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Polysaccharide-based chiral stationary phases as efficient tools for diastereo- and enantioseparation of natural and synthetic *Cinchona* alkaloid analogs

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ABSTRACT

In this study, we present results obtained on the diastereo- and enantioseparation of some basic natural and synthetic *Cinchona* alkaloid analogs by applying liquid chromatographic (LC) and subcritical fluid chromatographic (SFC) modalities on amylose and cellulose *tris*-(phenylcarbamate)-based stationary phases using *n*-hexane/alcohol/DEA or CO_2/alcohol/DEA mobile phase systems. Seven chiral stationary phases in their immobilized form were employed to explore their stereoselectivity for a series of closely related group of analytes. The most important characteristics of LC and SFC systems were evaluated through the variation of the applied chromatographic conditions (*e.g.*, the nature and content of the alcohol modifier, the concentration of additives, temperature). The columns Chiralpak **IC** and **IG** turned out to be the best in both LC and SFC modalities. Temperature-dependence study indicated enthalpy-controlled separation in most cases; however, separation controlled by entropy was also registered.

1. Introduction

Both natural and synthetic *Cinchona* alkaloids have a rather complex structural pattern with more than thirty representatives, among these quinine (QN), quinidine (QD), cinchonidine (CD), and cinchonine (CN) represent the main components [1]. In addition to the pharmaceutical relevance of the main alkaloids and derivatives thereof, they may also serve as catalysts in stereo-directed organic synthesis [2], and as chiral selectors in the course of the development of chiral stationary phases (CSPs) for high-performance liquid chromatography (HPLC) [3]. Besides, quinine has a long tradition to be applied as a flavor component in bitter beverages. In Table 1 the chemical structures of natural and synthetic *Cinchona* alkaloids applied in this study are depicted. With the exception of racemic quinine, the other analyte pairs QN/QD, DHQN/DHQD, CD/CN, and epi-QN/epi-QD are diastereomers to each other, although often termed pseudo-enantiomers (Table 1). QN and QD as well as CD and CN are diastereoisomeric to each other which, in principle, eases their separation. Analytically, HPLC methods are often applied for this purpose using preferentially non-chiral stationary phases, as summarized earlier by McCalley [4]. Recently, capillary electrophoretic [5] and HPLC-based [1,6] methods have been described for the separation of the four major *Cinchona* alkaloids QN, QD, CD, and CN as well as dihydroquinine (DHQN) and dihydroquinidine (DHQD). Along this line, the quantitative determination of six major alkaloids implementing supercritical fluid chromatography (SFC) has recently also been reported [7].

In principle, it is not necessary to use CSPs to resolve the given four pairs of diastereoisomers (see Table 1). Nevertheless, it is still of interest to examine their stereoselective molecular recognition pattern in the context of the resolution of these analytes. However, for the resolution of enantiomeric analytes, the application of a CSP is mandatory. As we have had access to racemic quinine (rac. QN) produced through a novel, fully synthetic way [8], we had the opportunity to investigate the chromatographic resolution of rac. QN *versus* the diastereomeric pair QN/QD and of their epimers (9-epi-QN, 9-epi-QD). Therefore, the impact of the chiral environment of the diverse polysaccharide-type CSPs on the overall stereoselectivity parameters could be explored.

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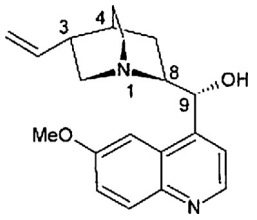
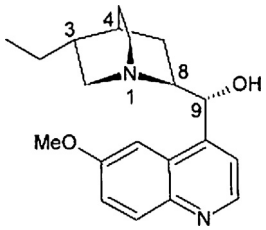
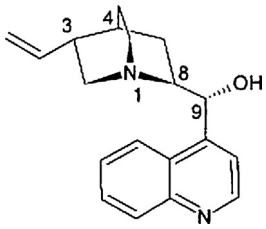
Table 1
Structure of natural and synthetic *Cinchona* alkaloids.

Name	Abbreviation	Configuration	Structure
(+) Quinine	(+) QN	(+) (1*R*,3*S*,4*R*,8*R*,9*S*)	
(-) Quinine	(-) QN	(-) (1*S*,3*R*,4*S*,8*S*,9*R*)	
(+) Quinidine	(+) QD	(+) (1*S*,3*R*,4*S*,8*R*,9*S*)	
(-) 9-epi-Quinine	(-) 9-epi-QN	(-) (1*S*,3*R*,4*S*,8*S*,9*S*)	
(+) 9-epi-Quinidine	(+) 9-epi-QD	(+) (1*S*,3*R*,4*S*,8*R*,9*R*)	
(-) Dihydroquinine	(-) DHQN	(-) (1*S*,3*R*,4*S*,8*S*,9*R*)	
(+) Dihydroquinidine	(+) DHQD	(+) (1*S*,3*R*,4*S*,8*R*,9*S*)	
(-) Cinchonidine	(-) CD	(-) (1*S*,3*R*,4*S*,8*S*,9*R*)	
(+) Cinchonine	(+) CD	(+) (1*S*,3*R*,4*S*,8*R*,9*S*)	

In an earlier paper Hoffmann et al. [9] studied the stereoselective resolution of QN/QD and CD/CN on a chiral cation exchanger followed by another recent investigation by Bajtai et al. [10] applying chiral zwitterionic CSPs. In terms of intermolecularly driven interaction mechanism between the chiral selector (SO) motifs of the CSPs and the chiral analytes (selectands, SAs), the ion exchanger-type CSPs investigated previously and the polysaccharide-type CSPs examined in the present study (see Fig. S1) belong to structurally entirely different CSPs with respect to retention and stereoselectivity characteristics. In this study, we aimed to trace the specifics of the polysaccharide CSPs used in normal-phase liquid chromatography (NP—LC) and in SFC modalities for the separation of a closely related group of *Cinchona* alkaloids (see Table 1).

Polysaccharide-based PS-CSPs usually display a great variety of enantio-, diastereo-, and chemoselectivity [11–17]. A variety of robust CSPs with very wide spectra of applications were obtained *via* the immobilization of substituted amylose- and cellulose-based selectors [14,12–17]. However, no data were found in the literature for the separation of some natural and synthetic *Cinchona* alkaloids and their derivatives on PS-CSPs. The main objective of the present paper is to reveal some general tendencies of a set of prominent PS-CSPs (Fig. S1) for the diastereo- and enantioseparation of the set of chiral analytes (Table 1) under NP-LC and SFC conditions. The investigation of stereoselectivity criteria of SOs and SAs was in the focus of this study. More specifically, of the three isobaric pairs of stereoisomers, QN/QD and 9-epi-QN/9-epi-QD are diastereomeric to each other, characterized by the change of only the stereogenic centers of C-8 and C-9, while the 1*S*, 3*R*, and 4*S* stereogenic centers of the quinuclidine ring residue remain constant. For the truly racemic QN, all five stereogenic centers are opposite to each other. For the two diastereomeric DHQN/DHQD and CD/CN pairs, the diastereomeric behavior of the analytes is the same as for QN/QD. See Table 1 for more details.

Methodological and experimental factors, such as the nature and the concentration of the different modifiers (alcohol, water, acid or base) and mobile phase compositions in NP-LC and SFC, as well as the structure of PS-CSPs and SAs and the temperature were evaluated on retention, selectivity, and resolution of stereoisomers with special attention with respect to elution sequences.

