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Original Article

Screening primary racemic amines for enantioseparation by derivatized polysaccharide and cyclofructan columns☆

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ABSTRACT

It is a challenge to separate the enantiomers of native chiral amines prone to deleterious silanol interactions. A set of 39 underivatized chiral primary amines was screened for enantiomeric separation. Seven recently introduced commercial chiral columns were tested. They included six polysaccharide based chiral stationary phases (CSP) with bonded derivatives, ChiralPak® IA, IB, IC, ID, IE and IF columns and a cyclofructan derivatized CSP, Larihc® CF6-P column. Both the normal phase (NP) mode with heptane/alcohol mobile phases and the polar organic (PO) mode with acetonitrile/alcohol were evaluated. It was found that the cyclofructan based CSP demonstrated the highest success rate in separating primary amines in the PO mode with only one chiral amine not resolved. It is shown that, when screening the columns, there is no standard optimal condition; an excellent mobile phase composition for one column may be poorly suited to another one. Although butylamine was a good mobile phase additive for the polysaccharide columns in both PO and NP modes, it was detrimental to the enantio-recognition capability of the cyclofructan column. Triethylamine was the appropriate silanol screening agent for this latter column.

1. Introduction

Chiral primary amines, either as native primary amines or protected amino acids or amino alcohols, are very important in chemical and pharmaceutical industries. They were first separated by derivatized crown ethers as described by Kyba et al. [1] and Ligenfelter et al. [2]. Bonded crown ethers make useful chiral stationary phases (CSPs) for high performance liquid chromatography (HPLC), which are specifically very efficient in separating primary amines with acidic aqueous mobile phases [3,4]. However, the use of strongly acidic aqueous mobile phases hindered scaling up the separations since the non-volatile perchloric acid additive was concentrated with the analyte in the purification process.

Derivatized polysaccharide polymer based CSPs are broadly selective chiral selectors which can separate some racemic primary amines with apolar mobile phases in the normal phase (NP) mode or non-aqueous polar mobile phases in the polar organic (PO) chromatographic mode while avoiding the highly acidic aqueous mobile phases required with crown ethers [5,6]. However, the first chiral columns of this sort were based on coated chiral stationary phases that had to be used with great care, since they were being fragile and possibly washed out by inappropriate solvents [7,8]. Bonded polysaccharide based CSPs were later introduced, allowing for the use of a large variety of solvents in all chromatographic modes including super/subcritical mobile phases. Their enantiomeric separation capabilities showed similar but not identical capabilities when compared to their coated counterparts [9,10].

Cyclofructans (CFs) are cyclic oligosaccharides consisting of β-2,1 linked d-fructofuranose units. They have been recently introduced as powerful chiral selectors after alkylation or aryl derivatization that released strain inside the native CF units, making the internal crown ether-like structure accessible [11]. These CF selectors were found to be very efficient in separating isomers of primary amines especially in the PO and NP modes [12–19].

In this work, the enantiomeric separation capabilities of six commercially-available bonded polysaccharide CSPs and a CF based CSP are evaluated in a screening process using a set of racemic primary amines and non-aqueous mobile phases, i.e., in the NP mode with apolar heptane:ethanol mobile phases and in the PO mode with acetonitrile:methanol or isopropyl alcohol mobile phases.

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Available online 22 July 2016

Table 1
Chiral primary amines and the best selectivity value for each.

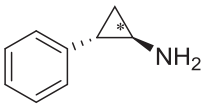
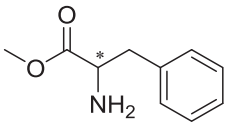
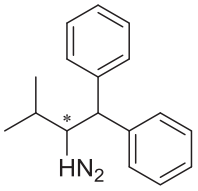
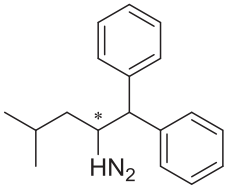
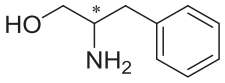
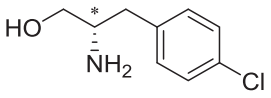
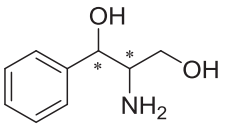
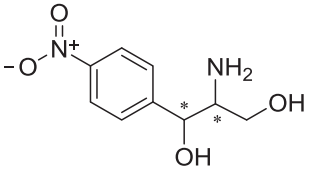
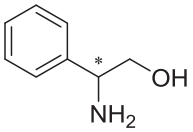
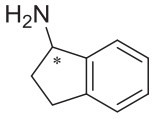
Code	Name	Structure	Best α (column, mode)
1	Trans-2-Phenylcyclopropylamine		1.23 (ChiralPak ID, 90:10:0.1 Hept/EtOH/BA)
2	DL-Phenylalanine methyl ester		1.76 (ChiralPak ID, 97:03:0.1 ACN/IPA/BA)
3	(±)-2-Amino-3-methyl-1,1-diphenylbutane		3.26 (ChiralPak IC, 90:10:0.1 Hept/EtOH/BA)
4	(±)-2-Amino-4-methyl-1,1-diphenylpentane		1.44 (ChiralPak IA, 90:10:0.1 Hept/EtOH/BA)
5	2-Amino-3-phenyl-1-propanol		1.17 (Larihc CF6-P, 60:40:0.3:0.2 Hept/EtOH/TFA/TEA)
6	DL-4-chlorophenyl alaninol		1.15 (Larihc CF6-P, 60:40:0.3:0.2 Hept/EtOH/TFA/TEA)
7	2-Amino-1-phenyl-1,3-propanediol (RR/SS)		1.86 (ChiralPak ID, 90:10:0.1 Hept/EtOH/BA)
8	(±)-2-Amino-1-(4-nitrophenyl)-1,3-propanediol (RR/SS)		1.23 (Larihc CF6-P, 90:10:0.3:0.2 ACN/MeOH/TFA/TEA)
9	2-Phenylglycinol		1.10 (Larihc CF6-P, 60:40:0.3:0.2 Hept/EtOH/TFA/TEA)
10	1-Aminoindan		1.20 (Larihc CF6-P, 90:10:0.3:0.2 ACN/MeOH/TFA/TEA)

