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Chiral separations in polar organic solvent chromatography: Updating a screening strategy with new chlorine-containing polysaccharide-based selectors☆

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ABSTRACT

The screening conditions of an existing column and mobile phase selection strategy for chiral drug substances in polar organic solvent liquid chromatography (POSC) were tested for their applicability on two new chlorine-containing polysaccharide-based stationary phases. The selectors of these phases are cellulose tris(3-chloro-4-methylphenylcarbamate) and amylose tris(5-chloro-2-methylphenylcarbamate). The enantioselectivity of these phases is compared to that of the four phases (Chiralpak® AD-RH, Chiralcel® OD-RH, Chiralpak® AS-RH and Chiralcel® OJ-RH) used in the earlier defined strategy. A test set of 62 structurally diverse chiral drug substances is analyzed using the screening conditions of the strategy on the six phases. The results confirm that the acetonitrile-based mobile phase provides a higher success rate and better resolutions than the methanol-based also on the new phases. However, the importance of the methanol-based mobile phase cannot be neglected for complementarity reasons; the two mobile phases insure enantioselectivity for different compounds. A third (ethanol-based) mobile phase, not part of the strategy, was also used to screen the two new phases. The joint results led to different possibilities to upgrade the current screening strategy so that improved success rates are obtained. The chlorine-containing chiral stationary phases demonstrated to have an added value to the screening process since they provided enantioresolution for compounds not resolved by non-chlorine-containing ones.

1. Introduction

Chirality has recently gained a lot of attention in pharmaceutical research. Drug substances administered as racemates can have severe side effects due to the activity of the distomer, i.e. the enantiomer that does not posses the therapeutic activity. In the best case distomers have no activity at all, but in many cases they prevent full activity of the eutomer, i.e. the therapeutically active enantiomer. They can also have an antagonistic or even a toxic effect when exposed to chiral environments. Because of the pharmacological, pharmacokinetic and toxicological differences between the distomer and the eutomer in a chiral environment, such as the human body, chiral analysis became mandatory in drug research [1,2].

Therefore, during drug development a thorough assessment of the potential chiral drug candidates is required. Several authorities, responsible for the authorization of human medicinal products, such as the Food and Drug Administration (FDA) in the US and the European Medicines Evaluation Agency (EMEA), have imposed guidelines to enable good assessment. According to these guidelines, new chiral drug molecules have to be tested in their various stages of development. This must be done with the racemate as well as with the single-enantiomer drug [3,4]. Hence, there is a continuous need for proper techniques and conditions enabling chiral separations to ensure the quality of a chiral drug. Different analytical and preparative separation techniques can be applied to separate chiral compounds [5–11]. The choices of technique, but also of chiral selector and of analysis conditions, such as mobile phase composition, use of additives or analysis temperature have to be made because these parameters may affect chiral resolution. All these factors and their combinations make the task of finding proper analysis conditions, ensuring enantioresolution, challenging.

To avoid time-consuming trial-and-error approaches, generic strategies have been developed in different techniques (NPLC, RPLC, POSC, SFC, CE, CEC) to help the analyst with chiral method development [12–18]. In most cases a strategy is built as a decision tree where each experimental step is defined based on results obtained

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in the previous step. The first step of the strategy is a screening consisting of a limited number of experiments that occasionally can be executed sequentially in a predefined sequence. Since the resolution (Rs) is the most important quality parameter for enantioseparation, the results of the screening step are evaluated by this parameter. Three situations possibly originating from the screening experiments are considered, resulting in three branches of the decision tree. The first branch deals with compounds that are not separated (Rs = 0). A second branch is defined for the compounds that show a partial separation or a beginning of separation ($0 < Rs < 1.5$), while a third branch handles the compounds that are baseline separated ($Rs \geq 1.5$). For each branch, limited optimization steps are defined to improve the results and to reach baseline separation. When compounds are already baseline separated after the screening ($Rs \geq 1.5$), an optimization step for the retention time (fast analysis) and/or the peak shape (sharp and symmetric peaks) can be included optionally. These strategies can also be applied for the development of a method to assay the distomer as impurity in single-enantiomer drug formulations. However, in these cases a resolution of 1.5 might be insufficient [14]. A further optimization of the resolution is then required. This can also be the case when a single-enantiomer formulation is administered and the enantiomer blood levels are measured for metabolization and/or racemization studies of the eutomer.

Whether analytical enantioseparations, preparative scale enantioseparations or impurity determinations are concerned, various strategies have been reported for analytical and preparative enantioseparation [12–19]. Within the HPLC technique, the analyst has various choices in regards of separation modes to be screened. Besides the well-known normal- (NPLC) and reversed phase (RPLC) modes [16], also in polar organic solvent chromatography (POSC) strategies are available [17–19]. This latter mode offers an alternative when no enantioseparation is achieved with NPLC or RPLC. Moreover pure organic solvents may provide a better solubility for some analytes, they can easily be evaporated, which is important in industrial-scale enantioseparation, and are less hazardous for human health and ecology than the apolar solvents used in NPLC [20,21]. It has also been observed that separations in POSC, in general, were faster than in NPLC [18,20–22]. In the POSC strategy defined by Matthijs et al. [18] the first step consists of a sequential screening of the tested compound on four polysaccharide-based chiral stationary phases (CSPs) with two different mobile phases. The first mobile phase is acetonitrile-based (ACN) while the second is methanol-based (MeOH). To each mobile phase, two additives are added, diethylamine (DEA) and trifluoroacetic acid (TFA). It is generally accepted that basic and acidic additives enhance the separation quality, both in terms of selectivity and peak shapes (efficiency) [23,24]. Thus the first mobile phase applied is ACN/DEA/TFA (100/0.1/0.1) (v/v/v), while the second is MeOH/DEA/TFA (100/0.1/0.1) (v/v/v). As mentioned above, both mobile phases are used on four polysaccharide-based CSPs in a predefined sequence, i.e. Chiralpak® AD-RH (amylose tris(3,5-dimethylphenylcarbamate)), Chiralcel® OD-RH (cellulose tris(3,5-dimethylphenylcarbamate)), Chiralpak® AS-RH (amylose tris[(S)-α-methylbenzylcarbamate]) and Chiralcel® OJ-R (cellulose tris(4-methylbenzoate)). These CSPs are actually the most widely used selectors because of their broad enantiorecognition ability [25,26].