2. Materials and methods

2.1. Chemicals and reagents

(–)QN, (+)QD, and (–)-1011-(–)DHQN were purchased from Buchler (Braunschweig, Germany). (+)-1011-DHQD, (+)-CN, and (–)-CD were from Sigma-Aldrich (Vienna, Austria). Racemic QN [1:1 mixture of (–)QN and (+)QN] was a generous gift from N. Maulide synthesized as described in [8]. C9-Epiquinine (–)*epi*-QN and C9-epiquinidine (+)*epi*-QD were synthesized as described in [18] (Table 1).

Methanol (MeOH), ethanol (EtOH), and *n*-hexane of HPLC grade were purchased from VWR International (Radnor, PA, USA). The alcohol additives 1-propanol (1-PrOH), 2-propanol (2-PrOH), the base additive diethylamine (DEA), and the acid additive acetic acid (AcOH) all analytical reagent grades, were from VWR. Liquid CO_2 was from Messer (Budapest, Hungary). Ultrapure water was obtained from Ultrapure Water System, Puranity TU UV/UF (VWR International).

2.2. Apparatus and chromatography

Three chromatographic systems were applied in this study. The first one was a Waters Breeze apparatus consisting of a 1525 binary pump, a 487 dual-channel absorbance detector, a 717 plus autosampler, and a column thermostat. For data collection 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 used to regulate column temperature.

The second liquid chromatographic system was from Shimadzu, and it contained a low-pressure quaternary pump (LC-20AD), a

photodiode array detector (SPD-M20A), a Model 7125 injector with a 20-μl loop (Rheodyne, Cotati, CA, USA), and LC Solution data acquisition system (Shimadzu Corporation, Tokyo Japan). All experiments in normal-phase mode (NP) were carried out under isocratic conditions at a flow rate of 0.6 mL min^{-1} and at a column temperature of 25 °C (if not otherwise stated).

The third device, a Waters Acquity Ultra Performance Convergence Chromatography™ (UPC², Waters Corporation) system was applied for SFC studies with a binary solvent pump, an autosampler, a backpressure regulator, a column oven, and a photodiode array detector. An Empower 2 software was used to system control and data acquisition. In every case SFC was performed in isocratic mode at a flow rate of 2.0 mL min^{-1} and a column temperature of 40 °C (if not otherwise stated). The outlet pressure was set at 150 bar. The mobile phases applied in SFC consisted of liquid CO_2 and MeOH, EtOH, 1-PrOH or 2-PrOH in different ratios (*v/v*) containing different acid and base additives.

Stock solutions of the analytes were prepared by dissolving the solid *Cinchona* samples in MeOH or 2-PrOH in 1.0 mg mL^{-1} concentration and further diluted when necessary. An injection volume of 20 μL was applied in LC and 7 μL in SFC. In LC the dead-time of columns (t_0) was determined by injection of tri-*t*-butylbenzene, while in SFC mode the first negative signal by injecting MeOH was used. Analytes were detected by their UV absorption at 215–230 nm.

Polysaccharide-based columns amylose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak **IA**), amylose *tris*-(3-chlorophenylcarbamate) (Chiralpak **ID**), amylose *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak **IE**), amylose *tris*-(3-chloro-4-methylphenylcarbamate) (Chiralpak **IF**), and amylose *tris*-(3-chloro-5-methylphenylcarbamate) (Chiralpak **IG**) as well as cellulose *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak **IB)** and cellulose *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak **IC**) all with the same size (250 mm × 4.6 mm I.D., 5-μm particle size) were generous gifts from Chiral Technologies Europe (Illkirch, France). All CSPs employed in this study belong to the immobilized PS-type columns. The structures of selectors are presented in Fig. S1.

3. Results and discussion

3.1. Effects of mobile phase composition in NP-LC and in SFC

In the case of PS-CSPs, the generally accepted recognition mechanism is based on the inclusion of chiral solutes into the chiral cavities of the polysaccharide-type selector driven by additional attractive forces such as H-bonding, dipole–dipole and π–π interactions. In addition, the role of steric "hindrance" for the given SAs to enter deep into the chiral groves should also be considered [11–17]. To regulate the overall chromatographic retention, the nature and concentration of an alcohol modifier in both normal phase LC (NP—LC) [19] and SFC [20] are often varied. To explore the possible effects of the alcohol modifier in NP—LC and SFC, two columns were selected: the cellulose-based Chiralpak **IC** column (as the most effective CSP in the screening process for the investigated conformationally restricted analytes) and the amylose-based Chiralpak **IE** column, both possessing the same carbamate modification (*tris*-3,5-dichlorophenylcarbamate moiety) of the two different polysaccharide backbones.

With variation of the nature of the alcohol for the studied analytes on the 7 CSPs in NP-LC modality, a relatively similar but slight increase in k_1 was registered in the EtOH<1-PrOH<2-PrOH sequence with the exception of *epi*-QN/*epi*-QD, which was retained more significantly (Fig. S2). The best stereoselectivity performances (higher α and R_S) could generally be achieved with 2-PrOH on Chiralpak **IC**. In SFC modality on the same columns, slightly higher k_1 values were obtained on mobile phases containing MeOH and 2-PrOH compared to EtOH (especially for *epi*-QN/*epi*-QD). For α and R_S the variations observed were quite similar to those in NP-LC; namely, Chiralpak **IE** exhibited much less effectiveness than Chiralpak **IC**. As expected, under NP-LC and SFC conditions an increase of the apolar character of the alcohol, *i.e.*, applying alcohols with a longer or branched chain usually resulted in an enhanced analyte retention, selectivity, and resolution, especially on Chiralpak **IC** (Fig. S2). It should be mentioned, that opposite behaviors had also been reported in the literature [21,22]. The change in enantio- and diastereoselectivity resulting from changing the alcohol modifier was previously rationalized as a result of alteration of the steric environment of the chiral cavities by different alcohol modifiers due to intra- and inter-molecular solvation effects [22]. On the basis of these results, further experiments were carried out with the application of EtOH and 2-PrOH in NP-LC as well as MeOH and 2-PrOH in SFC conditions.

For a more thorough study of the effects of alcohol concentration on chromatographic parameters in NP—LC, *n*-hexane/EtOH/DEA and *n*-hexane/2-PrOH/DEA (60/40/0.1–95/5/0.1 *v/v/v*) mobile phases were applied. Under SFC conditions, the MeOH and 2-PrOH content in liquid CO_2 was varied from 10 to 60 v% (with 20 mM DEA in all experiments). The amylose- and cellulose-based columns applied earlier with the same selector (Chiralpak **IC** and Chiralpak **IE**, see Fig. S1) were selected for this study. Regarding the retentive characteristics, a typical NP behavior was observed for both NP—LC and SFC modalities: increasing the ratio of apolar *n*-hexane or CO_2 resulted in an increased k_1, especially for the *epi*-QN/*epi*-QD pair (Fig. 1). It is noteworthy, that with the increase of the mobile phase polarity, the strength of the H-bonds between the analytes and the selector decreases and the solubility of the analytes in the mobile phase increases [23].

