Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 414–423

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Chiral separations in normal phase liquid chromatography: Enantioselectivity of recently commercialized polysaccharide-based selectors. Part I: Enantioselectivity under generic screening conditions

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ARTICLE INFO

Article history:
Received 26 November 2010
Received in revised form 3 February 2011
Accepted 10 February 2011
Available online 17 February 2011

Keywords:
Chiral separation
Chiral selectors
Enantioselectivity
Screening
Polysaccharides

ABSTRACT

Four recently commercialized polysaccharide-based chiral stationary phases, Sepapak® 1, Sepapak® 2, Sepapak® 3, and Sepapak® 4, now called Lux® Cellulose-1, Lux® Cellulose-2, Lux® Amylose-2 and Lux® Cellulose-4, respectively, were examined for their enantioselectivity on a set of 61 racemic compounds by applying the screening conditions of a previously developed chiral screening strategy in normal phase liquid chromatography (NPLC) [N. Matthijs et al., J. Chromatogr. A 1041 (2004) 119–133]. The enantioselectivity on these phases was compared to that on the initial set of polysaccharide-based phases, Chiralpak® AD-H, Chiralcel® OD-H, and Chiralcel® OJ-H, used in the earlier defined strategy. The results showed that 53 compounds out of 61 (86.9%) were resolved on the initial set of chiral stationary phases (CSPs) using two mobile phases per compound, either heptane–ethanol–diethylamine (DEA) or heptane–isopropanol–DEA for testing basic compounds and heptane–ethanol–trifluoroacetic acid (TFA) or heptane–isopropanol–TFA for acidic, bifunctional and neutral compounds. The recently commercialized set of columns gave 54 separations in total (88.5%). Our results indicated that ethanol (EtOH) as polar modifier provides a higher success rate and better resolutions than isopropanol (IPA) on both sets of stationary phases. However, the usefulness of the mobile phase with IPA as polar modifier cannot be neglected for complementarity reasons. It was found that the screening is improved by the introduction of the recently commercialized polysaccharides based CSPs since they provided enantioseparation for compounds that were not resolved by the traditional CSPs. The combination between the initial and the recently commercialized CSPs showed enantioresolution for 55 compounds out of 61 (90%), among which 47 were baseline resolved.

1. Introduction

Chromatographic enantioseparations are under constant development, both from a methodology point of view and an application one. The most important of these applications can be found in various fields, such as pharmaceutical, environmental and clinical analysis, where the optical purity of drugs, toxins, and pollutants may have very important implications on human health and/or therapeutic effectiveness. Different analytical and preparative separation techniques can be applied to achieve chiral resolution [1–6].

Many chromatographic techniques, for example, gas chromatography (GC), supercritical-fluid chromatography (SFC), capillary electrophoresis (CE), and high-performance liquid chromatography (HPLC) can be used for chiral separations. The liquid chromatographic separation of enantiomers on chiral stationary phases (CSPs) has emerged as the most effective and convenient way of determining the enantiomeric composition of many chiral compounds, including a variety of chiral drugs [7,8]. Chiral separation is performed in different modes of chromatography, such as normal-phase LC (NPLC), reversed-phase LC (RPLC) and polar organic solvents chromatography (POSC). The use of these chiral stationary phases enables the direct separation of the chiral analytes without preliminary derivatization with chiral reagents [9].

In chiral liquid chromatography, polysaccharide-based chiral stationary phases are the most popular [10–19]. Among all chiral stationary phases the acetate ester, benzoate ester or phenylcarbamate derivatives of cellulose and amylose have shown broad enantioselectivity [20–22]. From those derivatives, three, i.e. cellulose tris-(3,5-dimethylphenylcarbamate), amylose tris-(3,5-dimethylphenylcarbamate) and cellulose tris-(4-methylbenzoate), demonstrate significant complementary selectivity and numerous publications have proved their ability to achieve chiral resolution of more than 80% of the drugs currently available on the market [23–30]. A fourth one, with selector amylose tris-

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doi:10.1016/j.jpba.2011.02.015

((S)-α-methylbenzylcarbamate) also is regularly used, though its enantioselectivity is more specific compared to the other three phases. Those CSPs are known under the commercial names, Chiralcel® OD, Chiralpak® AD, Chiralcel® OJ and Chiralpak® AS, respectively. However, there is no universal chiral selector which separates all possible compounds and hence the need to develop new selectors remains [31,32]. According to the literature, several new cellulose and amylose derivatives have been synthesized and tested [33–36]. Especially the commercialized chlorine-containing cellulose and amylose phenylcarbamate derivatives were investigated [37–45].

The selection of the appropriate selector is often done by a trial and error approach which is too time-consuming to be of industrial interest. Therefore, there is a need for simple strategies with a fast screening of combinations of CSP-mobile phase conditions to provide chiral resolution for the compound(s) of interest. The aim of a screening strategy is to analyze rapidly and successfully large series of very diverse molecules. Thus, the first step in the development of such strategy is the selection of a limited number of chiral selectors with very broad enantiorecognition abilities so that most enantiomers can be resolved with one of them. Additionally, a small set of experimental conditions has to be defined, i.e. a few mobile phases to be combined with the selected selectors. If possible, no prior knowledge about the physico-chemical properties of the analytes should be required in order to keep the screening step as simple and as fast as possible. Short analysis times are clearly needed. At this stage, no optimal conditions are sought for unresolved compounds; the objective of this step is to quickly determine if acceptable recognition can be achieved under the generic screening conditions, part of the strategy. In a further optimization step, resolution efficiency and selectivity for partially or not resolved compounds might be improved by adjusting the factors that influence resolution e.g. mobile phase composition, additive concentration, temperature or flow rate. Separation strategies for the separation of chiral molecules by capillary electrophoresis (CE) [46], normal-phase liquid chromatography (NPLC) [47,48], reversed-phase liquid chromatography (RPLC) [48,49], polar organic solvents chromatography (POSC) [50] and capillary electochromatography (CEC) [51] have already been developed in our department.

In the context of an established separation strategy in NPLC, novel CSPs should be evaluated under the conditions specific to this strategy and also compared with regards to their success rate to the initially included CSPs. In order to potentially update the current NPLC separation strategy [48], the novel CSPs should be first evaluated under the generic conditions part of the separation strategy to see whether they are applicable. However, alternative mobile phase conditions, potentially more suitable for the novel CSPs could also be explored. The purpose of this evaluation is to find out whether the novel CSPs show either broader or complementary enantioselectivity to the CSPs already included in the screening step. In case of a positive outcome, the novel CSPs can be included in the screening step or may replace CSPs already part of the strategy. When a new CSP has a broader enantioselectivity with other conditions than those already included in the strategy, a new screening step or a new branch in the decision tree of the strategy can be defined.

