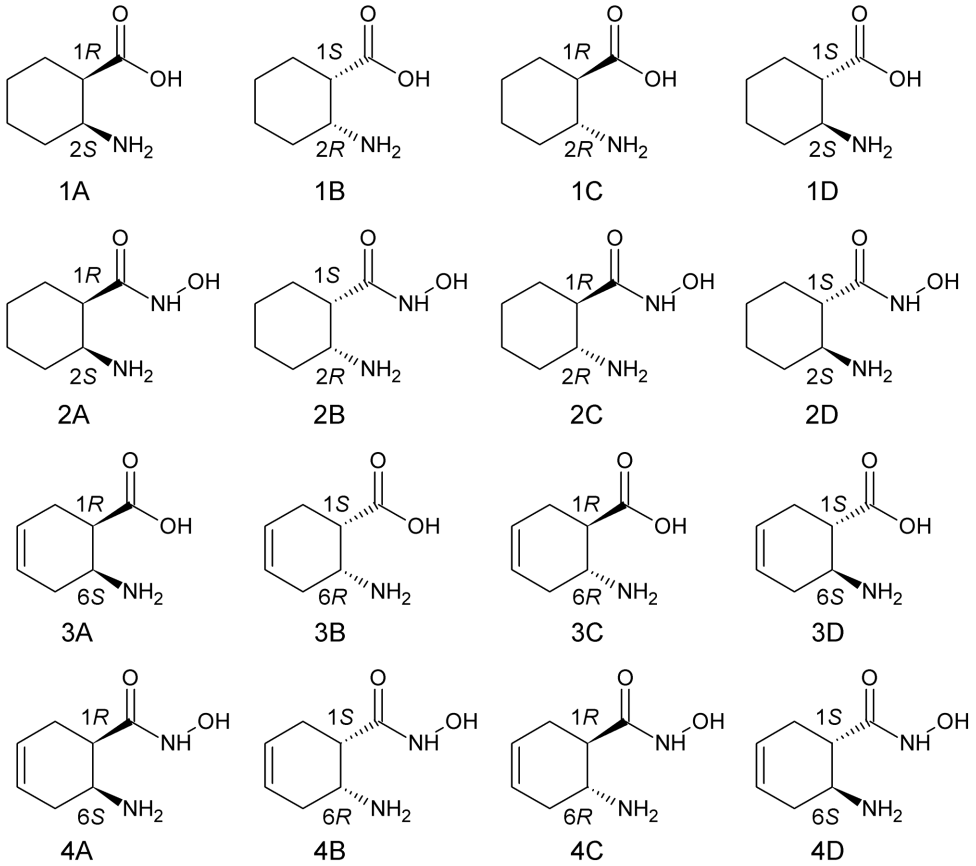

FIGURE 1 Chemical structures of cyclic β-amino acids and cyclic β-aminohydroxamic acids

Stock solutions of all analytes (ca. 1.0 mg/mL) were prepared by dissolution in the mobile phase or MeOH. The dead-times (t_0) of the columns were determined by injection of acetone dissolved in MeOH. The flow rate was 0.6 mL/min and the column temperature was 25°C, if not otherwise stated.

3 | RESULTS AND DISCUSSIONS

3.1 | Influence of bulk solvent composition

A mixture of MeOH as a protic solvent and ACN as an aprotic solvent in the presence of acid and base additives have been successfully applied for the enantioseparations on *Cinchona* alkaloid-based zwitterionic CSPs. The effect of composition of bulk solvent on the chromatographic parameters for all SAs were studied on the four ZWIX(+)^{TM}, ZWIX(–)^{TM}, ZWIX(+A), and ZWIX(–A) columns using MeOH/ACN (75:25, 50:50, or 25:75 v/v) mobile phase systems containing 25 mM DEA and 50 mM AcOH. The obtained results are summarized in Supporting Information Table S1. To simplify the presentation, only some of the results, obtained on ZWIX(–)^{TM} and ZWIX(+A) are depicted in Fig. 2. In all cases, increased retention was observed with increasing ACN content in the MeOH/ACN mixtures (Fig. 2). In the ACN-rich mobile phase both the solvation and solvation shell of the ionizable compounds decreased, resulting in an increased retention. Since the solvation of polar compounds at higher ACN content is less effective, stronger electrostatic interactions are expected, resulting in higher retention.