Table 1 *(continued)*

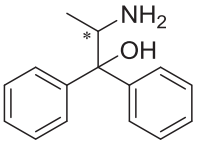
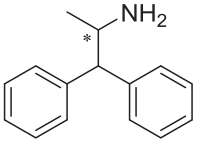
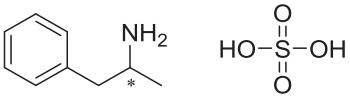
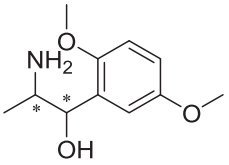
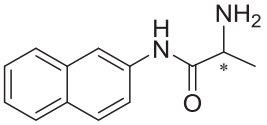
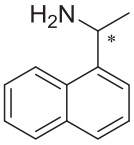
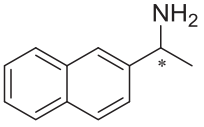
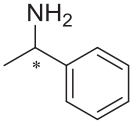
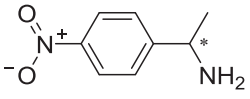
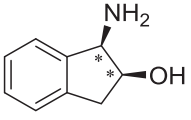
Code	Name	Structure	Best α (column, mode)
11	2-Amino-1,1-diphenyl-1-propanol		1.54 (ChiralPAk IA, 97:03:0.1 ACN/IPA/BA)
12	(±)-1,1-Diphenyl-2-aminopropane		1.38 (ChiralPak IF, 90:10:0.1 Hept/EtOH/BA)
13	DL-Amphetamine sulfate salt		1.46 (ChiralPak IC, 90:10:0.1 Hept/EtOH/BA)
14	α-(1-Aminoethyl)-2,5-dimethoxybenzyl alcohol (methoxamine)		3.11 (ChiralPak IF, 97:03:0.1 ACN/IPA/BA)
15	DL-alanine-β-naphthylamide		1.47 (ChiralPAk IC, 97:03:0.1 ACN/IPA/BA)
16	1-(1-Naphthyl)ethylamine		2.31 (ChiralPak IA, 97:03:0.1 ACN/IPA/BA)
17	1-(2-naphthyl) ethyl amine		1.22 (Larihc CF6-P, 60:40:0.3:0.2 Hept/EtOH/TFA/TEA)
18	α-Methylbenzylamine		2.15 (ChiralPAk IA, 97:03:0.1 ACN/IPA/BA)
19	α-Methyl-4-nitrobenzylamine		1.14 (Larihc CF6-P, 60:40:0.3:0.2 Hept/EtOH/TFA/TEA)
20	(±) cis-1-Amino-2-indanol		1.17 (ChiralPak IC, 90:10:0.1 Hept/EtOH/BA)

Table 1 *(continued)*

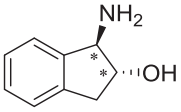
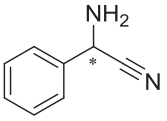
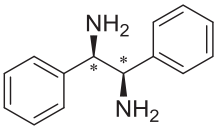
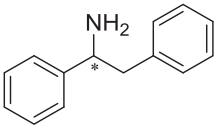
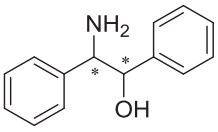
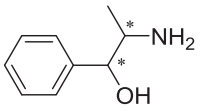
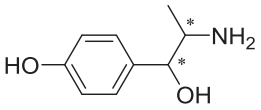
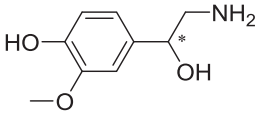
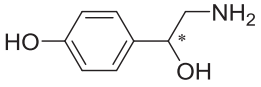
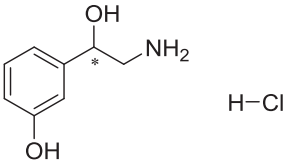
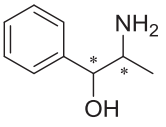
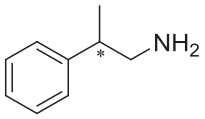
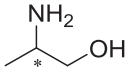
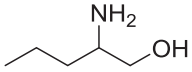
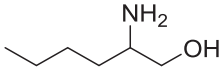
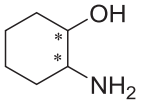
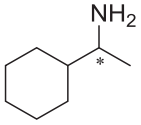
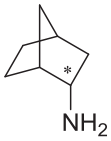
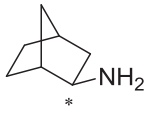
Code	Name	Structure	Best α (column, mode)
21	(±) trans-1-Amino-2-indanol		1.57 (ChiralPak ID, 90:10:0.1 Hept/EtOH/BA)
22	2-Phenylglycinonitrile		2.26 (Larihc CF6-P, 60:40:0.3:0.2 ACN/MeOH/AA/TEA)
23	(±)-1,2-Diphenylethylenediamine (RR/SS)		2.58 (ChiralPak IA, 97:03:0.1 ACN/IPA/BA)
24	1,2-Diphenylethylamine		1.25 (Larihc CF6-P, 60:40:0.3:0.2 ACN/MeOH/AA/TEA)
25	2-Amino-1,2-diphenylethanol (RS/SR)		2.64 (ChiralPak IA, 90:10:0.1 Hept/EtOH/BA)
26	(±)-Phenylpropanolamine (RS/SR)		2.24 (ChiralPak IA, 90:10:0.1 Hept/EtOH/BA)
27	(±)-alpha-(1-Aminoethyl)-4-hydroxybenzyl alcohol (RS/SR)		2.81 (ChiralPak ID, 97:03:0.1 ACN/IPA/BA)
28	DL-Normetanephrine		1.64 (ChiralPak IA, 90:10:0.1 Hept/EtOH/BA)
29	DL-Octopamine		2.53 (ChiralPak ID, 90:10:0.1 Hept/EtOH/BA)
30	Norphenylephrine hydrochloride		2.76 (ChiralPak ID, 97:03:0.1 ACN/IPA/BA)
31	(1R/S, 2S/R)-(-/+)-Norephedrine		2.58 (ChiralPak IF, 97:03:0.1 ACN/IPA/BA)