In this paper, four recently commercialized polysaccharide-based CSPs, Lux® Cellulose-1/Sepapak-1 (cellulose tris(3,5-dimethylphenylcarbamate)), Lux® Cellulose-2/Sepapak-2 (cellulose tris(3-chloro-4-methylphenylcarbamate)), Lux® Amylose-2/Sepapak-3 (amylose tris(5-chloro-2-methylphenylcarbamate)) and Lux® Cellulose-4/Sepapak-4 (cellulose tris(4-chloro-3-methylphenylcarbamate)), also called Lux columns, are examined. It can be noticed that Lux® Cellulose-1 and Chiralcel® OD have the same chiral selector. These CSPs are tested with the screening mobile phases of the NPLC strategy of Matthijs et al. [48]. The enantioselectivities of these recently commercialized CSPs were compared to those of the three initially included Daicel columns, Chiralcel OD, Chiralpak AD and Chiralcel OJ, which are already part of the screening step in the strategy.

2. Experimental

2.1. Chemicals and reagents

The test set consists of 61 racemic compounds, i.e. acebutolol, alprenolol, atenolol, atropine, betaxolol, chlorthalidone, diltiazem, ephedrine, fenoprofen, ibuprofen, ketoprofen, labetalol, mandelic acid, nadolol, naproxen, naringenin, oxazepam, pindolol, praziquantel, promethazine, procyclidine, sulpiride, suprofen, tetramisole, timolol and warfarin (all from Sigma Aldrich, Steinheim, Germany), acenocoumarol and dimethindene (from Novartis, Basel, Switzerland), nimodipine, nisoldipine and nitrendipine (Bayer, Leverkusen, Germany), oxprenolol (Cynamid Benelux, Brussels, Belgium), propranolol and verapamil (Fluka, Neu-Ulm, Switzerland), ambucetamide (Janssen Pharmaceutica, Beerse, Belgium), bopindolol (Sandoz, Holskirchen, Germany), carvedilol (Boehringer, Mannheim, Germany), esmolol (Du Pont de Nemours, Saconnex, Switzerland), flurbiprofen (ICN Biomedicals, OH, USA), mebeverine (Duphar, Amsterdam, The Netherlands), metoprolol (Astra Hassle AB, Lund, Sweden), morphine and cocaine (Bios Coutelier, Brussels, Belgium), nicardipine (UCB, Brussels, Belgium), sotalol (Merck, Darmstadt, Germany), terbutaline (Astra-Draco AB, Lund, Sweden), bupranolol, carazolol, salbutamol, salmeterol, bisoprolol, methadone, carbinoxamine, chlorphenamine, hexobarbital, isothipendyl, mepindolol, meptazinol, mianserin, propiomazine and tertatolol were gifts from different origins.

HPLC grade n-heptane was purchased from BDH (Poole, UK). Ethanol (EtOH) absolute extra pure and 2-propanol (IPA) HPLC grade were purchased from Merck (Darmstadt, Germany). Diethylamine (DEA) was obtained from UCB (Brussels, Belgium) and trifluoroacetic acid (TFA) from Sigma (Steinheim, Germany).

All sample solutions had a concentration of about 0.1 mg/ml either in ethanol or isopropanol depending on the organic modifier used in the mobile phase.

2.2. Chromatographic conditions

The chromatographic system consisted of a HP 1050 automatic injector and a UV detector (Agilent Technologies, Palo Alto, CA), a HP 1100 quaternary pump (Agilent Technologies) and a membrane degasser. The columns were thermostatted using an electric oven (Jones chromatography model 7956, Glamorgan, U.K.).

The injection volume of each sample was 5 μl. The analyses were executed at a temperature of 20 °C with a mobile phase flow rate of 1 ml/min. The detection wavelength was set at 220 nm to ensure that every compound is detected. The Lux® and Sepapak-4 columns were supplied by Phenomenex (Torrance, CA, USA) and the three other, Chiralpak® AD-H, Chiralcel® OD-H and Chiralcel® OJ-H by Chiral Technologies Europe (Illkirch, France). The particle size in all columns is 5 μm and the dimensions are 25 × 0.46 cm. Since the analysis times at the tested conditions could not be predicted, run times were limited to 60 min.

2.3. Data processing

Analytical data were acquired and treated with the Hewlett-Packard Chemstation for LC software package (Agilent Technologies).

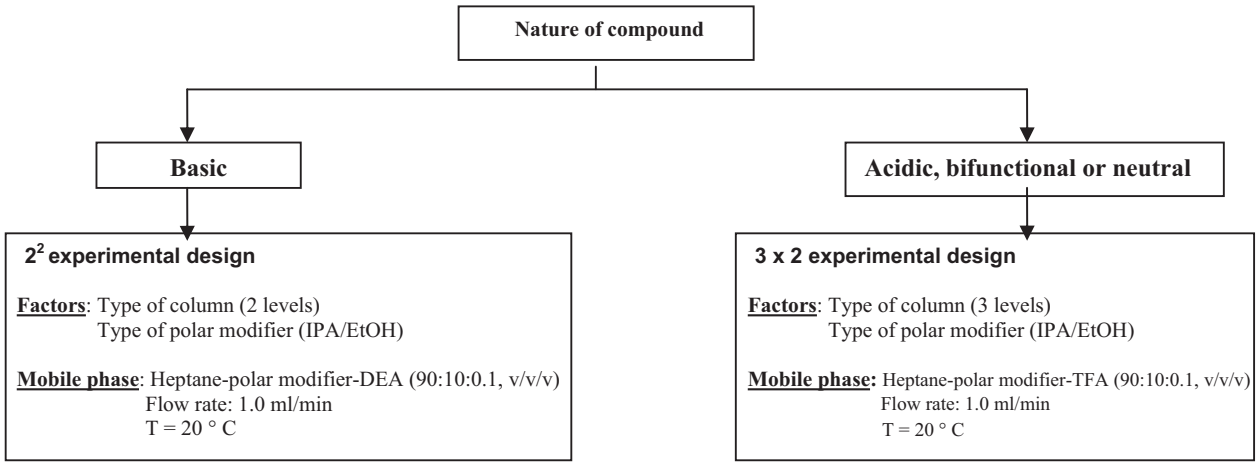

Fig. 1. General screening in NPLC extracted from [48].

Resolution values (*Rs*) were calculated according to the United States Pharmacopeia [52]:

$$Rs = \frac{2(tr_2 - tr_1)}{w_1 + w_2} \quad (1)$$

where tr_1 and tr_2 are the retention times in minutes of the first and the last eluting peak of a pair, respectively, while w_1 and w_2 are the baseline widths in minutes of these peaks (determined by the tangent method).

3. Results and discussion

In the normal-phase liquid chromatography (NPLC) strategy defined by Matthijs et al. [48], the screening step consists of a sequential screening of three polysaccharide-based CSPs in combination with two mobile phases, with the purpose of achieving chiral recognition for the test compound(s) at hand. The first mobile phase is heptane–IPA, and the second heptane–EtOH. Each mobile phase contained either DEA or TFA as additive, depending on the nature of the tested compound. It is generally accepted that basic and acidic additives enhance the separation quality, both in terms of selectivity and peak shape efficiency [53,54]. In the current study, hexane was replaced by heptane because of its lower toxicity and because retention and resolution data are very similar [48]. Thus the mobile phases applied are heptane–IPA–DEA (90:1:0.1, v/v/v) and heptane–EtOH–DEA (90:10:0.1, v/v/v) for the analysis of basic compounds, and heptane–EtOH–TFA (90:10:0.1, v/v/v) and heptane–IPA–TFA (90:10:0.1, v/v/v) for acidic, bifunctional or neutral compounds. As mentioned above, in this strategy both mobile phases are used in combination with three CSPs in the following predefined sequence: Chiralpak® AD-H, Chiralcel® OD-H and Chiralcel® OJ-H, respectively. A fourth Daicel phase, Chiralpak AS, is not involved in the proposed strategy because a previous study showed that both in NPLC and RPLC only few separations are obtained on this CSP [55]. Moreover, these same separations can also be achieved with the other three columns: AD, OD and OJ [48].