With the increase in the ACN content, selectivity values changed in different ways. On ZWIX(–)^{TM} no general trend could be observed, while on ZWIX(+A) and ZWIX(–A) selectivity reached its maximum value at a MeOH/ACN ratio of 50:50 v/v in most cases. In the case of ZWIX(+)^{TM} for SAs possessing *trans* configuration (**1C**, **1D**, **2C**, **2D**, **3C**, **3D,** and **4C**, **4D**), α increased, while for SAs with *cis* configuration (**1A**,**1B**, **2A**,**2B**, **3A**,**3B,** and **4A**,**4B**), α decreased slightly with increasing ACN content (Supporting Information Table S1). The increase in selectivity was probably due to additional H-bonding interactions and/or steric factors. Values of R_S on ZWIX(–)^{TM}, ZWIX(+A), and ZWIX(–A) CSPs generally reached their maximum at a MeOH/ACN ratio of 50:50 v/v with increasing ACN content. On ZWIX(+)^{TM}, in turn, a continuous slight increase was registered. In summary, the best separation performances were achieved applying the mobile phase composition MeOH/ACN 50:50 v/v containing 25 mM DEA and 50 mM AcOH. Consequently, most of the experiments were performed by using the latter mobile phase system.

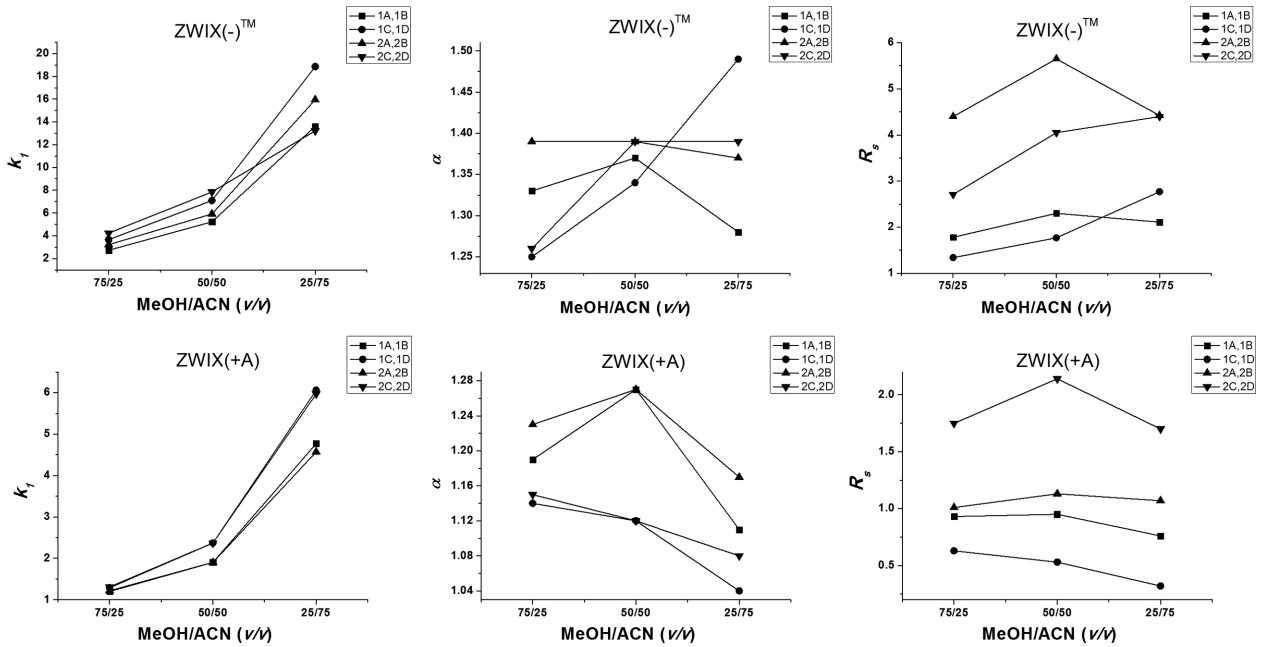

FIGURE 2 Influence of the bulk solvent composition on the chromatographic parameters, k_1, α, and R_S, for all analytes on ZWIX(–)^{TM} and ZWIX(+A) CSPs. Chromatographic conditions: column ZWIX(–)^{TM} and ZWIX(+A) CSPs; mobile phase, MeOH/ACN (75:25, 50:50, or 25:75 v/v) containing 25 mM DEA and 50 mM AcOH; flow rate, 0.6 mL/min; detection, corona detector; temperature, ambient; symbols, for analytes 1A,1B: ■, for 1C, 1D: ●, 2A,2B: ▲, and for 2C,2D: ▼

3.2 | Influence of counter-ion concentration

For the present zwitterionic CSPs in PI mode, a double ion-pairing retention mechanism has been described for the ampholytic analytes, which fits to an ion-exchange chromatographic mode [24,35]. In the ion-pairing process, the counter-ions present in the mobile phase act as competitors, hence retention can be regulated by varying their concentrations. In such cases, the retention can be described by the stoichiometric displacement model [41]. The plot of log *k* versus log *c* indicates a linear relationship, where the slope of the straight line is proportional to the effective charge involved in the ion-exchange process. The effects of counter-ion concentration were investigated in the MeOH/ACN 75:25 v/v mobile phase system containing AcOH and DEA. The concentrations