On Chiralpak **IE** the stereoselectivity exhibited only a small enhancement in both NP-LC and SFC modalities with increasing *n*-hexane or CO_2 content. However, on Chiralpak **IC** in NP-LC in *n*-hexane/EtOH/DEA mobile phase a slight increase, whereas in *n*-hexane/2-PrOH/DEA mobile phase a moderate enhancement in the α value was registered. In SFC on Chiralpak **IC** α generally slightly decreased or did not change significantly with increasing CO_2 content. The *epi*-QN/*epi*-QD pair, again, was an exception showing a slight increase on Chiralpak **IC** in CO_2/MeOH. It should be noted, that slightly higher α values were registered in CO_2/2-PrOH than in CO_2/MeOH mobile phases.

Regarding R_S values, in both NP-LC and SFC, they increased significantly on Chiralpak **IC** and slightly on Chiralpak **IE** with increasing of *n*-hexane or CO_2 content (although an unexpected exception was found in SFC modality for DHQN/DHQD on Chiralpak **IE**, Fig. 1). It is worth mentioning that the change in the chromatographic performance caused by the alcohol modifier depended on the structure of the chiral selector as well; the cellulose-based selector for the investigated basic analytes outperformed the amylose-based one.

The alcohol may be incorporated into the polysaccharide structure, either into the cavities or between the polymer chains, affecting the tertiary structure of the chiral polymer itself *via* solvation effects [24]. Under LC conditions, the main adsorbing sites are considered to be the polar carbamate residues [25] and the different involvement of the NH and CO groups in the H-bonding process were found to be responsible for the differences observed in the stereoselective and enantioselective binding process [26]. It is important to note, that in this study 10–60 v% of alcoholic modifier was employed under SFC conditions, *i.e.*, SFC is operated under subcritical conditions, where significant deviations due to the difference of the set values and actual operational conditions cannot be expected [27]. Besides affecting the physical properties of the

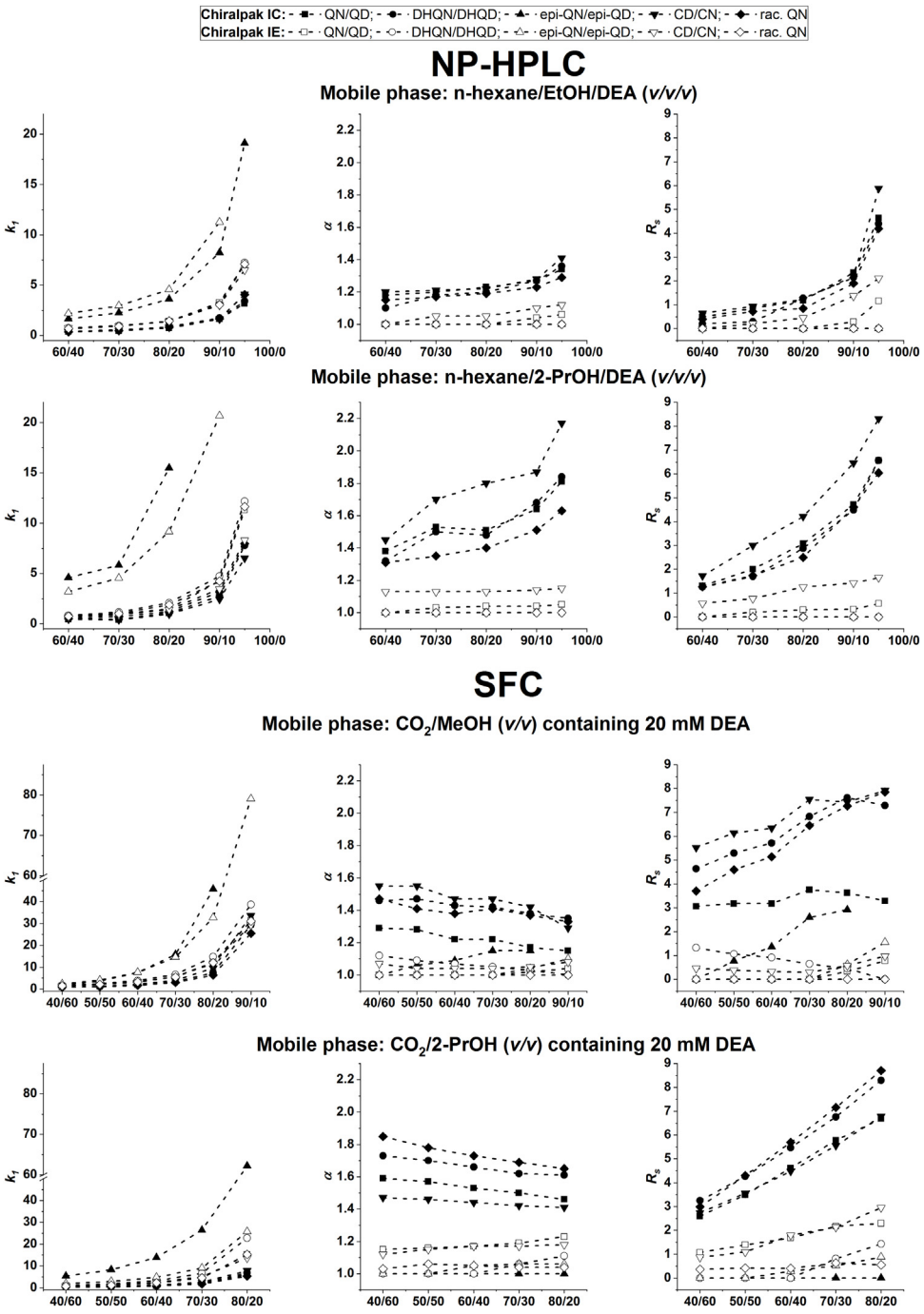


Fig. 1. Effect of mobile phase composition on chromatographic parameters, retention factor (k), separation factor (α) and resolution (R_S) for the separation of *Cinchona* alkaloid analogs on Chiralpak **IC** and **IE** columns in NP-LC and SFC modalities.
Chromatographic conditions: columns, Chiralpak **IC** and Chiralpak **IE**; mobile phase, for normal phase, *n*-hexane/EtOH/DEA and *n*-hexane/2-PrOH/DEA (60/40/0.1–95/5/0.1 *v*/*v*/*v*); for SFC, CO_2/MeOH (40/60–90/10 v/v) containing 20 mM DEA and CO_2/2-PrOH (40/60–80/20 v/v) containing 20 mM DEA; flow rate, 1.0 mL min^{-1}; detection at 220 nm; temperature, 25 °C; symbols for Chiralpak **IC,** ■, QN/QD, ●, DHQN/DHQD, ▲, *epi*-QN/*epi*-QD, ▼, CD/CN and ◆, rac. QN, for Chiralpak **IE**, –, QN/QD; ○, DHQN/DHQD; △, *epi*-QN/*epi*-QD; ▼, CD/CN and ◇, rac. QN.

eluent (*e.g.*, polarity, viscosity, and density), an alteration of the adsorption layer should also be considered under SFC conditions [27].