The general screening strategy [48] is presented in Fig. 1. The screening conditions depend on the nature of the compound (basic on the one hand and acidic, bifunctional or neutral on the other). The non-basic compounds are screened using a 3×2 experimental design. This means that the design includes two factors: one at three levels (column type) and the other at two (type of organic modifier). Two modifiers are screened because it is difficult to predict the optimal organic modifier for a given column and analyte [46,56].

For basic compounds, the same factors are investigated, but now a 2^2 full factorial design is applied because the Chiralcel® OJ-H column is not included in this design because of its low enantioselectivity towards these compounds [47,48]. After this screening step, optimization steps might follow in order to enhance resolution, selectivity and efficiency.

In the current study, four recently commercialized CSPs and the three initial CSPs are used as chiral selectors for a test set consisting of 61 compounds with different pharmaceutical activities and from different chemical classes (acidic, basic, bifunctional and neutral). These compounds were analyzed following the screening step of the separation strategy, which means a sequential testing of the two mobile phases on each of the columns, i.e. four non-chlorinated (including one Lux column) and three chlorinated columns. It is important to note that in this study every partially resolved test compound ($Rs > 0$), i.e. for which enantioselectivity was observed, was counted as successfully resolved in the further discussion. However, in the context of the strategy comparisons, a clear distinction is always made between the total number of partially and baseline resolved compounds. The number of partially resolved compounds is considered of significance because chiral selectivity observed under generic screening conditions carries the possibility of full separation upon subsequent method optimization. First, we investigated the chiral recognition ability of the Daicel columns (which are already part of the separation strategy) followed by that of the recently introduced CSPs. In the following part, a comparison is made between the two sets of results and possible updates are proposed to the screening strategy.

3.1. Enantioselectivity of the initial CSPs

3.1.1. Analysis of basic compounds

Resolution values obtained for 49 basic compounds on Chiralpak AD and Chiralcel OD are given in Table 1. The mobile phase containing ethanol induced the broadest enantioselectivity towards the set of test compounds on both columns and resulted in the chiral recognition of 33 compounds on Chiralpak AD and 23 on Chiralcel OD (Fig. 2).

The mobile phase with IPA as polar modifier separated 29 compounds on Chiralpak AD and 19 on Chiralcel OD. It was found that the use of IPA as polar modifier allowed the enantioseparation of five additional compounds (meptazinol, methadone, nicardipine, praziquantel and promethazine) on Chiralpak AD and four (bopindolol, isothipendyl, nicardipine and nimodipine) on Chiralcel OD relative to the use of ethanol as polar modifier. This shows the complementary character of the two mobile phases. Chiralpak AD column with both mobile phases showed a success rate of 38 out of 49 (77.5%) whereas it is 27 out of 49 (55%) on Chiralcel OD col-

Table 1
Resolution values (*Rs*) for basic test compounds on two of the initial CSPs, obtained with two mobile phases: (a) heptane–IPA–DEA and (b) heptane–EtOH–DEA, both (90:10:0.1, v/v/v).

	Chiralpak AD-H		Chiralcel OD-H	
	a	b	a	b
Acebutolol	0.00	2.58	**1.50**	0.90
Alprenolol	3.93	3.16	0.00	3.38
Ambucetamide	2.74	2.31	0.00	9.11
Atenolol	n.p.	0.00	0.00	**2.23**
Atropine	0.64	2.20	0.00	0.00
Betaxolol	2.66	4.37	6.38	4.05
Bisoprolol	1.70	2.71	3.97	2.53
Bopindolol	1.01	5.97	2.43	0.00
Bupranolol	2.75	2.35	0.00	1.17
Carazolol	0.87	5.46	0.00	1.52
Carbinoxamine	3.03	1.34	2.94	0.83
Carvedilol	n.p.	n.p.	0.00	0.00
Chloorfenamine	1.76	0.95	0.00	0.00
Cocaïne	0.00	0.00	0.00	0.00
Diltiazem	0.00	0.00	0.00	0.00
Dimethindeen	0.00	1.21	0.87	1.14
Ephedrine	0.61	2.35	1.95	1.00
Esmolol	1.78	4.03	4.82	3.33
Isothipendyl	4.12	0.79	0.69	0.00
Labetalol	n.p.	0.75/0.91/1.63	0.00	0.00
Mebeverine	0.66	1.09	3.20	1.96
Mepindolol	0.66	0.33	0.00	0.00
Meptazinol	0.64	0.00	0.99	0.67
Methadon	0.87	0.00	0.00	0.00
Metoprolol	2.03	4.11	6.98	5.52
Mianserine	0.00	0.00	1.08	1.79
Morphine	0.00	0.00	0.00	0.00
Nadolol	**0.61/3.74**	**4.33**	0.00	0.62/4.54/1.59
Naringenin	n.p.	n.p.	0.00	0.00
Nicardipine	0.81	0.00	0.70	0.00
Nimodipine	0.00	0.00	0.66	0.00
Nisoldipine	3.85	3.17	0.81	2.09
Nitrendipine	1.03	1.04	0.00	0.00
Oxprenolol	4.48	5.45	0.00	9.49
Pindolol	1.08	3.74	0.00	0.00
Praziquantel	**2.17**	n.p.	0.00	**3.44**
Procyclidine	0.00	0.00	0.00	0.00
Promethazine	1.04	0.00	0.00	0.00
Propiomazine	1.32	0.75	0.00	0.00
Propranolol	n.p.	2.91	7.56	3.74
Salbutamol	n.p.	0.65	0.00	0.00
Salmeterol	n.p.	0.88	0.00	0.00
Sotalol	n.p.	**7.33**	0.00	0.00
Sulpiride	n.p.	n.p.	0.00	**0.58**
Terbutaline	n.p.	**1.93**	0.00	0.00
Tertatolol	0.00	1.82	**15.50**	10.54
Tetramisol	2.05	2.86	1.63	1.77
Timolol	0.00	0.00	0.00	0.00
Verapamil	1.70	1.17	0.00	0.00

The substances in bold have a retention time exceeding 20 min. n.p., no peak observed after 60 min of analysis.

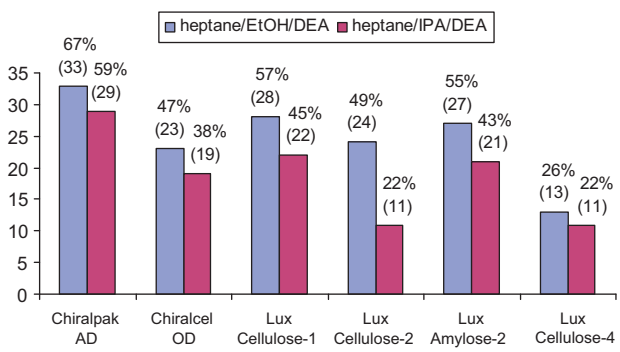

Fig. 2. Enantioselectivity obtained on the different systems for basic compounds, expressed in percentages and absolute number of separations.