of AcOH and DEA were varied from 12.5 to 200 mM and from 6.25 to 100 mM, respectively, while the acid to base ratio was kept constant at 2:1. As the data depicted in Supporting Information Fig. S2 illustrate, the decrease of retention factors on all four columns was a consequence of the increasing counter-ion concentration, as expected for ion-exchangers. Under the studied conditions, practically identical slopes were obtained for each enantiomer on all four CSPs, thus the individual enantioselectivity characteristics remained almost constant when the counter-ion concentration changed.

It is interesting to note that the slopes of the log *k* versus log *c* plots were changed in a very narrow range, between −0.20 and −0.30 for all the SAs studied, in accordance with slopes obtained for zwitterionic columns working in zwitterionic mode [22,35]. The amino acids is expected to have zwitterionic character, and able to bind to the SO through the formation of double ion-pairs. Hydroxamic acids, which are formally also termed as *N*-hydroxamic acid type compounds are weak acids with pK_a values around 8–9 (in aqueous solutions) [42]. However, this does not rule out an electrostatic interaction with a positively charged site with a pK_a > 9.5 with the support of a hydrogen bonding. To gain more experimental evidence for this possibility a separate study is currently in progress.

3.3 | Functional and structural effects of structurally related selectands on chromatographic parameters

The structure of the SAs may influence the retention and chiral recognition. To investigate the structure–retention (selectivity) relationships, the same mobile phase composition (MeOH/ACN 50:50 v/v containing 25 mM DEA and 50 mM AcOH) was applied (Table 1). Free cyclic β-amino acids were observed to be less retained than the related cyclic β-aminohydroxamic acids (exception was on ZWIX(–A) **1A → 2A**). An extra weak H-bond ability may contribute to increased retention, although the effect is only moderate.

The *cis* or *trans* configuration exerts a marked influence on retention. For compounds with *trans* configuration, k_1 values were generally higher. In this context, the elution sequence (stereoselectivity) is to be discussed separately. The steric arrangement in *trans*-isomers of the SAs is assumed to favor

TABLE 1 Comparison of separation performances and elution sequences of amino acids and aminohydroxamic acids on ZWIX(–)^{TM}, ZWIX(+)^{TM}, ZWIX(–A), ZWIX(+A) columns