Table 1 *(continued)*

Code	Name	Structure	Best α (column, mode)
32	β-phenethylamine, 1-amino-2-phenylpropane		2.10 (Chiralpak ID, 97:03:0.1 ACN/IPA/BA)
	Primary Amines lacking chromophore		
33	2- amino-1-propanol		1.14 (Larihc CF6-P, 90:10:0.3:0.2 ACN/MeOH/TFA/TEA)
34	2-amino-1-pentanol		4.33 (ChiralPak IA, 97:03:0.1 ACN/IPA/BA)
35	2-amino-1-hexanol		1.59 (ChiralPak IB, 90:10:0.1 Hept/EtOH/BA)
36	Aminocyclohexanol (RR/SS)		1.31 (Larihc CF6-P, 90:10:0.3:0.2 ACN/MeOH/TFA/TEA)
37	Cyclohexylethylamine		1.29 (Larihc CF6-P, 90:10:0.3:0.2 ACN/MeOH/TFA/TEA)
38	2-aminonorbornane		1.36 (Larihc CF6-P, 90:10:0.3:0.2 ACN/MeOH/TFA/TEA)
39	exo-2-amino-norbornane		1.83 (ChiralPak IB, 97:03:0.1 ACN/IPA/BA)

RR/SS or RS/SR: the amine has two stereogenic centers, hence four possible enantiomers, the indicated enantiomers were considered. Hept: heptane; IPA: isopropyl alcohol; BA: butylamine; ACN: acetonitrile; MeOH: methanol; AA: acetic acid; TEA: triethylamine; EtOH:ethyl alcohol. All mobile phase compositions are given in % v/v.

2. Materials and methods

2.1. Chemicals

All of chiral analytes tested in this study were purchased from Sigma–Aldrich (St Louis, MO, USA) and Anichem (North Brunswick, NJ, USA). Acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), n-heptane (hept), and isopropyl alcohol (IPA) of HPLC grade were obtained from VWR (Sugarland, Texas), and used as received. Butylamine (BA), acetic acid (AA), trifluoroacetic acid (TFA) and triethylamine (TEA) were obtained from Sigma. Table 1 lists the structures of the 39 racemic primary amines used as test compounds. The chiral amines were sorted according to the four different substituents on their asymmetric center.

2.2. Columns

Table 2 lists the properties of the seven chiral columns used with non-aqueous mobile phases. The ChiralPak® columns were obtained from Chiral Technologies Inc. (West Chester, Pennsylvania, USA, a division of the Daicel Group, Tokyo, Japan). The Larihc® CF6-P column was obtained from AZYP (Arlington, Texas, USA). All columns were 4.6 mm in internal diameter, 25 cm long, and packed with bonded 5 μm fully porous silica particles.

2.3. Chromatographic procedure

The chromatographic system was an Agilent 1260 (Agilent Technologies, Santa Clara, CA, USA), with a 1260 quaternary pump,

Table 2
Characteristics of the 25 cm (0.46 cm i.d.) chiral columns and mobile phase compositions used in the screening procedure.

Name	Particle size (μm)	Bonded selector	Mobile phases[a]	Separated amines[b]	Baseline separation[c]
ChiralPak IA	5	Amylose tris (3, 5 dimethyl phenylcarbamate	NP: hept/EtOH (90:10, v/v) with 0.1% BA and	19 (50%)	9
ChiralPak IB	5	Cellulose tris (3, 5 dimethyl phenylcarbamate		11 (28%)	1
ChiralPak IC	5	Cellulose tris (3, 5 dichloro phenylcarbamate		16 (41%)	4
ChiralPak ID	5	Amylose tris (3 chloro phenyl carbamate	PO: ACN/IPA (97:3, v/v) with 0.1% BA	18 (46%)	15
ChiralPak IE	5	Amylose tris (3, 5 dichloro phenylcarbamate		17 (44%)	12
ChiralPak IF	5	Amylose tris (3 chloro, 4 methyl phenylcarbamate		26 (67%)	18
Larihc CF6- P	5	Isopropyl carbamoylated cyclofructan 6	NP: hept/EtOH (60:40, v/v) with 0.3% TFA+0.2% TEA and PO: ACN/MeOH (60:40, v/v) with 0.3% AA+0.2% TEA and (90:10, v/v) with 0.3% TFA + 0.2% TEA	38 (97%)	21

[a] NP: normal phase mode; PO: polar organic mode; hept: heptane; IPA: isopropyl alcohol, BA: butylamine; ACN: acetonitrile; MeOH: methanol; AA: acetic acid; TEA: triethylamine. All mobile phase compositions are given in volume percentage. 0.1% (v/v) BA is 10 mM; 0.3% (v/v) TFA is 40 mM; 0.3% (v/v) AA is 52 mM; 0.2% (v/v) TEA is 14 mM.
[b] Amines listed in Table 1, cumulating partial and baseline separations on all mobile phases (PO+NP). Percentage in parenthesis refers to the test set of 39 chiral primary amines (Table 1).
[c] Resolution factor between enantiomers equal or higher than 1.5 with all mobile phases.

a 1260 autosampler, thermostated column compartment, and a 1260 diode array detector (DAD). Data was evaluated using the Agilent Chemstation software. A Shimadzu SCL 10A refractive index detector (Shimadzu, Columbia, MA, USA) was used to detect the seven primary amines lacking UV chromophores (Solutes #33 to #39 in Table 1).