Besides the nature and concentration of the alcohol components, other additives (*e.g.*, acid, base, water) are also frequently applied to modify "chiral" resolution on different types of CSPs. In SFC, beyond improvement in peak shape, additives may also have an impact on retention [28] and on the number of theoretical plates [29]. Fig. 2 depicts the effects of AcOH (**A**) and H_2O (**B**) additives on the chromatographic performance of Chiralpak **IC** with eluent systems CO_2/MeOH (60/40 v/v) containing 20 mM DEA, and 0.0–40 mM AcOH (**A**) or 0.0–5.0 v% H_2O (**B**) applying the QD/QN pair of diastereomers as test compounds. Upon increasing the concentration of the additive retention decreased in case of AcOH and slightly increased in case of water in parallel with a minor decrease of α and a marked reduction in R_S values (Fig. 2), while the change in the efficiency was usually in the range of 10–15% (data not shown). The slight change caused by the addition of water was probably

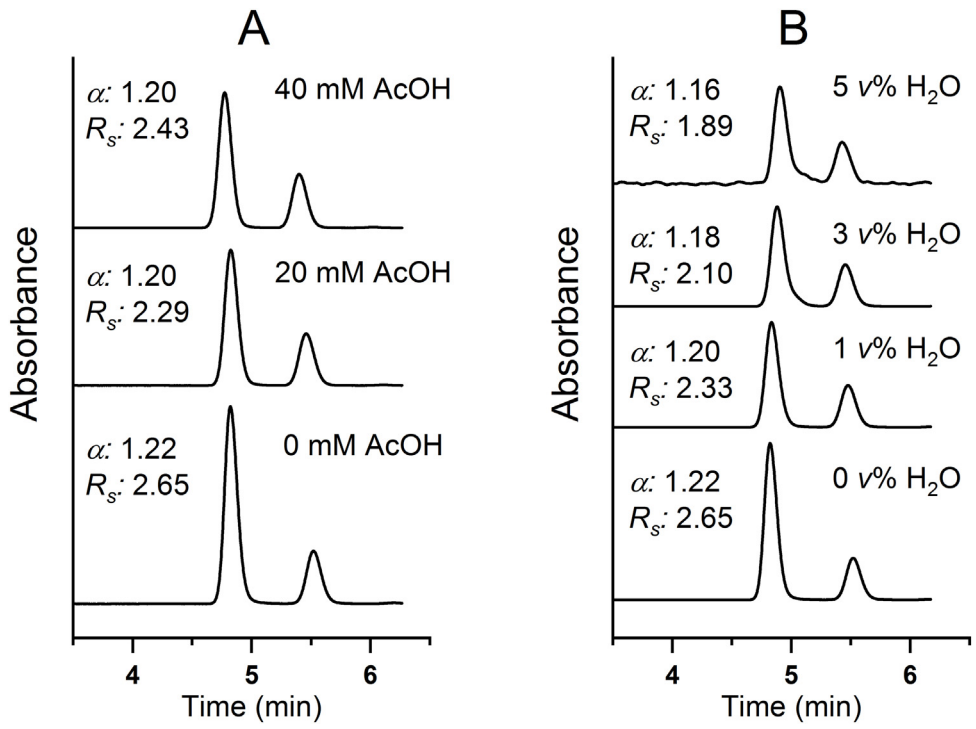


Fig. 2. Effect of AcOH and H_2O content on the chromatographic performance of Chiralpak **IC** for analyte QD/QN under SFC conditions. Chromatographic conditions: mobile phase, CO_2/MeOH (60/40 *v/v*) containing 20 mM DEA and **A,** 0 mM, 20 mM and 40 mM AcOH; **B**, 0. 0 v%, 1.0 v%, 3.0 v% and 5.0 v% H_2O; flow rate, 1.0 mL min^{-1}; detection at 220 nm; temperature, 25 °C.

due to the less polar character of the CSP studied as observed by Armstrong et. al [29].

3.2. Effect of the structure of polysaccharide-type selectors

The stereoselectivity characteristics of the studied *Cinchona* alkaloids on the seven PS-CSPs were studied with different mobile phase systems, and the corresponding data are summarized in Tables 2–5. Due to the difference in linkage of glycopyranose moieties in amylose- and cellulose-based selectors, the interactions between analyte and selector may change resulting in different chromatographic behaviors, which may even affect the elution order of the resolved diastereomers and enantiomers. The contribution of the polysaccharide backbone can be evaluated by the comparison of the chromatographic data obtained on the same carbamate residue; *i.e.*, *tris*-(3,5-dimethylphenylcarbamate) (Chiralpak **IA** *vs.* Chiralpak **IB**) and *tris*-(3,5-dichlorophenylcarbamate) (Chiralpak **IE** *vs.* Chiralpak **IC**), linked to amylose or cellulose, respectively. To facilitate the comparison between different columns, chromatographic conditions were kept constant, applying the *n*-hexane/EtOH/DEA and the *n*-hexane/2-PrOH/DEA (80/20/0.1 v/v/v) mobile phase compositions in NP-LC. In a few cases when a partial resolution occurred, the separation was further optimized. Data summarized in Table 2 show that higher retention could be obtained on amylose- than on cellulose-based CSPs in most cases. Exceptions, however, do exist especially for mobile phases containing 2-PrOH.

It should be noted, that in NP-LC, despite shorter retention times, Chiralpak **IC** proved to be the most effective CSP in the separation of the diastereomeric pairs of both *Cinchona* alkaloids and of rac. QN. In SFC modality the effect of the polysaccharide backbone was also evaluated on the same pairs of CSPs applying CO_2/MeOH and CO_2/2-PrOH (60/40 *v/v*) mobile phases all containing 20 mM DEA (Table 3). Regarding retention, the results with Chiralpak **IE** and **IC** were similar to the data obtained in NP-LC; *i.e.*, the amylose backbone offered higher retentions. Chiralpak **IA** and **IB**, in turn, gave opposite results, with the cellulose-based CSP providing higher retentions. Among the above-mentioned four columns under SFC conditions, the best separations were achieved with Chiralpak **IC**, similar to those found in NP-LC.

The fundamental structural differences between amylose- and cellulose-based *tris*-(3,5-dimethylphenylcarbamate) or *tris*-(3,5-dichlorophenylcarbamate) were found to be reflected in the stereochemical recognition patterns even for some diastereomeric analytes. Reversal of elution order between amylose- and cellulose-based CSPs containing the same substituents was registered in NP-LC containing EtOH as mobile phase for analyte CN/CD on Chiralpak **IA** *vs.* **IB**, and in mobile phases containing EtOH and 2-PrOH on Chiralpak **IE** *vs.* **IC** (Table 4). Similar behaviors were registered in SFC modality for QN/QD and CN/CD on Chiralpak **IE** *vs.* **IC** (Table 2B). Examples of reversed elution orders of enantiomeric analytes on amylose- or cellulose-based columns have been described previously [19].

Applying constant chromatographic conditions in NP-LC and SFC modality, data listed in Table 2 and 3 can provide opportunity to evaluate the effect of the nature of the substituted phenylcarbamate moiety. By comparing the data obtained with Chiralpak **IA** *vs.* **IE** (both are amylose-based CSPs), and **IB** *vs.* **IC** (both are cellulose-based CSPs), higher retentions can clearly be identified for all analytes on CSPs with *tris*-(3,5-dichlorophenylcarbamate) moiety. The higher α and R_S values, observed generally in the case of CSPs with *tris*-(3,5-dichlorophenylcarbamate), suggest more pronounced SO–SA interactions of the studied analytes. This may be attributed to a π–π-type interaction increment of the acidic phenylcarbamate and the π-basic-type quinoline moiety of *Cinchona* alkaloids. However, in a few cases, in particular for *epi*-QN/*epi*-QD, lower α and R_S were registered on Chiralpak **IE** and Chiralpak **IC** than on Chiralpak **IA** and Chiralpak **IB**. Nevertheless, results showed that the *tris*-(3,5-dichlorophenylcarbamate) CSPs, in most cases, outperform other SO types.