Column	Compound	k_1	α	R_S	Elution sequence
ZWIX(–)^{TM}	1A,1B	5.22	1.37	2.30	1B < 1A
	1C, 1D	7.10	1.34	1.77	1D < 1C
	2A,2B	5.92	1.39	5.65	2B < 2A
	2C,2D	7.86	1.39	4.05	2D < 2C
	3A,3B	6.62	1.32	1.94	3B < 3A
	3C,3D	6.18	1.42	2.62	3D < 3C
	4A,4B	7.39	1.34	6.04	4B < 4A
	4C,4D	6.82	1.49	6.49	4D < 4C
ZWIX(+)^{TM}	1A,1B	4.22	1.25	2.17	1A < 1B
	1C, 1D	6.03	1.08	0.58	1C < 1D
	2A,2B	4.67	1.19	3.25	2A < 2B
	2C,2D	6.58	1.13	1.75	2C < 2D
	3A,3B	5.30	1.17	1.39	3A < 3B
	3C,3D	5.13	1.24	1.53	3C < 3D
	4A,4B	5.61	1.23	3.27	4A < 4B
	4C,4D	5.50	1.22	2.12	4C < 4D
ZWIX(−A)	1A,1B	2.56	1.33	2.22	1A < 1B
	1C, 1D	2.96	1.38	2.12	1D < 1C
	2A,2B	1.90	1.27	2.14	2A < 2B
	2C,2D	2.37	1.12	0.65	2D < 2C
	3A,3B	1.97	1.16	0.77	3A < 3B
	3C,3D	2.00	1.11	0.53	3D < 3C
	4A,4B	2.03	1.14	1.08	4A < 4B
	4C,4D	2.13	1.24	2.19	4D < 4C
ZWIX(+A)	1A,1B	1.90	1.18	0.96	1B < 1A
	1C, 1D	2.22	1.09	0.53	1C < 1D
	2A,2B	1.90	1.27	2.14	2B < 2A
	2C,2D	2.37	1.12	0.65	2C < 2D
	3A,3B	1.97	1.16	0.77	3B < 3A
	3C,3D	2.00	1.11	0.53	3C < 3D
	4A,4B	2.03	1.14	1.08	4B < 4A
	4C,4D	2.13	1.24	2.19	4C < 4D

Chromatographic conditions: columns, ZWIX(–)^{TM}, ZWIX(+)^{TM}, ZWIX(−A), ZWIX(+A); mobile phase, MeOH/MeCN (50/50 *v/v*) containing 50 mM AcOH and 25 mM DEA; flow rate, 0.6 mL/min; detection, Charged Aerosol Detector; temperature 25°C.

more tight interactions with the cationic and anionic sites of the SO. However, on ZWIX(–)^{TM} and ZWIX(+)^{TM} for SAs possessing an unsaturated ring, a higher retention was measured for the *cis* analogs (**3A**,**3B** versus **3C**,**3D** and **4A**,**4B** versus **4C**,**4D**).

Selectivities changed in different ways, but in a relatively narrow range of 1.09–1.49. For cyclic β-aminohydroxamic acids, α was generally higher than for the related free cyclic β-amino acids, and no general rule could be observed regarding SAs with *cis* or *trans* configurations.

Comparing the four SOs, ZWIX(–)^{TM}, and ZWIX(+)^{TM} CSPs, in most cases, were more selective for the separation of the studied SAs than ZWIX(–A) and ZWIX(+A). Hence, they exhibited higher k_1, α, and R_S values. In most cases ZWIX (–)^{TM} provided more effective separation for both the free carbocyclic β-amino acids and carbocyclic β-aminohydroxamic acids. However, these values need to be inspected and put in the context of the elution sequences of the enantiomeric pairs, which turned out to depend on subtle structural features.

3.4 | Elution sequences

Chiral SOs based on *Cinchona* alkaloids [quinine (QN) and quinidine (QD)] and *trans*-2-aminocyclohexanesulfonic

acids [(*S,S*)- and (*R,R*)-ACHSA], namely, the ZWIX(+) and ZWIX(–) as well as ZWIX(+A) and ZWIX(–A) CSPs are usually considered as pseudo-enantiomeric in nature. In fact, they are actually diastereoisomers because the stereochemical centers of the quinuclidine residue of QN and QD remain constant.