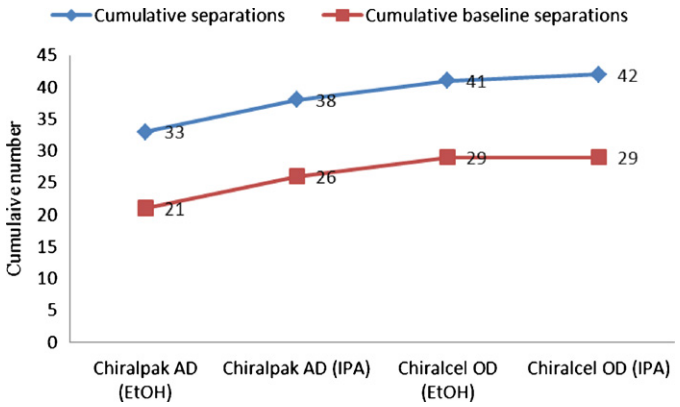

Fig. 3. Cumulative separations and cumulative baseline separations of basic compounds on the initial polysaccharide CSPs using two mobile phases, heptane–EtOH–DEA (EtOH) and heptane–IPA–DEA (IPA).

umn. The success rate of a separation strategy is a reflection of the success of the generic screening conditions. The more generic the selected screening conditions, the higher the success rate. Particular CSPs may be able to resolve given compounds under other than generic mobile phase conditions, but for time considerations such experiments cannot be included in the screening step of a generic strategy.

After screening the test set of 49 basic compounds with the prescribed mobile phases on the two CSPs, the preferred column sequence was Chiralpak AD > Chiralcel OD which is the same as suggested by Matthijs et al. [48] but with the preference of ethanol as polar modifier (IPA was preferred by Matthijs). Fig. 3 shows the cumulative number of (partly) resolved compounds as well as cumulative number of baseline separations when the two Daicel CSPs and the two mobile phases were combined. In this plot, systems are ranked in such a way that the first system with the highest success rate was presented followed by the system which increases the cumulative percentage most, etc. Such plot allows judging the effectiveness of the considered screening.

Chiralpak AD then should be first examined because of its broadest enantioselectivity towards the tested basic chiral pharmaceuticals with the two mobile phases, starting with ethanol as polar modifier. Afterwards, Chiralcel OD is considered with the same sequence of mobile phases. The cumulative number of separated basic compounds on both columns is 42 (85.7%). The above is somewhat different from the screening step proposed by Matthijs et al. [48] where the first mobile phase was tested on all columns before the introduction of the second.

3.1.2. *Analysis of acidic, bifunctional and neutral compounds*

A total of 12 acidic, bifunctional and neutral compounds are examined on Chiralpak® AD, Chiralcel® OD, Chiralcel® OJ columns with two mobile phases. The resolution values are shown in Table 2. Again, the broadest enantioselectivity is seen with the ethanol containing mobile phase. Chiralcel OJ gave the highest separation percentage (66.6%), i.e. eight out of 12 compounds, whereas Chiralpak AD gave six separations and Chiralcel OD five (Fig. 4). The enantioseparation of seven compounds on Chiralcel OJ, seven on Chiralpak AD and six on Chiralcel OD was achieved with the IPA containing mobile phase. The combined results of both mobile phases led to eight separations on Chiralcel OJ and seven on both Chiralpak AD and Chiralcel OD. The cumulative numbers of separations on Chiralcel OJ, Chiralpak AD and Chiralcel OD is 11/12 (91.6%) of which nine are baseline resolved (Fig. 5). Here it can be concluded that the use of mobile phase b (with IPA) had no added value and for this set of compounds and was in fact redundant. Since the EtOH containing mobile phase was able to separate 11 out of

Table 2
Resolution values (*Rs*) for non-basic test compounds on the initial set of CSPs, obtained with two mobile phases: (a) heptane–IPA–TFA and (b) heptane–EtOH–TFA, both (90:10:0.1, v/v/v).

	Chiralpak AD-H		Chiralcel OD-H		Chiralcel OJ-H	
	a	b	a	b	a	b
Acenocoumarol	0.00	0.00	1.80	**2.62**	n.p	n.p
Chloorthalidon	n.p.	n.p.	n.p.	0.93	n.p	n.p
Fenoprofen	3.01	1.56	0.70	0.00	3.43	0.64
Flurbiprofen	4.33	4.51	0.64	0.00	0.00	1.09
Hexobarbital	4.43	0.00	1.19	1.14	**1.81**	**0.57**
Ibuprofen	0.00	0.00	0.00	0.00	0.82	1.28
Ketoprofen	2.96	13.15	0.00	0.00	4.40	1.68
Mandelic acid	2.24	0.93	2.97	2.51	1.56	**1.78**
Naproxen	0.00	0.00	0.00	0.00	0.00	0.00
Oxazepam	0.00	n.p.	**2.90**	4.33	**3.36**	**3.72**
Suprofen	1.00	1.66	0.00	0.00	**4.69**	**4.61**
Warfarine	**9.89**	**8.20**	0.00	0.00	n.p	n.p

The substances in bold have a retention time exceeding 20 min. n.p., no peak observed after 60 min of analysis.

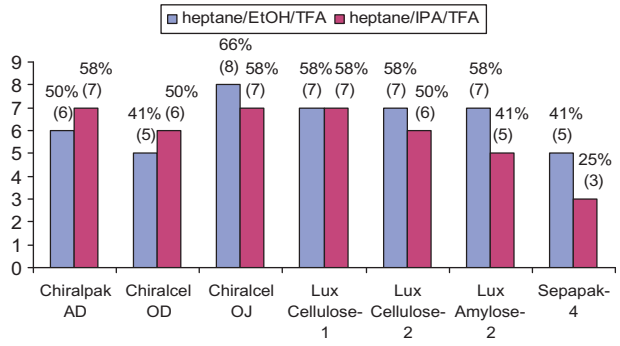

Fig. 4. Number of successful separations obtained on the different systems for non-basic compounds expressed in percentages and absolute number of separations.

12 compounds. The preferred sequence for screening is Chiralcel® OJ > Chiralpak® AD > Chiralcel OD.