The mobile phase compositions are listed in Table 2. The selected screening mobile phases were those recommended by the column manufacturer. In NP mode, hept/EtOH mobile phases were used with BA as the silanol-screening additive to enhance peak shapes for all six ChiralPak CSPs and with a combination of TFA or AA and TEA for the single Larihc CF6-P column. In PO mode, BA was also added to the ACN/IPA mobile phases with the ChiralPak separations. Two PO mobile phases were tested with the Larihc CF6-P column: one used the TFA and TEA additives and the other used AA and TEA additives as discussed in the following text. All separations were performed at 2.0 mL/min flow rate.

3. Results and discussion

3.1. Screening procedures

The enantiomers of chiral native primary amines are often challenging to separate. A recent application guide by Chiral Technologies Inc. presents close to 1100 enantiomeric separation examples. Of these 1100 separations, it is striking to find that only seven primary amines were separated on bonded CSPs [20]. Herein, a screening procedure was established to evaluate the capabilities of the newly introduced bonded CSPs based on bonded derivatized polysaccharides [5–10] (trade name ChiralPak® with Ix codes). A recently introduced chiral column based on a bonded derivatized CF selector [13–19] (trade name Larihc®) was also evaluated.

Why a particular enantiomeric pair is separated by a selector but not by another one is a question that has no answer yet. Interactions of enantiomers with chiral selectors involve a variety of different forces with some being enantioselective and others being achiral [21]. All interactions combine to give the enantiomer retention at the column exit. The two enantiomers are separated only if there are differences between the enantioselective interactions. Considering the molecular structures of the enantiomers to be separated, it is possible to guess that a particular chiral selector will not be effective. However, most often there is no other way to obtain a successful chiral separation than setting up an experiment with an available chiral column to test if it will be able to separate the particular enantiomeric pair. A change in mobile phase composition may ruin or otherwise enhance a chiral separation [21]. Since the number of experiments must be limited, in a screening procedure, a maximum number of solutes must be tested under a selected number of experimental conditions.

The experimental conditions must be adapted to the CSP tested. The screening procedure applied to the six bonded polysaccharide CSPs: ChiralPak IA, IB, IC, ID, IE and IF is listed in Table 2 and consists of one NP mobile phase and one PO mobile phase. The cyclofructan-6 bonded Larihc CF6-P was also tested with one NP mobile phase, but two PO mobile phases were evaluated (Table 2). The addition of 0.1% (v/v) BA to the non-aqueous mobile phases used with ChiralPak columns is recommended by the manufacturer [11,20]. It did work well in producing efficiencies in the 4000–8000 plate range or height equivalent to a theoretical plate between 30 and 60 μm. Such efficiencies are not impressive but sufficient for the desired separations where primary amines are notably known to produce poor efficiencies, poor peak shapes and peak tailing that were not seen with BA additive. However, BA interacts too strongly with the active site of the CF chiral selector [16–19], so it must not be added to mobile phases used with the Larihc® column. TEA should be the silanol screening agent. Fig. 1 illustrates the resulting separation of a chiral primary amine when using the CF based CSP with either the ChiralPak suggested mobile phase (Fig. 1A) or the Larihc suggested NP mobile phase (Fig. 1B). Using the correct mobile phase (Fig. 1B) resulted in a baseline separation with an enantiomeric selectivity value of 1.30, whereas there was no selectivity observed in Fig. 1A. If BA is a good silanol screening agent, the 6200 plates of Fig. 1A are useless since the enantioselectivity is lost (α=1.0, Rs=0). The 3600 plates obtained with the TEA screening agent are associated to an excellent 1.3 enantioselectivity (Fig. 1B) giving baseline separation (Rs=1.7) of the two enantiomers. This shows that it is critical to follow the manufacturer's suggested mobile phase compositions. Also, the recommendation for one particular column may not be valid for the other one.

It is important to realize that such screening procedures described here allow for quick determination of the appropriate selector able to discriminate the two primary amine enantiomers. The enantiomeric separation obtained may not be perfect and could be optimized once the effective CSP is identified. The optimization step is beyond the scope of this work and the presented results must not be considered as the best results that can be obtained with the tested CSPs.

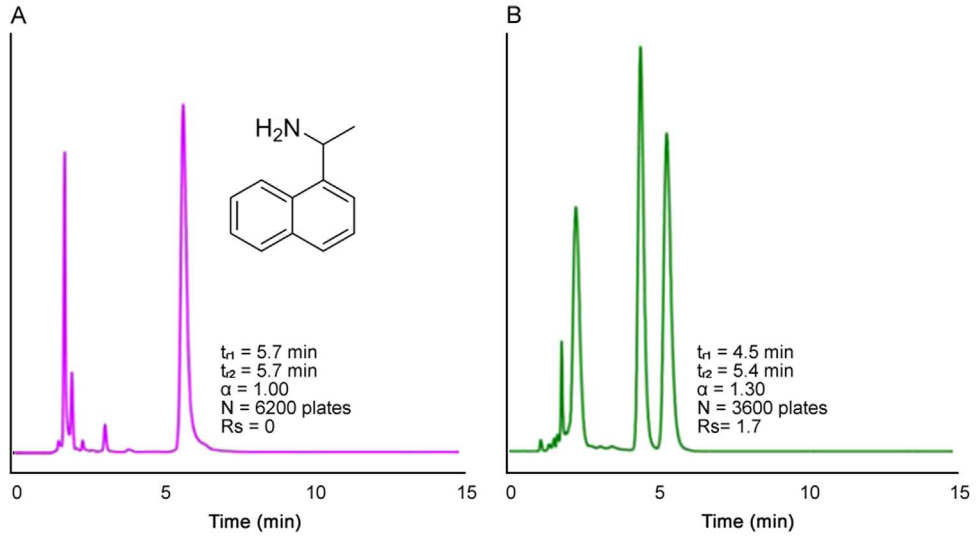


Fig. 1. Separation of the enantiomers of 1-(1-Naphthylethylamine) (Solute #16 in Table 1) on a 25 cm Larihc CF6-P column using (A) ChiralPak suggested mobile phase: Hept/EtOH/BA (90:10:0.1, v/v/v) and (B) Larihc CF6-P suggested mobile phase: Hept/EtOH/TFA/TEA (60:40:0.3:0.2, v/v/v/v).