In this context, only the switch of the stereocenters of (8*S*,9*R*) for QN and of (8*R*,9*S*) for QD leads to the pseudo-enantiomeric behavior of QN- and QD-derived selectors (see Supporting Information Fig. S1). As further variants of ZWIX(+) involving QN and (*S,S*)-ACHSA as chiral subunits of the entire SO, we designed the ZWIX(+A) SO by fusing QN and (*R,R*)-ACHSA. Similarly, we combined QD with (*S,S*)-ACHSA for the ZWIX(–A) selector. Consequently, the overall stereochemistry of these four selector variants may determine the elution sequence of the enantiomers of the investigated analytes (Fig. 1). Thanks to the availability of all stereochemically fully assigned SA standards, the chromatographic data, and the elution sequences (summarized in Table 1) could be fully explored.

First, we observed that by switching from the QN-based CSPs [ZWIX(+)^{TM} and ZWIX(+A)] to the QD-based CSPs [ZWIX(–)^{TM} and ZWIX(–A)], the elution sequences were predominantly reversed. In other words, ZWIX(+) and ZWIX(–) as well as ZWIX(+A) and ZWIX(–A) behave pseudo-enantiomerically to each other. However, exceptions could, in principle, still be expected.

Secondly, it became evident that the *cis*- and *trans*-configured SAs can behave differently when changing from ZWIX(+) to ZWIX(+A) and from ZWIX(–) to ZWIX(–A). The *cis* analytes, in contrast to *trans* analytes, showed a reversal of elution order, which can only happen when the underlying binding mechanism between the SOs and SAs changes fundamentally. Specifically, the combination of QD and (*R,R*)-ACHSA shows the same elution sequence for *cis*-hydroxamic acid analytes as the combination of QN and (*R,R*)-ACHSA. In other words, the absolute configuration of the (*R,R*)-ACHSA site dominates over the cinchona site (either QN or QD) driving the overall enantioselectivity.

It is important to note that the configuration of the chiral subunit is not the only factor that determines the relative-binding strength of the SO–SA associates. Another contributing feature might be the structural characteristics of the chiral analytes. In summary, a switch from *cis* to *trans* configuration can lead to a reversal of the elution sequence of the enantiomers. However, all characteristics contributing to such changes are not yet fully understood.

As a basis for all these pronounced effects related to the overall observed chromatographic stereoselectivity, it should be stressed that for the zwitterionic amino acids and for the hydroxamic acids we are dealing with a double ion-pairing binding mechanism between the charged sites of the ampholytic SO and SA molecules occurring simultaneously. The strong, long-range electrostatic interactions of the charged sites of SO and SA and the stereochemically demanding substitution pattern around these sites determine the stereochemically driven discrimination.

Selected chromatograms indicating the elution sequence are depicted in Supporting Information Fig. S3.

3.5 | Temperature effect and thermodynamic parameters

The effect of temperature was investigated between 5 and 50°C on ZWIX(–), ZWIX(+), ZWIX(–A), and ZWIX(+A) CSPs and experimental data are depicted in Supporting Information Table S2. Partial or baseline separation could be achieved with the mobile phase MeOH/ACN 75:25 v/v containing 25 mM DEA and 50 mM AcOH. However, in some cases [for **1C**, **1D** on ZWIX(+) and for **3A**,**3B** on ZWIX(–A)], no separation could be observed at higher temperature. In all cases, the retention decreased with increasing temperature. Transfer of the SA from the mobile phase to the stationary phase is ordinarily an exothermic process, that is, k_1 decreases with increasing temperature. The changes observed in the selectivity with increasing temperature were consistent; in general, α decreased with increasing temperature. Resolution usually decreased with the increase in temperature, while in a few cases slightly higher R_S values were registered at 10°C in comparison to those found at 5°C.

To calculate thermodynamic parameters $\Delta(\Delta H°)$, $\Delta(\Delta S°)$, and $\Delta(\Delta G°)$, van't Hoff plots were constructed. Data presented in Supporting Information Table S3 illustrate that $\Delta(\Delta H°)$ ranged from −0.3 to −2.2 kJ/mol and these values strongly depended on the structures of SOs and SAs. $\Delta(\Delta S°)$ ranged from −0.1 to −6.5 J mol^{-1} K^{-1}. The relative contribution of the enthalpic and entropic terms to the free energy of adsorption can be visualized by the enthalpy/entropy ratios $Q = \Delta(\Delta H°)/298 \times \Delta(\Delta S°)$ (Supporting Information Table S3). A comparison of the Q values for the investigated analytes revealed that enantioselective separation, in all cases, was driven by enthalpy.