3.2. Enantioselectivity of the recently commercialized CSPs

3.2.1. Analysis of basic compounds

Forty-nine basic compounds have been screened on the recently commercialized CSPs with the two mobile phases. Lux® Cellulose-2 (Sepapak-2) and Lux® Amylose-2 (Sepapak-3) succeeded to separate two compounds that were not resolved on the initial CSPs,

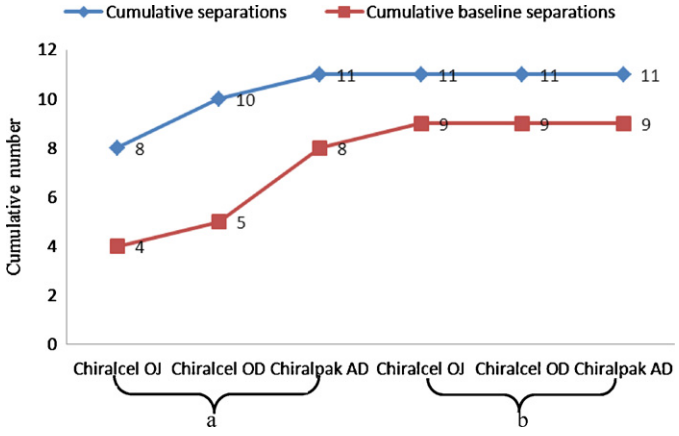

Fig. 5. Cumulative number of separations and cumulative number of baseline separations of non-basic compounds on the initial polysaccharide CSPs using mobile phases: (a) heptane–EtOH–TFA and (b) heptane–IPA–TFA.

Table 3
Resolution values (*Rs*) for basic compounds on the recently commercialized CSPs, obtained with two mobile phases: (a) heptane–IPA–DEA and (b) heptane–EtOH–DEA, both (90:10:0.1, v/v/v).

	Sepapak-1 (Lux-C1)		Sepapak-2 (Lux-C2)		Sepapak-3 (Lux-A2)		Sepapak-4 (Lux-C4)	
	a	b	a	b	a	b	a	b
Acebutolol	**1.92**	1.52	n.p	**1.18**	n.p.	**6.25**	n.p.	0.00
Alprenolol	12.78	8.46	0.00	0.87	0.00	0.62	0.90	0.99
Ambucetamide	19.66	19.90	n.p	0.00	5.79	4.32	n.p.	0.00
Atenolol	n.p.	4.46	n.p	n.p.	n.p.	**3.04**	n.p.	n.p.
Atropine	6.40	0.00	n.p	6.20	n.p.	2.24	n.p.	4.71
Betaxolol	11.40	8.93	n.p	2.47	1.02	0.00	0.00	0.00
Bisoprolol	8.85	5.32	0.00	3.41	2.77	1.98	0.00	0.00
Bopindolol	2.94	0.00	2.92	10.46	0.00	4.41	0.71	7.07
Bupranolol	6.89	3.12	0.66	0.00	0.60	1.44	0.00	n.p.
Carazolol	**3.44**	**3.49**	0.00	3.42	1.71	1.67	0.00	1.62
Carbinoxamine	3.60	1.95	1.58	0.64	2.58	3.66	1.07	0.00
Carvedilol	n.p.	n.p.	n.p	n.p.	n.p.	**1.56**	n.p.	n.p.
Chloorfenamine	0.00	0.00	0.00	0.00	4.35	0.00	0.00	0.00
Cocaïne	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Diltiazem	0.00	0.00	n.p	0.00	n.p.	0.00	n.p.	0.00
Dimethindeen	1.64	3.29	0.00	0.66	1.04	0.55	0.00	0.00
Ephedrine	2.08	2.54	n.p	2.00	n.p.	n.p.	2.42	1.70
Esmolol	0.00	6.80	0.00	2.81	3.82	0.00	0.00	0.00
Isothipendyl	2.56	1.09	0.00	0.00	1.32	0.00	0.00	0.00
Labetalol	n.p.	n.p.	n.p	n.p.	n.p.	0.56	n.p.	n.p.
Mebeverine	4.23	3.15	2.88	0.00	0.00	1.29	0.00	0.00
Mepindolol	n.p.	0.00	n.p	7.09	0.63	2.51	n.p.	4.09
Meptazinol	1.02	0.54	0.00	n.p.	0.00	n.p.	0.00	n.p.
Methadone	0.00	0.00	n.p	0.73	1.33	n.p.	0.00	1.29
Metoprolol	0.00	10.36	n.p	1.64	0.80	3.67	0.00	0.00
Mianserine	2.68	5.11	0.00	0.00	0.00	0.68	0.00	n.p.
Morphine	n.p.	0.00	n.p	0.00	n.p.	0.00	n.p.	0.00
Nadolol	n.p.	3.10	n.p	0.00	n.p.	0.87	n.p.	0.00
Naringenin	n.p.	n.p.	n.p	n.p.	n.p.	n.p.	n.p.	n.p.
Nicardipine	1.17	0.62	1.13	0.00	0.56	0.00	0.93	0.00
Nimodipine	1.39	0.59	0.00	1.75	2.35	1.84	0.58	0.00
Nisoldipine	1.54	0.61	2.54	n.p.	0.00	n.p.	n.p.	0.00
Nitrendipine	0.00	0.00	0.00	0.00	0.00	0.00	0.62	0.00
Oxprenolol	0.00	16.70	1.99	0.00	1.87	2.45	0.92	0.00
Pindolol	n.p.	0.00	0.00	6.85	1.40	5.33	**7.29**	3.59
Praziquantel	n.p.	4.70	n.p	n.p.	n.p.	n.p.	n.p.	n.p.
Procyclidine	n.p.	0.00	0.65	0.00	0.00	n.p.	0.00	n.p.
Promethazine	0.00	0.00	0.00	n.p.	0.97	1.38	0.00	n.p.
Propiomazine	0.66	0.00	0.00	0.00	1.27	1.81	0.00	0.00
Propranolol	**16.79**	6.56	4.17	1.88	0.00	n.p.	1.59	0.61
Salbutamol	n.p.	0.00	n.p	2.10	n.p.	0.00	n.p.	1.71
Salmeterol	n.p.	n.p.	n.p	**3.80**	n.p.	0.00	n.p.	n.p.
Sotalol	n.p.	0.55	n.p	**4.02**	n.p.	**6.62**	n.p.	n.p.
Sulpiride	n.p.	**0.90**	n.p	n.p.	n.p.	n.p.	n.p.	n.p.
Terbutaline	n.p.	0.00	n.p	**1.34**	n.p.	0.00	n.p.	**1.12**
Tertatolol	0.00	19.06	3.36	1.62	1.05	3.77	0.91	0.63
Tetramisol	4.68	4.00	**7.14**	6.76	**6.26**	7.72	0.00	**5.73**
Timolol	0.00	0.00	n.p	0.00	0.00	0.00	n.p.	n.p.
Verapamil	0.00	0.00	0.00	0.89	0.00	4.69	0.00	0.00

The substances in bold have a retention time exceeding 20 min. n.p., no peak observed after 60 min of analysis.

i.e. carvedilol and procyclidine. The four recently commercialized columns not only achieved two extra separations, but they were also able to separate all compounds resolved by the initial polysaccharide CSPs (Table 3). The use of ethanol as polar modifier resulted again in a broader enantioselectivity on the tested columns. It resulted in 28 separations on Lux® Cellulose-1, 24 separations on Lux® Cellulose-2, 27 separations on Lux® Amylose-2 and 13 on Sepapak-4 (Fig. 2). The second mobile phase, heptane–IPA–DEA, separated 22 compounds on Lux® Cellulose-1 (Sepapak-1), of which three were additional to the EtOH containing mobile phase. Further, Lux® Cellulose-2 separated 11 compounds of which six were additional to the EtOH containing mobile phase. Lux® Amylose-2 separated 21 compounds of which six were additional to the EtOH containing mobile phase, and finally Lux® Cellulose-4 (Sepapak-4) separated 11 compounds with five addi-