3.2. Normal phase mode

The NP mode uses alkane/alcohol mobile phases and polarity is adjusted with the alcohol content. The ChiralPak columns have bonded polysaccharide selectors (Table 2), allowing to use practically all usual solvents [10,11]. Likewise, the Larihc CF6-P phase is also bonded and can be used in all chromatographic modes with a variety of polar solvents. For the screening procedure, the selected NP mobile phase was hept/EtOH (90:10, v/v) with 0.1% BA. The Larihc CF6-P column can also operate in the NP mode. However, the mobile phase composition was hept/EtOH (60:40, v/v) with 0.3% TFA and 0.2% TEA.

Fig. 2 compiles the results from the NP screening evaluation presenting the number of enantiomeric separations for each column with a thin bar giving the number of baseline separations. The two columns which performed the best were the Larihc CF6-P and the ChiralPak IF. Both could separate 20 primary chiral amines or 52% of the test set shown in Table 1. The Larihc CF6-P had 11 baseline separations, the greatest of all tested columns. Some chiral amines were separated by a CSP and not by another, showing some complementary nature between the polysaccharide based columns. Considering the full set of six ChiralPak columns, the enantiomers of seven chiral amines could not be resolved by any polysaccharide CSP in the NP mode. Of these seven chiral amines, four were separated in the NP mode by the Larihc CF6-P column. This left only three amines (Solutes # 32, 35, and 39) that could not be resolved in the NP mode with the screening mobile phases used by any of the seven tested columns (Fig. 3).

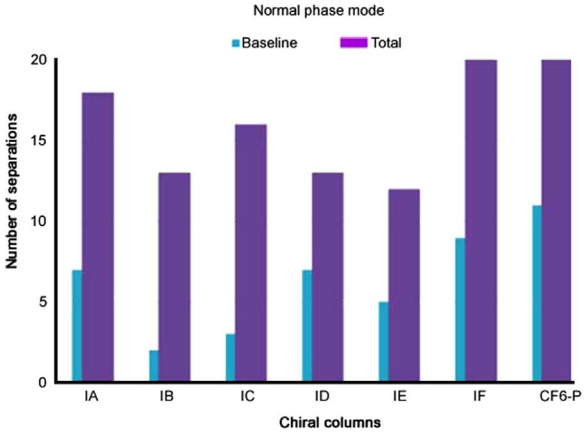


Fig. 2. Efficacy of the six 25 cm (0.46 cm i.d.) ChiralPak and the Larihc CF6-P bonded columns in separating the set of 39 racemic primary amines (Table 1) in NP mode. ChiralPak mobile phase: heptane/ethanol/BA (90:10:0.1, v/v/v); Larihc CF6-P mobile phase: heptane/ethanol/TFA/TEA (60:40:0.3:0.2, v/v/v/v). Flow rate: 2 mL/min.

3.3. Polar organic mode

Amines are relatively polar molecules, so the PO mode is often a good choice for screening such compounds. Figs. 4 and 5 summarize the separation results from screening all seven columns in PO mode. In this mode, the Larihc CF6-P column performed remarkably well being able to separate the enantiomers of 33 primary amines (85% of the test set shown in Table 1 with 18 being baseline) with the ACN/MeOH (90:10, v/v, with 0.3% TFA and 0.2% TEA) PO mobile phase. The next most successful column was the ChiralPak IF column separating 25 chiral amines (65% with 14 being baseline) with the ACN/IPA (97:3, v/v with 0.1% BA) PO mobile phase.

Complementary separation capabilities were again observed in the six polysaccharide columns. Fig. 4 shows the results individually obtained for the 32 UV absorbing amines screened with the ACN/IPA (97:3, v/v) PO mobile phase with 0.1% BA. The most effective column was the ChiralPAK IF, which separated 21 chiral amines or 66% of the UV absorbing set. It was followed by the ChiralPAK IA and

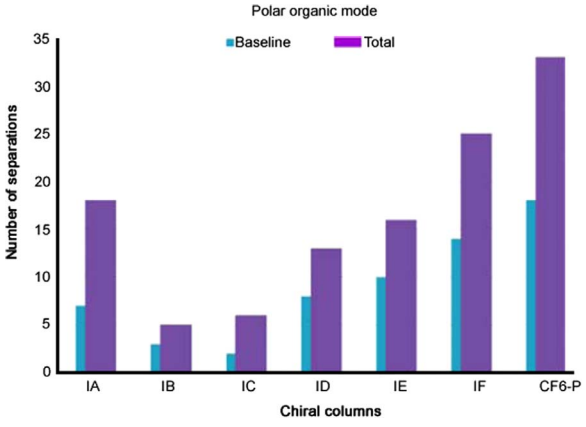


Fig. 3. Efficacy of the six 25 cm (0.46 cm i.d.) ChiralPak and the Larihc CF6-P bonded columns in separating the set of 39 racemic primary amines (Table 1) in PO mode. ChiralPak mobile phase: acetonitrile/isopropyl alcohol/BA (90:3:0.1, v/v/v); Larihc CF6 P mobile phase: acetonitrile/methanol/TFA/TEA (90:10:0.3:0.2, v/v/v/v). Flow rate: 2 mL/min.