Table 2
Effect of EtOH and 2-PrOH content in *n*-hexane as bulk solvent for the chromatographic data, k_1, α, R_S of *Cinchona* alkaloids in normal phase modality.

Column	Mobile phase	k_1, α, R_S	QN/QD	rac. QN	DHQN/DHQD	*epi*-QN/*epi*-QD	CD/CN
IA	**a**	k_1	0.59	0.59	0.60	2.47	0.76
		α	1.18	1.00	1.00	1.00	1.45
		R_S	1.02	0.00	0.00	0.00	2.47
	b	k_1	0.60	0.64	0.62	2.45	0.77
		α	1.19	1.00	1.00	1.00	1.00
		R_S	0.70	0.00	0.00	0.00	0.00
IB	**a**	k_1	0.51	0.52	0.45	1.15	0.56
		α	1.00	1.00	1.00	1.28	1.22
		R_S	0.00	0.00	0.00	1.12	0.47
	b	k_1	1.02	1.01	0.99	2.72	0.99
		α	1.00	1.00	1.00	1.46	1.00
		R_S	0.00	0.00	0.00	1.26	0.00
IE	**a**	k_1	7.12**	1.45	1.40	4.57	3.00*
		α	1.06**	1.00	1.00	1.00	1.10*
		R_S	1.16**	0.00	0.00	0.00	1.40*
	b	k_1	1.48	1.89	2.10	9.15	1.42
		α	1.00	1.00	1.00	1.00	1.13
		R_S	0.85	0.00	0.00	0.00	1.25
IC	**a**	k_1	0.73	0.82	0.86	3.61	0.83
		α	1.23	1.19	1.20	1.00	1.22
		R_S	1.18	0.84	1.27	0.00	1.22
	b	k_1	1.05	1.02	1.09	15.48	0.97
		α	1.52	1.56	1.48	1.00	1.80
		R_S	3.07	2.67	2.88	0.00	4.20
ID	**a**	k_1	0.68	0.68	0.69	2.68	0.76
		α	1.21	1.08	1.16	1.14	1.39
		R_S	1.49	0.22	0.94	1.62	2.55
	b	k_1	0.80	0.97	1.08	5.37	0.99
		α	1.13	1.00	1.00	1.14	1.00
		R_S	0.18	0.00	0.00	1.45	0.00
IF	**a**	k_1	0.99	0.99	0.96	4.17	1.15
		α	1.00	1.00	1.00	1.00	1.18
		R_S	0.00	0.00	0.00	0.00	1.22
	b	k_1	1.37	1.41	1.53	5.73	1.18
		α	1.00	1.00	1.00	1.03	1.24
		R_S	0.00	0.00	0.00	0.27	1.00
IG	**a**	k_1	0.98	0.98	0.98	5.02	1.36
		α	1.27	1.00	1.10	1.08	1.22
		R_S	2.27	0.00	0.22	1.25	2.14
	b	k_1	1.06	1.07	1.08	6.64	1.16
		α	1.10	1.00	1.18	1.09	1.18
		R_S	0.46	0.00	0.48	1.28	1.64

Chromatographic conditions: columns, Chiralpak **IA-IG**; mobile phase, **a**, *n*-hexane/EtOH/DEA (80/20/0.1 v/v/v), * *n*-hexane/EtOH/DEA (90/10/0.1 v/v/v) and ** *n*-hexane/EtOH/DEA (95/5/0.1 v/v/v), **b**, *n*-hexane/2-PrOH/DEA (80/20/0.1 v/v/v); flow rate, 1.0 mL min^{-1}; detection, 230–250 nm; temperature, 25 °C.

As mentioned earlier, the structure of SO may affect the sequence of elution too. In this study, a reversal of elution sequence was registered in NP-LC modality in EtOH-containing mobile phases for analytes QN/QD using Chiralpak **IA** *vs.* **IE** (Table 4). The reversal of elution sequence by changing the chemical structure of substituents on the *tris*-(phenylcarbamate) moiety was already indicated in earlier publications [19,30,31].

The effect of the position of the substituents of the carbamate moiety of the PS-CSPs on the chromatographic performance for the given analytes was investigated by comparison of chromatographic data obtained on Chiralpak **IF**, **IG**, and **ID** columns in both NP-LC and SFC modalities (see Fig. 1S). In general, higher retention and better α and R_S values were obtained on Chiralpak **IG** than on **IF**. Chiralpak **ID** seems to be as efficient as Chiralpak **IG** in NP-LC modality in the presence of EtOH as bulk solvent component, and less efficient in all other cases. The secondary structure of the *tris*-(3-chloro-5-methylphenylcarbamate)-based CSP (Chiralpak **IG**) offers stronger retentive interactions with the analytes. Regarding elution sequences of QN/QD and rac. QN, no change was registered by altering these CSPs. In contrast, for *epi*-QN/*epi*-QD and CD/CN a reversal of elution sequence was registered in NP-LC in the presence of 2-PrOH as eluent constituent. The strong dependence of the sequence of elution as a function of the mobile phase (eluent composition and SO structure), observed in all cases, draws attention to the importance of identification of each peak in the case of PS-CSPs. For chiral ion-exchangers investigated previously, this scattered tendency was not seen [10].

In the enantio- and diastereoseparation of the investigated *Cinchona* alkaloids in SFC modality, Chiralpak **IC** and **IG** columns performed significantly better. However, in NP-LC, the 3-chloro-substitution in Chiralpak **IC** and **ID** promotes an increased stereodiscrimination but with lower effectiveness than in SFC modality.

The enantio- and diastereoselectivities of the seven chiral selectors for the five pairs of *Cinchona* alkaloid stereoisomers are summarized in Tables 4 and 5. For the separation of enantiomers, consistencies have been noticed numerous times with an elution sequence (+)QN < (−)QN, but a reversed elution sequence was registered on Chiralpak **ID** and **IF** in SFC modality applying 2-PrOH as alcohol modifier.

The situation becomes even more complicated for the resolution of the diastereomeric pairs QN/QD, DHQN/DHQD, CD/CN, and *epi*-QN/*epi*-QD, where a clear trend cannot be seen. In both NP-LC and SFC modalities on the seven columns, the elution sequence QN < QD, DHQN < DHQD, and CD < CN and its reversal can also be observed, as a clear indication of the difficulty to interpret enantioselectivity *vs* diastereoselectivity (Tables 2 and 3). Unexpected reversals of the elution order of the diastereomeric (often

Table 3
Comparison of the effect of MeOH and 2-PrOH content in CO_2 as bulk solvent for the chromatographic data, k_1, α, R_S of *Cinchona* alkaloids in SFC modality.