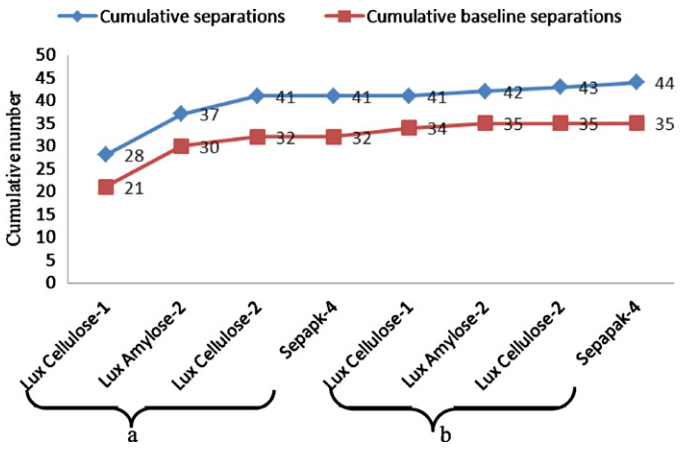

Fig. 6. Cumulative number of separations and cumulative number of baseline separations of basic compounds on the recently commercialized CSPs using two mobile phases: (a) heptane–EtOH–DEA and (b) heptane–IPA–DEA.

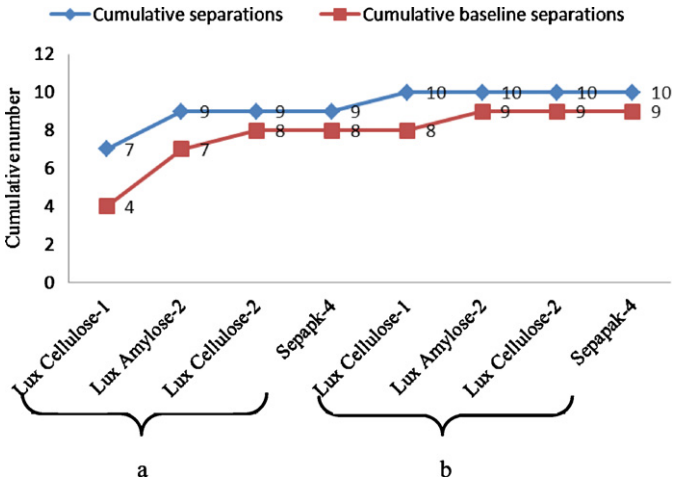

Fig. 7. Cumulative number of separations and cumulative number of baseline separations of non-basic compounds on the recently commercialized CSPs using two mobile phases: (a) heptane–EtOH–TFA and (b) heptane–IPA–TFA.

tional separations compared to the EtOH containing mobile phase. Table 3 emphasizes the complementarity between the two mobile phases, i.e. the IPA containing mobile phase succeeded in the enantioseparation of some compounds that were not resolved with the EtOH containing mobile phase. The preferred column sequence for this dataset was Lux® Cellulose-1 > Lux® Amylose-2 > Lux® Cellulose-2 > Lux® Cellulose-4. The cumulative numbers of separations and baseline resolved analytes on the four recently commercialized columns is 44 out of 49 (89.8%) (see Fig. 6).

3.2.2. Analysis of acidic, bifunctional and neutral compounds

The twelve non-basic compounds were also examined on the Lux columns. Resolution values are given in Table 4. In EtOH containing mobile phase seven separations were achieved on each of Lux® Cellulose-1, Lux® Cellulose-2 and Lux® Amylose-2, and five separations on Lux® Cellulose-4, whereas in IPA containing mobile phase less enantioselectivity was noticed. Still, one additional separation could be achieved on each of Lux® Cellulose-1, Lux® Cellulose-2 and Lux® Cellulose-4 (Fig. 4). The preferred sequence for screening is Lux® Cellulose-1 > Lux® Cellulose-2 > Lux® Amylose-2 > Lux® Cellulose-4. The cumulative numbers of separated analytes and baseline resolutions on Lux columns is 10 out of 12 (83.3%) and is shown in Fig. 7. The maximum enantioseparation of acidic, bifunctional and neutral compounds for the considered test set was reached using only two columns (Lux Cellulose-1 and Lux Amylose-2) and the two mobile phases

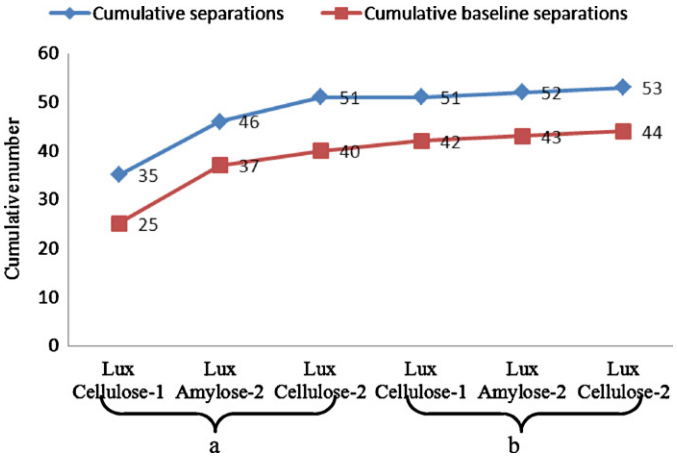

Fig. 8. Cumulative number of separations and cumulative number of baseline separations from entire test set on three of the recently commercialized CSPs using two mobile phases: (a) heptane–EtOH–additive and (b) heptane–IPA–additive.

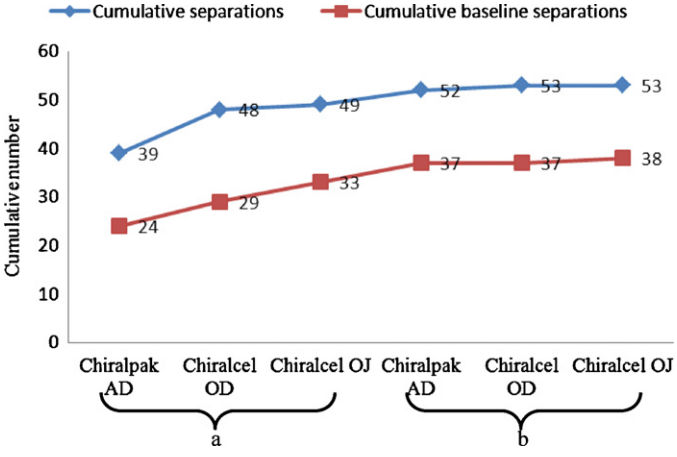

Fig. 9. Cumulative number of separations and cumulative number of baseline separations from entire test set on the initial CSPs using two mobile phases: (a) heptane–EtOH–additive and (b) heptane–IPA–additive.

Table 4
Resolution values (*Rs*) for non-basic compounds on the recently commercialized CSPs, obtained with two mobile phases: (a) heptane–IPA–TFA and (b) heptane–EtOH–TFA, both (90:10:0.1, v/v/v).