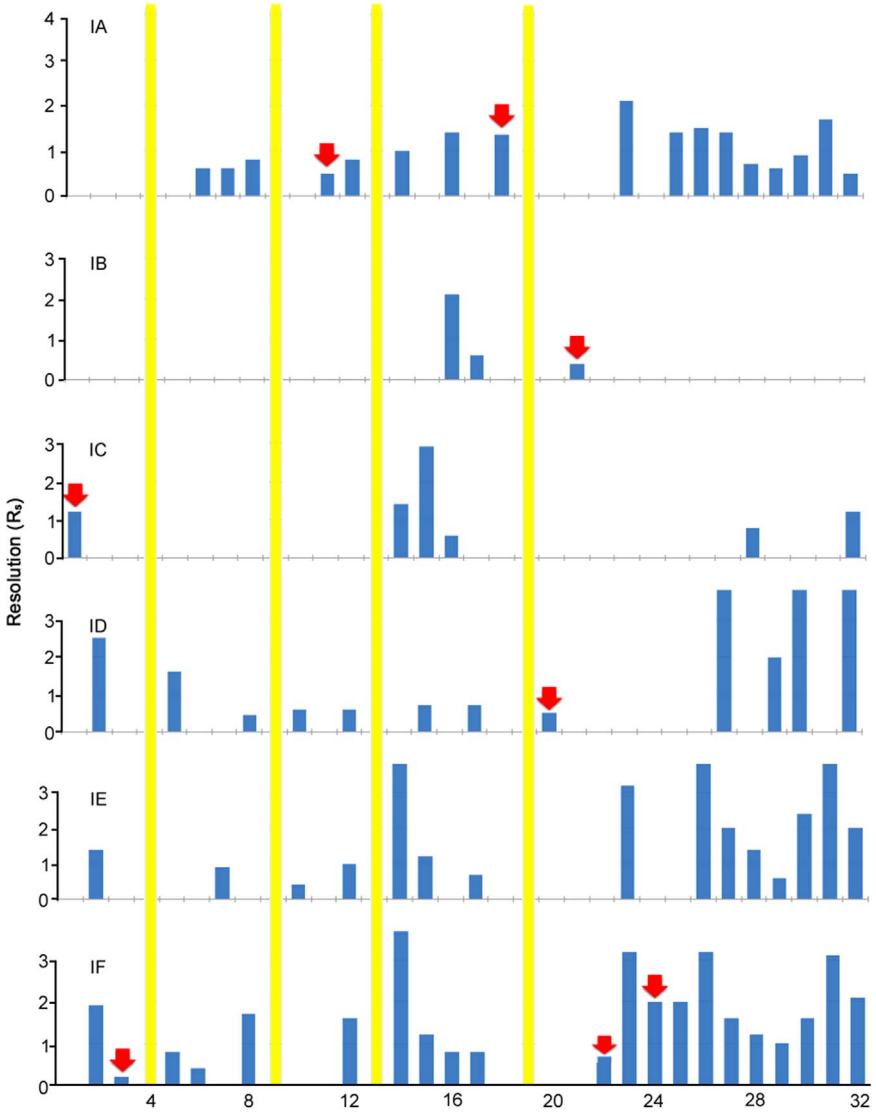


Fig. 4. Resolution factors for 32 UV absorbing primary amines (see Table 1 for compound names) obtained on 6 ChiralPak columns (Table 2) with the PO mobile phase made of acetonitrile/isopropyl alcohol (97:3, v/v) with 0.1% BA as a silanol screening agent. Red arrows indicate separations obtained on only one ChiralPack column. Vertical yellow bands indicate no chiral separation on the 6 columns.

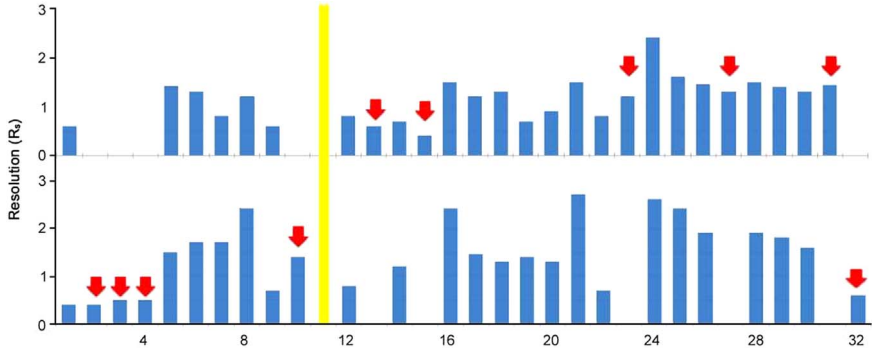


Fig. 5. Resolution factors for the same 32 UV absorbing primary amines as in Fig. 4 (see Table 1 for compound names) obtained on the Larihc CF6-P column with the PO mobile phases made of acetonitrile and methanol as indicated in the diagrams. Red arrows indicate separations obtained with one mobile phase only. The vertical yellow band indicates the only chiral amine (Compound 11) whose enantiomers were not separated by this column.

ChiralPak IE CSPs separating 18 (55%) and 15 (46%) UV absorbing native chiral amines, respectively. Fig. 4 shows that 21 amines, or 2/3 of the set, could be separated by two polysaccharide CSPs or more. The red arrows point to the eight amines that could only be separated by a single polysaccharide CSP (not including the cyclofructan based column) and the four thin vertical bars mark the amines (13%) that could not be separated by any polysaccharide CSPs in this PO mode condition.