Column	Mobile phase	k_1, α, R_S	QN/QD	rac. QN	DHQN/ DHQD	*epi*-QN/ *epi*-QD	CD/CN
IA	a	k_1	0.38	0.33	0.37	0.83	0.39
		α	1.21	1.38	1.46	1.11	1.20
		R_S	1.16	1.91	2.43	0.74	1.03
	b	k_1	0.30	0.29	0.33	1.16	0.42
		α	1.41	1.00	1.23	1.00	1.26
		R_S	1.43	0.00	0.79	0.00	0.65
IB	a	k_1	0.41	0.41	0.40	1.47	0.50
		α	1.00	1.00	1.00	1.00	1.07
		R_S	0.00	0.00	0.00	0.00	0.21
	b	k_1	0.52	0.52	0.54	1.55	0.69
		α	1.00	1.00	1.00	1.18	1.00
		R_S	0.00	0.00	0.00	0.53	0.00
IE	a	k_1	3.20	3.20	3.81	7.57	3.21
		α	1.00	1.00	1.07	1.00	1.04
		R_S	0.00	0.00	0.91	0.00	0.32
	b	k_1	2.28	2.27	3.25	4.83	2.42
		α	1.17	1.05	1.00	1.05	1.17
		R_S	1.68	0.40	0.00	0.28	1.79
IC	a	k_1	1.87	1.66	1.89	7.52	2.19
		α	1.22	1.38	1.43	1.09	1.47
		R_S	3.18	5.14	5.71	1.36	6.34
	b	k_1	0.90	0.80	0.97	13.86	1.23
		α	1.53	1.73	1.66	1.00	1.44
		R_S	4.62	5.69	5.47	0.00	4.48
ID	a	k_1	0.95	0.91	1.14	1.74	0.89
		α	1.08	1.00	1.00	1.00	1.08
		R_S	0.91	0.00	0.00	0.00	0.73
	b	k_1	0.71	0.71	1.01	2.63	0.88
		α	1.31	1.08	1.08	1.00	1.00
		R_S	2.89	0.00	0.83	0.00	0.00
IF	a	k_1	1.14	1.08	1.27	2.95	1.12
		α	1.00	1.00	1.00	1.00	1.08
		R_S	0.63	0.00	0.00	0.00	0.76
	b	k_1	0.70	0.72	0.85	2.62	0.91
		α	1.34	1.13	1.28	1.00	1.13
		R_S	1.89	0.68	1.56	0.00	0.74
IG	a	k_1	1.26	1.03	1.45	2.08	0.99
		α	1.12	1.38	1.45	1.33	1.46
		R_S	1.32	3.84	4.68	3.81	4.37
	b	k_1	0.71	0.72	1.18	2.92	0.87
		α	1.87	1.00	1.49	1.18	1.47
		R_S	5.98	0.00	4.20	2.49	3.82

Chromatographic conditions: columns, Chiralpak **IA-IG**; mobile phase, **a**, CO_2/MeOH (60/40 *v/v*) containing 20 mM DEA, **b**, CO_2/2-PrOH (60/40) containing 20 mM DEA; flow rate, 2.0 mL min^{-1}; detection, 215−230 nm; temperature, 40 °C; back pressure, 150 bar.

termed pseudo-enantiomeric) pairs can easily happen as a function of the composition of the mobile phase. In this context, it became particularly interesting that on Chiralpak **IG** applying 2-PrOH as eluent component under LC conditions the elution orders (+)DHQD < (−)DHQN and CN < CD changed in SFC modality to (−)DHQN < (+)DHQD and CD < CN (Table 4 and 5). This is another strong indication about the role of solvation of the selector and selectand moieties on the overall diastereoselectivity.

Representative chromatograms for the resolution of racemic quinine and four diastereomers of *Cinchona* alkaloids in SFC mode are depicted in Fig. 3 and Fig. S3 on Chiralpak **IC** and **IG** applying 2-PrOH and MeOH as alcohol modifiers. Full separation and identifications of five pairs of enantiomers and diastereomers can only be achieved in two chromatographic runs.

3.3. Effect of the structure of analyte

Analytes, except DHQN/DHQD, possess an unsaturated side chain (vinyl *versus* ethyl group) on the quinuclidine moiety, which may influence interactions between SA and CSP, despite small differences in size and polarity. Surveying the data in Tables 2 and 3 revealed, that, in general, at a given mobile phase composition retentions in NP—LC or SFC modalities differ only very slightly. In some cases, however, the difference is more significant. As a result,

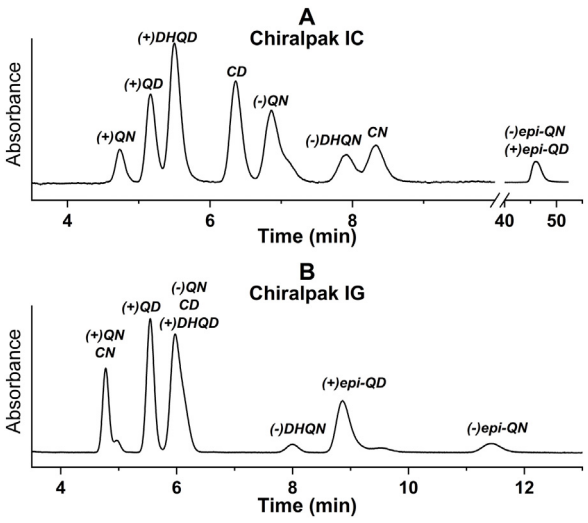


Fig. 3. Chromatograms for separation of diastereomers and enantiomers of natural and synthetic *Cinchona* alkaloid analogs.
Chromatographic conditions: columns, **A**, Chiralpak **IC**; mobile phase, CO_2/2-PrOH (70/30 v/v) containing 20 mM DEA, **B**, Chiralpak **IG**; mobile phase, CO_2/MeOH (70/30 v/v) containing 20 mM DEA; flow rate, 1.0 mL min^{-1}; detection at 220 nm; temperature, 25 °C.

Table 4
Elution sequences of *Cinchona* alkaloids in *n*-hexane as bulk solvent containing EtOH or 2-PrOH in normal phase modality.

Mobile phase: *n*-hexane/EtOH (80/20 *v*/*v*) containing 0.1% DEA							
Column	IA	IB	IE	IC	ID	IF	IG
QN/QD	*(-)QN < (+)QD*	–	*(+)QD < (-)QN*	*(+)QD < (-)QN*	*(-)QN < (+)QD*	–	*(-)QN < (+)QD*
rac. QN	–	–	–	*(+)QN < (-)QN*	*(+)QN < (-)QN*	–	–
DHQN/ DHQD	–	–	–	*(+)DHQD < (-)DHQN*	*(-)DHQN < (+)DHQD*	–	*(-)DHQN < (+)DHQD*
***epi*-QN/ *epi*-QD**	–	*(+)epi-QD < (**-**)epi-QN*	–	–	*(**-**)epi-QN < (+)epi-QD*	–	*(**-**)epi-QN < (+)epi-QD*
CD/CN	*CD < CN*	*CN < CD*	*CD < CN*	*CN < CD*	*CD < CN*	*CD < CN*	*CD < CN*
Mobile phase: *n*-hexane/2-PrOH (80/20 *v*/*v*) containing 0.1% DEA							
QN/QD	*(-)QN < (+)QD*	–	*(-)QN < (+)QD*	*(+)QD < (-)QN*	*(-)QN < (+)QD*	–	*(-)QN < (+)QD*
rac. QN	–	–	–	*(+)QN < (-)QN*	–	–	–
DHQN/ DHQD	–	–	–	*(+)DHQD < (-)DHQN*	–	–	*(+)DHQD < (-)DHQN*
***epi*-QN/ *epi*-QD**	–	*(+)epi-QD < (**-**)epi-QN*	–	–	*(**-**)epi-QN < (+)epi-QD*	*(**-**)epi-QN < (+)epi-QD*	*(+)epi-QD < (**-**)epi-QN*
CD/CN	–	–	*CD < CN*	*CN < CD*	–	*CD < CN*	*CN < CD*

Chromatographic conditions: columns, Chiralpak **IA-IG**; flow rate, 1.0 mL min^{-1}; detection, 230–250 nm; temperature, 25 °C.