4 | CONCLUDING REMARKS

The HPLC-based enantioseparation of cyclic β-amino acids and cyclic β-aminohydroxamic acids were performed on *Cinchona* alkaloid and sulfonic acid-based zwitterionic chiral stationary phases in a comparative fashion. The influence of structural elements of the chiral selectors and that of the analytes were studied on the chromatographic performance in polar ionic mode. The elution sequence was found to be reversed by the change of the QN-based CSPs to the QD-based CSPs offering a good possibility for the determination of enantiomeric excesses. Evidence was provided for the

decisive effect of the selectors subunits; both the QN or QD moiety and the ACHSA subunit may take a determining role in the chiral recognition. In addition to the structural features of the selector geometrical isomerism of the analytes was shown to play an important role in the formation of diastereomeric complexes for a successful enantioseparation.

By variation of the mobile phase composition enantioseparations were optimized and baseline separation could be achieved. Thermodynamic parameters derived from the ln α versus 1/*T* curves were calculated, indicating enthalpy-driven separations in all studied cases.

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REFERENCES

1. Codd, R., Traversing the coordination chemistry and chemical biology of hydroxamic acids. *Coord. Chem. Rev.* 2008, *252*, 1387–1408.
2. Mishra, R. C., Tripathi, R., Katiyar, D., Tewari, N., Singh, D., Tripathia, R. P., Synthesis of glycosylated β-amino hydroxamates as new class of antimalarials. *Bioorg. Med. Chem.* 2003, *11*, 5363–5374.
3. Zarember, K. A., Cruz, A. R., Huang, C., Gallin, J. I., Antifungal activities of natural and synthetic iron chelators alone and in combination with azole and polyene antibiotics against *Aspergillus fumigatus*. *Antimicrob. Agents Chemother.* 2009, *53*, 2654–2656.
4. Gupta, A. K., Ciclopirox: an overview. *Int. J. Dermatol.* 2001, *40*, 305–310.
5. Slingerland, M., Guchelaar, H. J., Gelderblom, H., Histone deacetylase inhibitors: an overview of the clinical studies in solid tumors. *Anti-Cancer Drugs* 2014, *25*, 140–149.
6. Tanaka, K., Matsuo, K., Nakanishi, A., Hatano, T., Izeki, H., Ishida, Y., Mori, W., Syntheses and anti-inflammatory and analgesic activities of hydroxamic acids and acid hydrazides. *Chem. Pharm. Bull.* 1983, *31*, 2810–2819.
7. Mann, B. S., Johnson, J. R., He, K., Sridhara, R., Abraham, S., Booth, B. P., Verbois, L., Morse, D. E., Jee, J. M., Pope, S., Harapanhalli, R. S., Dagher, R., Farrell, A., Justice, R., Pazdur, R., Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. *Clin. Cancer Res.* 2007, *13*, 2318–2322.
8. Ohigashi, H., Kaji, M., Sakaki, M., Koshimizu, K., 3-Hydroxyuridine, an allelophatic factor of an African tree, *Baillonellatoxisperma*. *Phytochemistry* 1989, *28*, 1365–1368.
9. Wilson, B. R., Bogdan, A. R., Miyazawa, M., Hashimoto, K., Tsuji, Y., Siderophores in iron metabolism: from mechanism to therapy potential. *Trends Mol. Med.* 2016, *22*, 1077–1090.