Similarly, Fig. 5 shows the resolution factors obtained with the same 32 chiral UV-absorbing primary amines on the Larihc CF6-P column with two PO mobile phases. The elution strength of the ACN/MeOH (60:40, v/v) with 0.3% AA and 0.2% TEA is similar to that of the (90:10, v/v) with 0.3% TFA and 0.2% TEA mobile phase, producing comparable retention times. However, the chiral recognition ability of the two PO mobile phases is slightly different. Yet, both mobile phases resulted in the separation of 26 chiral amines, or 82% of the UV absorbing set, which is better than all polysaccharide based CSPs. The mobile phase effect, especially acid additive, is evident: the red arrows point to 10 chiral amines whose enantiomers were resolved by one PO mobile phase and not by the other, demonstrating the need to screen the AA and TFA acid additives as illustrated by the chromatograms in Fig. 6.

Fig. 6 compares the separations of the phenylpropanolamine (RS/SR) enantiomers (Solute #26, Table 1) on the Larihc CF6-P column in the PO mode. The PO mobile phases contained 0.3% acid additive and 0.2% TEA base additive. The 0.3% (v/v) acid was either 40 mM TFA or 52 mM AA; both amounts are three to four times higher than the 0.2% TEA (14 mM). The excess acid in the PO mobile phases favors the formation of the ammonium ion form of the chiral amines screened. This form interacts well with the crown ether of the CF chiral selector [15–19]. TFA often, but not always, results in a better resolution and/or a greater selectivity (Fig. 6A) than what can be obtained on the same selector with AA (Fig. 6B).

Solute #11, 2-amino-1,1-diphenyl-1-propanol, is the sole chiral amine not separated by the Larihc CF6-P column in PO mode. Overall, the CF based column was able to separate 97% of the chiral amine set in PO mode with two mobile phases (Table 2). Several of these PO separations resulted in the highest selectivity values found for a given analyte (Table 1).

The chromatograms of Figs. 7 and 8 illustrate the difference between polysaccharide and the CF based columns. Fig. 7A shows the separation of (±) trans-1-amino-2-indanol (Solute #21) on the ChiralPak IB column, the only polysaccharide column which was able to provide selectivity for enantiomers of this amine. Fig. 7B shows the baseline separation of the same amine on the Larihc CF6-P column with resolution of 2.7. Fig. 8 shows the separation of phenylpropanolamine (Solute #26) in PO mode on the Larihc CF6-P (Fig. 8A) and ChiralPak IE (Fig. 8B) columns. Both columns are able to resolve the Solute #26 RS and SR enantiomers, but the polysaccharide ChiralPak IE column is much more selective and efficient in giving sharp peaks with this compound. These two figures (Figs. 7–8) illustrate a general trend that was observed in this study. The CF based CSP separated far more chiral basic compounds than the polysaccharide columns, but when a given polysaccharide column did have selectivity, the separations were often very good (Table 1).

3.4. Non-UV absorbing chiral amines

Table 1 also lists seven non-UV absorbing compounds (Solutes #33–39) that were detected by a refractive index detector (RID) [22]. These amines have alkyl, cycloalkyl, and/or hydroxyl functionalities. They lack UV chromophores that are aromatic rings, carbonyl groups or double bonds with π electrons making their enantiomers more challenging to differentiate. In the NP mode, the ChiralPak IF column was the most effective CSP in separating three (Solutes #33, 38 and 39) of the seven non-UV absorbing amines. However, none of the six polysaccharide CSPs could separate amines Solutes #34 and #37 in the NP mode. Yet, the CF based column could separate cyclohexylethylamine (Solute #37) in the NP mode. Interestingly, it was the only non-UV absorbing chiral amine that this CSP could resolve in this mode.

However, with PO mobile phases, none of the six polysaccharide CSPs could separate amines Solutes #35 and #37. The ChiralPak IF column was again the most effective polysaccharide column separating four non-UV absorbing chiral amines (Solutes #33, 36, 38 and 39). On the other hand, the Larihc CF6-P column was much more effective. It separated all non-UV chiral primary amines working with both PO screening mobile phases. The resolution factors were between 0.4 and 1.8 (average value 0.85) with the AA additive (ACN/MeOH (60:40, v/v)) and between 0.5 and 2.0 (average value 1.06) with the TFA additive (ACN/MeOH (90:10, v/v)).

4. Conclusion

The results listed in Table 2 cumulate all enantiomeric separations (all compounds and both PO and NP modes). It clearly shows that the single Larihc CF6-P column had the greatest success rate in separating

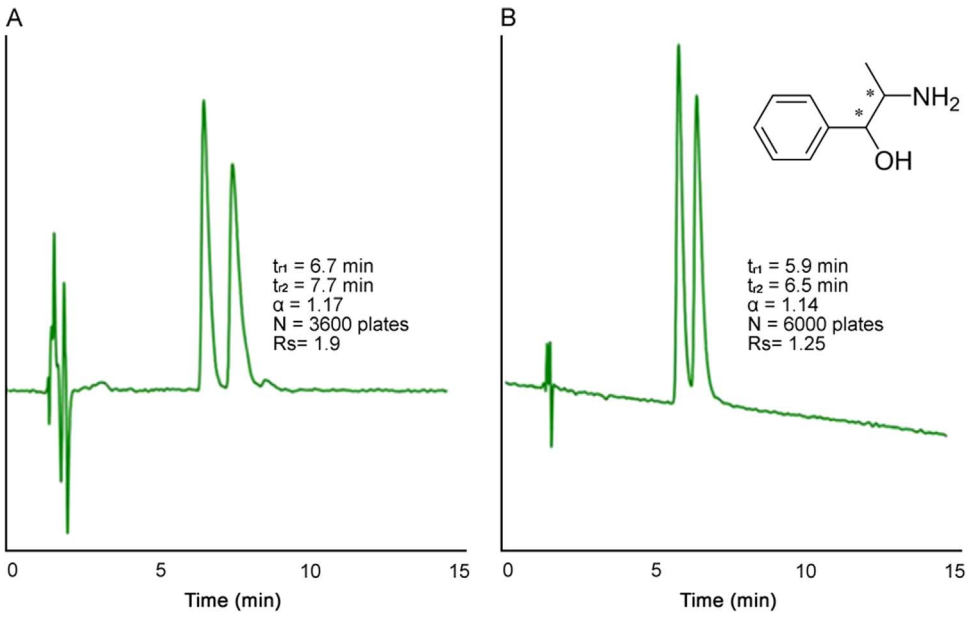


Fig. 6. Separation of the enantiomers of RS/SR phenylpropanolamine (Solute #26 in Table 1) on a 25 cm Larihc CF6-P column in PO mode with (A) ACN/ MeOH/TFA/TEA (90:10:0.3:0.2, v/v/v/v) and (B) ACN/MeOH/AA/TEA (60:40:0.3:0.2, v/v/v/v). Flow rate: 2 mL/min; detection UV: 254 nm.