Table 5
Elution sequences of *Cinchona* alkaloids in CO_2 as bulk solvent containing MeOH or 2-PrOH in SFC modality.

Mobile phase: CO_2/MeOH (90/10 v/v) containing 20 mM DEA							
Column	IA	IB	IE	IC	ID	IF	IG
QN/QD	*(+)QD < (-)QN*	–	–	*(+)QD < (-)QN*	*(-)QN < (+)QD*	–	*(+)QD < (-)QN*
rac. QN	*(+)QN < (-)QN*	–	–	*(+)QN < (-)QN*	–	–	*(+)QN < (-)QN*
DHQN/ DHQD	*(+)DHQD < (-)DHQN*	–	*(+)DHQD < (-)DHQN*	*(+)DHQD < (-)DHQN*	–	–	*(+)DHQD < (-)DHQN*
***epi*-QN/ *epi*-QD**	*(**-**)epi-QN < (+)epi-QD*	–	–	*(+)epi-QD < (**-**)epi-QN*	–	–	*(+)epi-QD < (**-**)epi-QN*
CD/CN	*CN < CD*	*CN < CD*	*CN < CD*	*CN < CD*	*CN < CD*	*CN < CD*	*CN < CD*
Mobile phase: CO_2/2-PrOH (80/20) containing 20 mM DEA							
QN/QD	*(-)QN < (+)QD*	–	*(-)QN < (+)QD*	*(+)QD < (-)QN*	*(-)QN < (+)QD*	*(-)QN < (+)QD*	*(-)QN < (+)QD*
rac. QN	–	–	–	*(+)QN < (-)QN*	*(-)QN < (+)QN*	*(-)QN < (+)QN*	–
DHQN/ DHQD	*(-)DHQN < (+)DHQD*	–	–	*(+)DHQD < (-)DHQN*	*(-)DHQN < (+)DHQD*	*(-)DHQN < (+)DHQD*	*(-)DHQN < (+)DHQD*
***epi*-QN/ *epi*-QD**	–	*(+)epi-QD < (**-**)epi-QN*	*(+)epi-QD < (**-**)epi-QN*	–	–	–	*(+)epi-QD < (**-**)epi-QN*
CD/CN	*CD < CN*	–	*CD < CN*	*CN < CD*	–	*CD < CN*	*CD < CN*

Chromatographic conditions: columns, Chiralpak **IA-IG**; flow rate, 2.0 mL min^{-1}; detection, 215–230 nm; temperature, 40 °C; back pressure, 150 bar.

the slightly less polar DHQN/DHQD analytes are more retained. This is not surprising, but the marked increase of retention of the pair of *epi*-QN/*epi*-QD diastereoisomers is striking. The increased retentions were accompanied with higher α and R_S values only in a few cases. A plausible explanation for this behavior is most probably related to the configuration of the five chiral centers and the resulting conformation of the sterically restricted analytes. Namely, for QN/QD, DHQN/DHQD, and CD/CN the chiral centers of the quinuclidine ring are identical [(1*S*), (3*R*), and (4*S*)], and only the two other chiral centers (C-8 and C-9 carbon atoms) are different [(*R*)/(*S*) for QD and (*S*)/(*R*) for QN]. There is a similar situation with respect to the absolute configuration of C-9 in 9-epi-QN [(9*S*)] and 9-epi-QD [(9*R*)]. This has a strong impact on the conformation of molecules with multiple chiral centers.

In the present case, only the overall retention but not the stereochemical differentiation of the diastereomeric analytes is affected markedly under the applied chromatographic conditions.

3.4. Effect of temperature and thermodynamic parameters

The temperature dependence of retention and enantioselectivity may provide some valuable information on the chiral recognition process [31–35]. Keeping in mind the limitations of the approach applied in this study [33,32–35], the difference in the change in standard enthalpy $\Delta(\Delta H^\circ)$ and entropy $\Delta(\Delta S^\circ)$ for the enantiomers were calculated on the basis of the van't Hoff equation:

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

where R is the universal gas constant, T is temperature in Kelvin, and α is the apparent selectivity factor.

In order to investigate the effects of temperature on the chromatographic parameters, a variable temperature study was carried out for five analytes in LC-NP and SFC modalities on four PS-CSPs (Chiralpak **IA, IB, IE**, and **IC**) in the temperature range 7.5–50 °C in mobile phases based on *n*-hexane/2-PrOH/DEA (80/20/0.1 v/v/v) and CO_2/MeOH (90/10, 80/20 and 70/30 v/v) all containing 20 mM DEA. The experimental data are summarized in Tables S1 and Table S2.

In both modalities on all four columns, the retention in most cases decreased with increasing temperature. The transfer of the analyte from the mobile phase to the stationary phase, in general, is an exothermic process. As a result, k and α as well as R_S decrease with increasing temperature. However, regarding separation factor and resolution, several exceptions were observed. On Chiralpak **IA** for CD/CN in the temperature range 30–50 °C and on Chiralpak **IC** for DHQN/DHQD and CD/CN in the temperature range 7.5–30 °C, α (and R_S) increased with increasing temperature (Table S2). Interestingly, in SFC modality for *epi*-QN/*epi*-QD on all investigated CSPs, k decreased, but α and R_S increased with increasing temperature in the studied temperature range (Table S2). Applying PS-CSPs, several examples were reported previously for the increase of selectivity with increasing temperature in chromatographic systems [14,19,33,35]. It should be noted, that several times in both modalities, R_S increased with increasing temperature independently of the change of α. This phenomenon can be attributed to the enhanced kinetic effect upon increasing temperature.

From the chromatographic data van't Hoff plots were constructed. As a general trend, ln α *vs. 1/T* curves gave clearly linear

Table 6
Thermodynamic parameters, $\Delta(\Delta H^o)$, $\Delta(\Delta S^o)$, $T x \Delta(\Delta S^o)$, $\Delta(\Delta G^o)$, correlation coefficients (R^2), T_{iso} and Q values of *Cinchona-alkaloid* analogs on Chiralpak **IA, IB, IC, IE** columns in normal-phase and SFC modalities.