	Sepapak-1 (Lux-C1)		Sepapak-2 (Lux-C2)		Sepapak-3 (Lux-A2)		Sepapak-4 (Lux-C4)	
	a	b	a	b	a	b	a	b
Acenocoumarol	**3.56**	5.03	n.p.	**1.74**	n.p.	n.p.	n.p.	**1.68**
Chloorthalidon	0.00	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.	n.p.
Fenoprofen	1.40	0.63	1.18	0.48	5.24	9.56	1.59	n.p.
Flurbiprofen	1.33	0.53	0.00	0.00	1.05	1.10	0.00	0.00
Hexobarbital	3.99	2.80	0.00	**10.42**	11.16	**23.14**	n.p.	**5.69**
Ibuprofen	0.83	0.79	1.62	0.00	0.00	0.00	0.00	0.00
Ketoprofen	0.67	0.00	1.23	0.35	1.06	2.13	0.00	n.p.
Mandelic acid	3.22	1.50	0.67	0.83	4.84	1.44	4.17	1.67
Naproxen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Oxazepam	0.00	**8.59**	n.p.	n.p.	n.p.	**1.87**	n.p.	n.p.
Suprofen	0.00	n.p.	1.51	1.28	0.00	**4.09**	2.51	3.65
Warfarine	0.00	n.p.	**6.80**	3.56	0.00	0.00	0.00	3.16

The substances in bold have a retention time exceeding 20 min. n.p., no peak observed after 60 min of analysis.

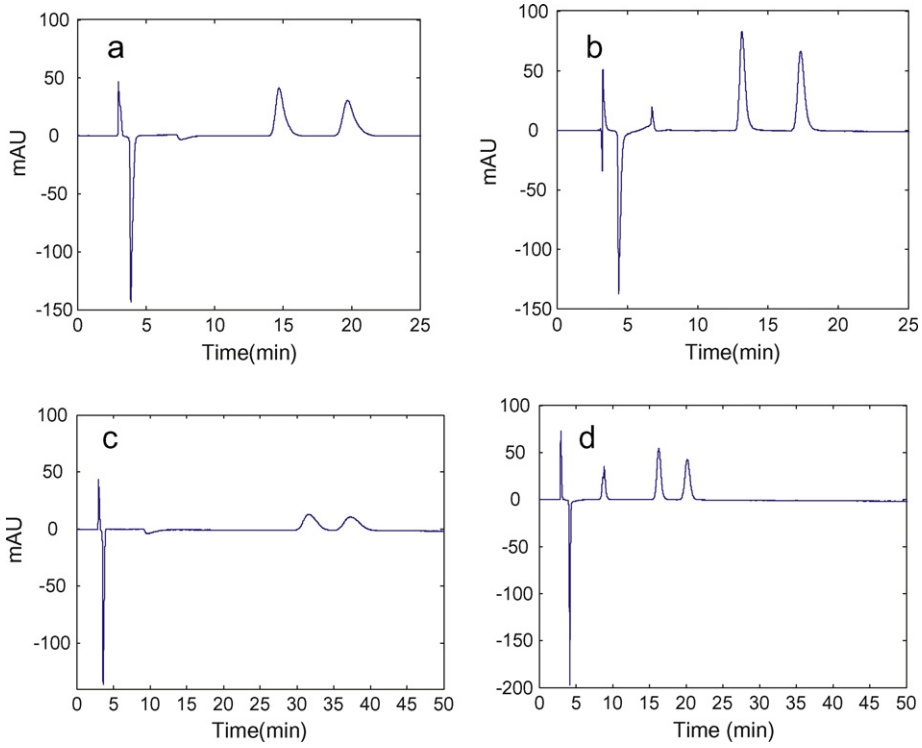

Fig. 10. Chromatograms showing the separation of pindolol on (a) Chiralpak AD and (b)Lux Amylose-2, and of carazolol on (c) Chiralcel OD and (d) Lux Cellulose-2. Mobile phase: heptane–EtOH–DEA (90:10:0.1, v/v/v), flow rate: 1 ml/min, temperature 20 °C.

(four chromatographic systems) while Lux Cellulose-2 and Lux® Cellulose-4 did not add any additional separations. IPA allowed for only one additional separation and could be omitted from the screening step for these analytes.

3.2.3. Comparison and combination between the initial and the recently commercialized CSPs

After screening both the initial and the recently commercialized set of CSPs, the total number of separations on the four recently commercialized polysaccharide based columns (4 × 2 systems) was found to be similar to that obtained on the initial set of columns part of the screening step (3 × 2 systems), i.e. 54 versus 53. When the evaluation is limited to only the best three of the recently commercialized CSPs, i.e. Lux® Cellulose-1, Lux® Cellulose-2 and Lux® Amylose-2, the total number of separations is found to be 53 (Fig. 8) which is the same as on the initial CSPs (Fig. 9). However, Lux columns had the advantage that more compounds were baseline separated. Forty-four drugs were baseline resolved with a Lux column, while only 38 compounds with the Daicel CSPs. The peak shape obtained with the Lux columns was also better than that obtained with the columns of the existing strategy (see Fig. 10 for some examples). The analysis times on the recently commercialized CSPs also can be considered advantageous over Daicel columns since most compounds eluted within 20 min. A limited number of compounds only eluted after 20 min, these substances are marked in bold in Tables 3 and 4 (most of them are baseline resolved). Another observation for the recently commercialized CSPs is that they show enantioselectivity for two compounds that were not resolved by the other CSPs. These compounds are carvedilol and

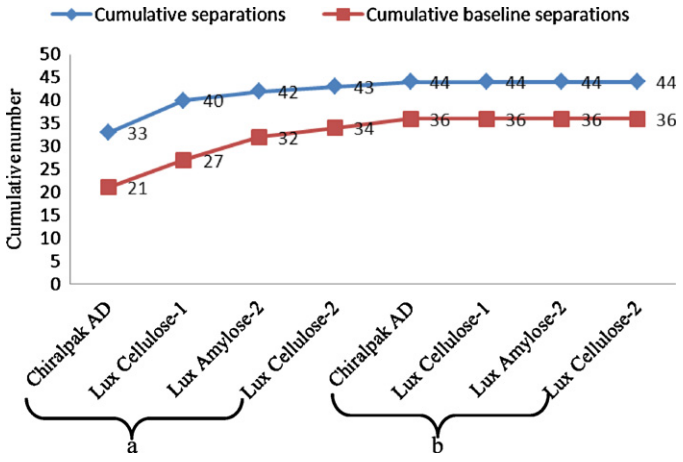

Fig. 11. Cumulative number of separations and cumulative number of baseline separations of basic compounds on the most successful CSPs using two mobile phases: (a) heptane–EtOH–DEA and (b) heptane–IPA–DEA.