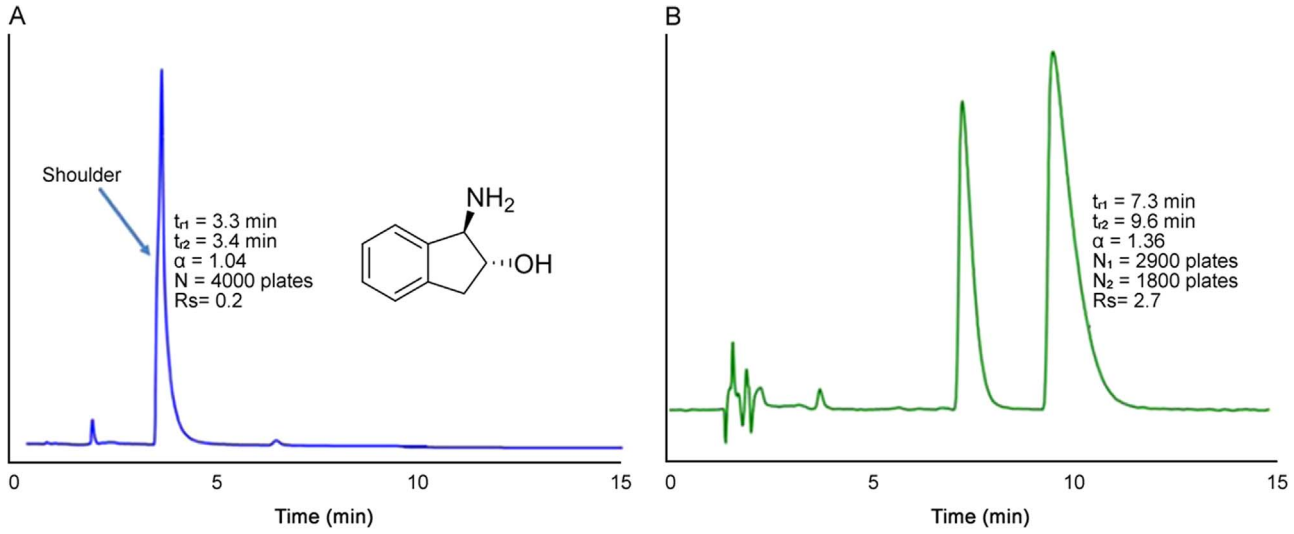


Fig. 7. Separation of the enantiomers of (±) trans-1-Amino-2-indanol (Solute #21 in Table 1) on a 25 cm column in PO mode as (A) ChiralPak IB column with ACN/IPA (97:3, v/v) mobile phase with 0.1% BA and (B) Larihc CF6-P column with ACN/MeOH (90:10, v/v) mobile phase with 0.3% TFA and 0.2% TEA ionic additives. Flow rate: 2 mL/min; detection UV: 254 nm.

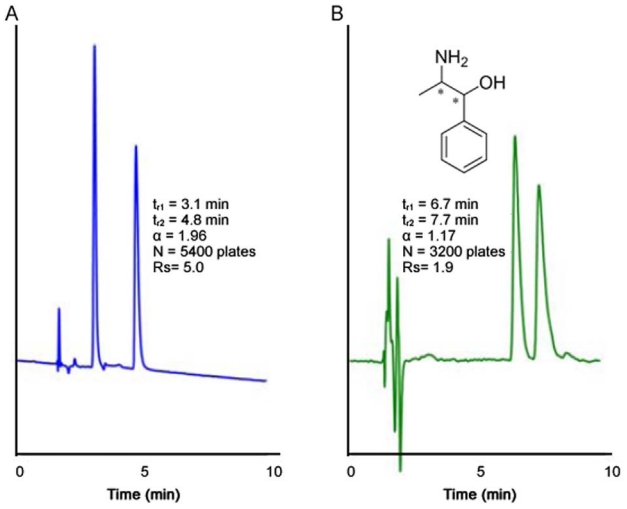


Fig. 8. Separation of the RS and SR enantiomers of phenylpropanolamine (Solute #26) on a 25 cm column in PO mode as (A) ChiralPak IE column with a ACN/IPA (97:3, v/v) mobile phase with 0.1% BA and (B) Larihc CF6-P column with a ACN/MeOH (90:10, v/v) mobile phase with 0.3%TFA and 0.2%TEA additives. Flow rate: 2 mL/min; detection UV: 254 nm.

enantiomers of primary amines. With this cyclofructan-based column, the PO mode with ACN/MeOH mobile phases containing either acetic or trifluoroacetic acid and triethylamine additives was significantly more effective than the NP mode. From a set of 39 chiral primary amines, the enantiomers of only one amine could not be separated by the CF column in the PO mode. In the same mode, the six polysaccharide CSPs together could not separate four amines. In this screening study, it is likely that many partial separations could be greatly improved by mobile phase composition optimization. It has been recently demonstrated that the CF6-P selector was also very effective in differentiating primary amine enantiomers in supercritical fluid chromatography [22].

Acknowledgments

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