10. Cooper, B., Bunn, H. F., Propper, R. D., Nathan, D. G., Rosenthal, D. S., Moloney, W. C., Treatment of iron overload in adults with continuous parenteral desferrioxamine. *Am. J. Med.* 1977, *63*, 958–966.
11. Li, Z., Yamamoto, H., Hydroxamic acids in asymmetric synthesis. *Acc. Chem. Res.* 2013, *46*, 506–518.
12. Ahlford, K., Adolfsson, H., Amino acid derived amides and hydroxamic acids as ligands for asymmetric transfer hydrogenation in aqueous media. *Catal. Commun.* 2011, *12*, 1118–1121.
13. Barlan, A. U., Zhang, U., Yamamoto, H., Development and application of versatile bis-hydroxamic acids for catalytic asymmetric oxidation. *Tetrahedron* 2007, *63*, 6075–6087.
14. Xiao, K., Lin, D. W., Miura, M., Zhu, R., Gong, W., Wasa, M., Yu, J., Palladium(II)-catalyzed enantioselective C(sp^3)–H activation using a chiral hydroxamic acid ligand. *J. Am. Chem. Soc.* 2014, *22*, 8138–8142.
15. Bhura, D. C., Tandon, S. G., Unsaturated N-arylhydroxamic acids as colorimetric reagents for vanadium(v): spectrophotometric determination with n-phenyl-3-styrylacrylo-hydroxamic acid. *Anal. Chim. Acta* 1971, *53*, 379–386.
16. Kumar, S. A., Pandey, S. P., Shenoy, N. S., Kumar, S. D., Matrix separation and preconcentration of rare earth elements from seawater by poly hydroxamic acid cartridge followed by determination using ICP-MS. *Desalination* 2011, *281*, 49–54.
17. Alburn, H. E., Clark, D. E., Grant, N. H., Lapidus, M., Hydroxamic acids of alicyclic amino acids. US patent: US 3997594 A, publ. date December 14, 1976.
18. Verleysen, K., Sandra, P., Enantiomeric separation of some amino acids and derivatives by capillary electrophoresis with 18-crown-6-tetracarboxylic acid as chiral selector, *J. Microcolumn. Sep.* 1999, *11*, 37–43.
19. Lippmann, T., Hartenstein, H., Sicker, D., Enantiomeric separation of close analogs to naturally occurring 1,4-benzoxazin-3-ones by liquid chromatography using *β*-cyclodextrin-modified stationary phases. *Chromatographia* 1993, *35*, 302–304.
20. Kokare, N. D., Shinde, D. B., N-[(diphenoxyphosphoryl)oxy]-2-phenyl-1H-benzimidazolas a versatile reagent for synthesis of O-alkylhydroxamic acids. *J. Heterocyclic Chem.* 2008, *45*, 981–986.
21. Rao, R. N., Maurya, P. K., Shinde, D. D., Development of a validated LC method for enantiomeric separation and determination of adrafinil and its related substances on a Chiralcel OJ-H column connected to PDA and polarimetric detectors in series. *Biomed. Chromatogr.* 2010, *24*, 1228–1233.
22. Rao, R. N., Shinde, D. D., Enantioselective separation and determination of adrafinil and modafinil on Chiralcel OJ-H column in rat serum and urine using solid-phase extraction followed by HPLC, *Biomed. Chromatogr.* 2009, *23*, 811–816.
23. Lajkó, G., Orosz, T., Grecsó, N., Fekete, B., Palkó, M., Fülöp, F., Lindner, W., Péter, A., Ilisz, I., High-performance liquid chromatographic enantioseparation of cyclic *β*-aminohydroxamic acids on zwitterionic chiral stationary phases based on Cinchona alkaloids. *Anal. Chim. Acta* 2016, *921*, 84–94.