Analyte		$-\Delta(\Delta H^o)$ (kJ/mol)	$-\Delta(\Delta S^o)$ (J/(mol*K)	Correlation coefficients (R^2)	$-Tx\Delta(\Delta S^o)_{298K}$ (kJ/mol)	$-\Delta(\Delta G^o)_{298K}$ (kJ/mol)	T_{iso} (^oC)	Q
NP-LC modality								
Chiralpak IA								
QN/QD		1.1	2.3	0.938	0.7	0.4	219	1.6
CD/CN		−2.3	−7.9	0.993§	−2.4	0.2	14	0.9
Chiralpak IB								
epi-QN/*epi*-QD		4.5	12.3	0.996	3.7	0.8	95	1.2
Chiralpak IE								
QN/QD		−0.4	−2.1	0.959	−0.6	0.2	−91	0.7
DHQN/DHQD		−0.7	−3.0	0.998♠	−0.9	0.2	−33	0.8
CD/CN		−0.2	−1.55	0.931	−0.5	0.3	−170	0.4
Chiralpak IC								
QN/QD		1.9	3.1	0.979	0.9	1.0	345	2.1
rac. QN		1.6	1.8	0.926	0.5	1.1	604	3.2
DHQN/DHQD		−0.5	−4.9	0.979♣	−1.5	1.0	30*	0.3
DHQN/DHQD		1.9	3.1	0.991♠	0.9	1.0		2.1
CD/CN		−1.0	−8.2	0.963♣	−2.4	1.4	29*	0.4
CD/CN		1.8	0.9	0.973♠	0.3	1.5		6.0
SFC modality								
Chiralpak IA								
QN/QD	**a**	3.4	9.0	0.990	2.7	0.7	108	1.3
rac. QN		5.4	14.1	0.990	4.2	1.2	111	1.3
DHQN/ DHQD		2.4	2.8	0.996	0.8	1.6	584	3.0
epi-QN/*epi*-QD		−1.4	−5.4	0.997	−1.6	0.2	−15	0.9
Chiralpak IB								
epi-QN/*epi*-QD	**b**	−4.2	−14.4	0.999	−4.3	0.1	20	0.9
Chiralpak IE								
DHQN/ DHQD	**c**	0.5	1.0	0.994	0.3	0.2	217	1.7
epi-QN/*epi*-QD		−1.9	−6.3	0.985	−1.9	4.4	24	0.9
Chiralpak IC								
rac. QN	**c**	1.0	0.4	0.997	0.1	0.9	>1000	10.0
DHQN/ DHQD		1.0	0.4	0.993	0.1	0.9	>1000	10.0
epi-QN/epi-QD		−5.5	−18.8	0.980	−5.6	0.1	18	0.9

Chromatographic conditions: column, **Chiralpak IA, IB, IC, IE**; mobile phase, in **NP-LC** modality, *n*-hexane/2-PrOH/DEA 80/20/0.1 (*v/v/v*), in **SFC** modality, **a**, CO_2/MeOH 80/20 (*v/v*) containing 20 mM DEA, **b**, CO_2/MeOH 90/10 (*v/v*) containing 20 mM DEA, **c**, CO_2/MeOH 70/30 (*v/v*) containing 20 mM DEA; in NP-LC modality, temperature range, §35–50 °C, ♣7.5–30 °C, ♠30–50 °C and *temperature of point of intersection (see Fig. S4); flow rate, in NP-LC, 1.0 mL min^{-1}, in SFC, 2.0 mL min^{-1}; back pressure in SFC, 150 bar; detection, 215–230 nm; T_{iso}, temperature where the enantioselectivity cancels; $Q = \Delta(\Delta H^o) / T x \Delta(\Delta S^o)_{298K}$.

van't Hoff plots, as indicated by the correlation coefficients listed in Table 6. The differences in the changes in standard enthalpy and entropy in NP-LC modality ranged between –4.5 to +2.3 kJ mol^{-1} and –12.3 to +8.1 J mol^{-1} K^{-1}, while in SFC modality between –5.4 to +5.5 kJ mol^{-1} and –14.1 to +18.8 J mol^{-1} K^{-1}. If k and α decrease with increasing temperature, negative $\Delta(\Delta H^{\circ})$ values for a pair of enantiomer or diastereomers accompanied by a negative $\Delta(\Delta S^{\circ})$ provide information on the relative ease of transfer of analytes from the mobile to the stationary phase. A negative $\Delta(\Delta S^{\circ})$ reflects an increase in the order/or loss in the degrees of freedom in the course of interaction between the stereoisomers and CSP, and the number of solvent molecules released from the chiral SO and the analyte when the molecule is associated with the CSP. If k decreased but α increased with increasing temperature, $\Delta(\Delta H^{\circ})$ and $\Delta(\Delta S^{\circ})$ were positive. In this case, the change in the adsorption enthalpy with increasing temperature has a positive effect on enantioselectivity. On the other hand, the positive $\Delta(\Delta S^{\circ})$ compensated the positive $\Delta(\Delta H^{\circ})$ and resulted in negative $\Delta(\Delta G^{\circ})$. An exceptional behavior in NP-LC on Chiralpak **IC** for DHQN/DHQD and CD/CN pairs was registered (Fig. S4); namely, in the temperature range 7.5–30 °C, α increased with increasing temperature, while above 30 °C α decreased with increasing temperature. That is, the separation is governed in the lower temperature range by entropy, while in the higher temperature range by enthalpy.

To estimate the enthalpy/entropy contribution to the free energy, Q values [$Q = \Delta(\Delta H^{\circ})/[298 \times \Delta(\Delta S^{\circ})]$ were calculated. According to the data in Table 6, the discrimination process was enthalpically or entropically driven depending on both the nature of analyte and the chiral selector. Under SFC conditions on all investigated CSPs, *epi*-QN/*epi*-QD in SFC modality exhibited entropy-controlled separation, but no other general trend could be observed.

4. Conclusions

In this comprehensive study we have investigated the performance of a set of chiral polysaccharide-based stationary phases for the separation of some closely related natural and synthetic *Cinchona* alkaloid analogs. As evidenced by chromatographic data summarized in Tables 2–4 and, several characteristic features can be extracted. Consequently, it was of interest to investigate the impact of seven different polysaccharide-type CSPs for their chromatographic resolution. Specific conclusions are as follows:

a) with respect to the effect of the nature of alcohol in NP-LC and SFC, the use of 2-PrOH and in some cases EtOH in NP-LC and MeOH in SFC were favored for this class of compounds;
b) the "fitting" of the studied analytes to the amylose- or cellulose-based polymeric chain-type selectors characterized by the shape and size of the chiral groves may markedly depend on the different solvation effects of the alcohol components of the mobile phase;
c) results showed that the *tris*-(3,5-dichlorophenylcarbamate)-based CSPs (Chiralpak **IC** and Chiralpak **IE**) always outperform the *tris*-(3,5-dimethylphenylcarbamate)-based ones (Chiralpak **IA** and Chiralpak **IB**);
d) the extremely high retentions of 9-*epi*-QN and 9-*epi*-QD compared to those of QN/QD observed in a few cases can be attributed to the change of the configuration of the C-9 atoms for the (–)*epi*-QN from (9*R*) to (9*S*) and for the (+)-*epi*-QD from (9*S*) to (9*R*) resulting in the full change in the overall steric configuration of the analytes; whereas it results in a stronger interaction with the selector sites of the CSP, it is not necessarily accompanied with an increased stereodifferentiation;
e) overall, the enantio- and diastereoselectivity of these chiral columns for the stereochemically rather restricted *Cinchona* alkaloids was moderate, which hints to a restricted adoptive conformation (induced fit phenomenon) of the analytes towards the chiral selectors and *vice versa*; it should be mentioned that a further screening of alternative mobile phase compositions may reveal some additional effects.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2020.113724.

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