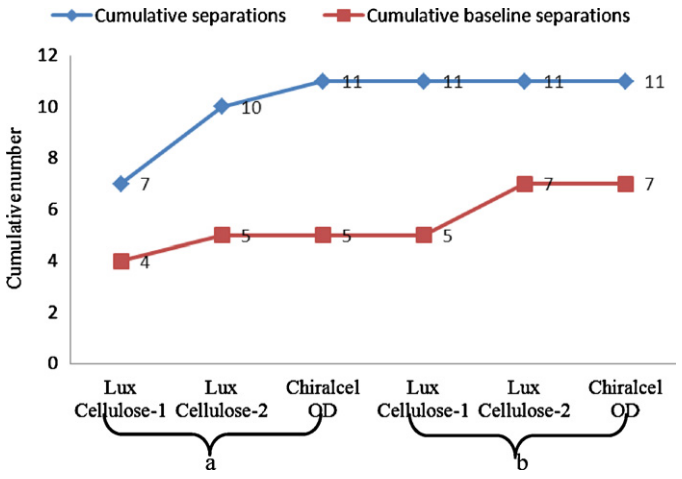

Fig. 12. Cumulative number of separations and cumulative number of baseline separations of basic compounds on the most successful CSPs using two mobile phases: (a) heptane–EtOH–TFA and (b) heptane–IPA–TFA.

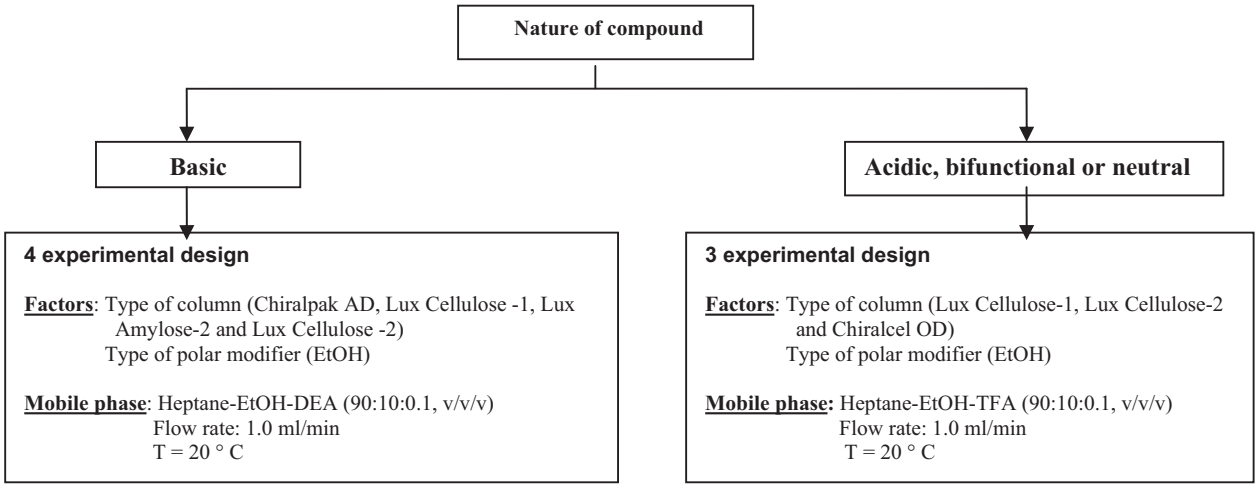

Fig. 13. Updated screening strategy in NPLC.

procyclidine, which could be separated on Lux® Amylose-2 and Lux® Cellulose-2, respectively.

Combination of the best Lux and Daicel CSPs for the screening of the tested basic compounds led to the separation of 44 compounds (Fig. 11). The IPA containing mobile phase (b) seems to have few benefits during the screening step. Thus 43/49 (89.8%) of the tested basic compounds were separated using four stationary phases with the EtOH containing mobile phase. A 44th compound was separated on Chiralpak AD column using the mobile phase with IPA. Thus by screening only four columns with one mobile phase, only for one compound enantioselectivity is not seen compared to the eight systems or even to the 12.

For the non-basic compounds, by combining the results on the most successful Lux and Daicel columns 11 compounds out of 12 (91.7%) can be separated (Fig. 12). In this case, the second mobile phase offers no additional benefit to the overall success of the strategy and therefore can be omitted (as before for the basic compounds). Hence, by selecting the most successful CSPs, 11 separations can be achieved with only three systems.

It is worth noticing that the results obtained with Lux® Cellulose-1, an alternative CSP to Chiralcel OD (both have the same chiral selector), are somewhat different from those on Chiralcel OD (see Figs. 2 and 4).

3.3. Screening strategy updates

The four recently commercialized chiral stationary phases, evaluated in this work, showed a high ability to separate the test set of chiral pharmaceutical compounds. The different CSPs have complementary chiral recognition ability leading to the higher overall success rates. Updating the current screening strategy might be done either by replacing the initial CSPs by new CSPs or by combining both sets of CSPs and selecting those that give the maximum number of separations for the test set studied. In both cases the mobile phase with ethanol will be the first choice because it ensured the highest enantioselectivity. Alternatively, the preferred sequence of CSPs to be screened can be determined based on the maximal enantioselectivity. If the second approach is chosen the sequence of the stationary phases is determined by the maximal cumulative increase in separations observed (see Figs. 11 and 12). The preferred sequence then is Chiralpak® AD > Lux® Cellulose-1 > Lux® Amylose-2 > Lux® Cellulose-2 for basic compounds and Lux® Cellulose-1 > Lux® Cellulose-2 > Chiralcel® OD for screening non-basic pharmaceutical chiral compounds. It can be noticed for the non-basic compounds (which is a minority of the drug molecules) that both Lux Cellulose-1 and Chiralcel OD (having the same selector) are included. This is a consequence of the different and complementary enantioselectivity of the two CSPs. Fig. 13 presents an updated screening strategy based on the above. In the updated strategy, EtOH is the only polar modifier used in the screening step since IPA added little value to the cumulative success rate. Therefore the use of IPA as polar modifier might be useful during the optimization phase of the strategy for compounds that do not show enantioselectivity with EtOH. Given the fact that Chiralcel OD has the same selector as Lux Cellulose-1, one might consider performing the screening for non-basic compounds on the two Lux columns (Lux Cellulose-1 and Lux Cellulose-2) and only test the Chiralcel OD column is a later optimization stage of the strategy.

4. Conclusion

Earlier described generic screening conditions in NPLC were applied on four recently commercialized CSPs and on the three initial CSPs to evaluate their enantioselectivity towards a set of 61 pharmaceutical compounds. The current outcome shows that the mobile phase with ethanol as polar modifier results in systems with the broadest enantioselectivity and suggests the elimination of the mobile phase containing IPA from the screening step because of only limited complementary selectivity. Under the initial screening conditions, the initial CSPs provided enantioseparation for 53 compounds, with 38 of them baseline separated whereas the recently commercialized CSPs gave 54 separations with 44 baseline separated. When limiting the screening to the four most successful CSPs, Chiralpak® AD, Lux® Cellulose-1 (Sepapak-1), Lux® Cellulose-2 (Sepapak-2) and Lux® Amylose-2 (Sepapak-3), the enantioseparation of 54 compounds out of 61 (88.5%) was achieved. The analysis times on the recently commercialized CSPs are considered advantageous since most compounds eluted within 20 min. Peak shapes observed with the recently commercialized CSPs are better than these obtained with the initial ones.

In summary, the recently commercialized polysaccharide-based chiral stationary phases show additional enantiorecognition abilities compared to the initial polysaccharide-based selectors. Including them in a screening strategy, either by themselves or in combination with the old phases, results in improved success rates compared to the initial protocol.

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