24. Hoffmann, C. V., Pell, R., Lämmerhofer, M., Lindner, W., Elucidation of the chiral recognition mechanism of Cinchona alkaloid carbamate-type receptors for 3,5-dinitrobenzoyl amino acids. *Anal. Chem.* 2008, *80*, 8780–8789.
25. Zhang, T., Holder, E., Franco, P., Lindner, W., Zwitterionic chiral stationary phases based on Cinchona and chiral sulfonic acids for the direct stereoselective separation of amino acids and other amphoteric compounds. *J. Sep. Sci.* 2014, *37*, 1237–1247.
26. Wernisch, S., Pell, R., Lindner, W., Increments to chiral recognition facilitating enantiomer separations of chiral acids, bases, and ampholytes using Cinchona-based zwitterion exchanger chiral stationary phases. *J. Sep. Sci.* 2012, *35*, 1560–1572.
27. Lajkó, G., Orosz, T., Ugrai, I., Szakonyi, Z., Fülöp, F., Lindner, W., Péter, A., Ilisz, I., Liquid chromatographic enantioseparation of limonene-based carbocyclic β-amino acids on zwitterionic Cinchona alkaloid-based chiral stationary phases. *J. Sep. Sci.* 2017, *40*, 3196–3204.
28. Ilisz, I., Péter, A., Lindner, W., State-of-the-art enantioseparations of natural and unnatural amino acids by high-performance liquid chromatography. *Trends Anal. Chem.* 2016, *81*, 11–21.
29. Schurig, V., Ossig, J., Link, R., Evidence for a temperature dependent reversal of enantioselectivity in complexation gas chromatography on chiral phases. *Angew. Chem.* 1989, *101*, 194–196.
30. Allenmark, S., Schurig, V., Chromatography on chiral stationary phases. *J. Mater. Chem.* 1997, *7*, 1955–1963.
31. Gotmar, G., Fornstedt, T., Guiochon, G., Retention mechanism of *ß*-blockers on an immobilized cellulose. Relative importance of the hydrophobic and ionic contributions to their enantioselective and nonselective interactions. *Anal. Chem.* 2000, *72*, 3908–3915.
32. Fornstedt, T., Sajonz, P., Guichon, G., A closer study of chiral retention mechanisms. *Chirality* 1998, *10*, 375–381.
33. Chester, T. L., Coym, J. W., Effect of phase ration on van't Hoff analysis in reversed-phase liquid chromatography, and phase-ratio-independent estimation of transfer enthalpy. *J. Chromatogr. A* 2003, *1003*, 101–111.
34. Gotmar, G., Fornstedt, T., Guiochon, G., Apparent and true enantioselectivity in enantioseparations. *Chirality* 2000, *12*, 558–564.
35. Grecsó, N., Forró, E., Fülöp, F., Péter, A., Ilisz, I., Lindner, W., 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* 2016, *1467*, 178–187.
36. Forró, E., Fülöp, F., The first direct enzymatic hydrolysis of alicyclic beta-amino esters: a route to enantiopure *cis* and *trans* beta-amino acids. *Chem. Eur. J.* 2007, *13*, 6397–6401.
37. Fülöp, F., Palkó, M., Forró, E., Dervarics, M., Martinek, T. A., Sillanpää, R., Facile regio- and diastereoselective syntheses of hydroxylated 2-aminocyclohexanecarboxylic acids. *Eur. J. Org. Chem.* 2005, *15*, 3214–3220.
38. Kiss, L., Forró, E., Fülöp, F., A new strategy for the regio- and stereoselective hydroxylation of trans-2-aminocyclohexenecarboxylic acid. *Tetrahedron. Lett.* 2006, *17*, 2855–2858.
39. Xu, D., Prasad, K., Repic, O., Blacklock, T. J., A practical synthesis of enantiopure ethyl cis-2-amino-1-cyclohexanecarboxylate via asymmetric reductive amination methodology. *Tetrahedron Asymmetry* 1997, *8*, 1445–1451.
40. Cimarelli, C., Palmieri, G., Stereoselective reduction of enantiopure β-enaminoesters by hydride: a convenient synthesis of both enantiopure β-aminoesters. *J. Org. Chem.* 1996, *61*, 5557–5563.
41. Kopaciewicz, W., Rounds, M. A., Fausnaugh, F., Regnier, F. E., Retention model for high-performance ion-exchange chromatography. *J. Chromatogr. A* 1983, *266*, 3–21.
42. Monzyk, B., Crumbliss, A. L., Acid dissociation constants (K_a) and their temperature dependencies (ΔH_a, ΔS_a) for a series of carbon- and nitrogen-substituted hydroxamic acids in aqueous solutions. *J. Org. Chem.* 1980, *45*, 4670–4675.

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