However, there is no universal chiral selector which separates all possible compounds and hence the need to develop new selectors remains [20,21]. According to the literature, several new cellulose and amylose derivatives have been synthesized and tested [27–30]. Especially the chlorine-containing cellulose and amylose phenylcarbamate derivatives were investigated [31,32]. It was already known that these chlorine-containing derivatives were selectors with high chiral recognition ability but due to stability problems when applying some solvents, these selectors were limited in use [21,31–34]. After overcoming these problems, high recognition abilities were shown with the tested mobile phases, being in most cases mixtures of alkanes (hexanes and heptanes) and alcohols. Hence cautiousness is advisable with other solvents. However, in order to fully evaluate their potential as CSP, new phases should be also tested in combination with other mobile phases. In the context of an established screening strategy, novel CSPs should be evaluated under the conditions specific to this strategy and also compared in regards of success rate to traditional CSPs. In order to update the strategy in current use [18], the novel CSPs should be first evaluated under the conditions specific to the current strategy if applicable, but also in combination with alternative experimental conditions which are more applicable with the novel phases. The purpose of this evaluation is to see if the novel CSPs show either broader or complementary enantioselectivity when compared to the CSPs already included in the screening strategy. In case of a positive evaluation the new CSPs may be included in the screening strategy or may replace a CSP already part of the strategy. When the new CSP gives better results with other conditions than those in the strategy, a new screening step or a new branch in the decision tree can be defined.

In this paper, two recently commercialized chlorine-containing polysaccharide-based CSPs, Sepapak®-2 (cellulose tris(3-chloro-4-methylphenylcarbamate)) and Sepapak®-3 (amylose tris(5-chloro-2-methylphenylcarbamate)) are examined. These phases are tested with the above mentioned mobile phases part of the POSC strategy of N. Matthijs et al. [18]. The enantioselectivity of these new CSPs will be compared to that of the four polysaccharide-based phases without chlorine substituents used in the strategy. In addition, a third ethanol-based mobile phase (EtOH/DEA/TFA (100/0.1/0.1) (v/v/v)), was tested.

2. Experimental

2.1. Chemicals and reagents

The test set consists of the racemic compounds acebutolol, alprenolol, atenolol, atropine, betaxolol, chlorthalidone, diltiazem, ephedrine, fenoprofen, ibuprofen, ketoprofen, labetalol, mandelic acid, nadolol, naproxen, naringenin, oxazepam, pindolol, praziquantel, promethazine, 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), leucovorin and 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, Ohio, 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.

All compounds were dissolved in the mobile phase's main solvent, i.e. acetonitrile, methanol or ethanol. When solubility problems occurred, mixtures of 50/50 (v/v) solvent/ethanol have been used. Exceptions were leucovorin and terbutaline, which

were dissolved in MilliQ-water and morphine which was dissolved in solvent/MilliQ-water 50/50 (v/v). Acetonitrile (ACN), methanol (MeOH) and ethanol (EtOH), all HPLC grade, were from Fisher Scientific (Loughborough, Leicestershire, UK), diethylamine (DEA) from Fluka Chemie (Buchs, Switzerland) and trifluoroacetic acid (TFA) from Sigma–Aldrich. MilliQ-water was prepared in house by a MilliQ-System (Millipore, Milford, MA, USA).

2.2. Chromatographic conditions

The chromatographic system was a Merck-Hitachi HPLC system (Tokyo, Japan) consisting of a L-7100 pump, a programmable autosampler L-7250 with a 100 μl loop, a L-7400 UV detector, a D-7000 interface and a L-7360 column oven. All chromatographic data were managed with the D-7000 HPLC System Manager software (Merck-Hitachi, Ltd. 1994–2001, version 4.1). All sample solutions had a concentration of about 250 μg/ml. 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 0.5 ml/min. The detection wavelength was set at 220 nm to ensure that every compound is detected. The Sepapak® columns were from Sepaserve (Münster, Germany) and the other four (Chiralpak® AD-RH, Chiralcel® OD-RH, Chiralpak® AS-RH and Chiralcel® OJ-R) from Chiral Technologies Europe (Illkirch, France). The particle size in all columns is 5 μm, the dimensions of the Sepaserve columns is 25 cm × 0.46 cm while these of the other columns were 15 cm × 0.46 cm.

2.3. Data processing

The resolution (Rs) has been calculated with the equation of the United States Pharmacopoeia [35]:

$$\mathrm{Rs} = \frac{2(t_{r2} - t_{r1})}{w_1 + w_2}$$

where t_{r1} and t_{r2} are the retention times in minutes of, respectively, the first and the last eluting peak of a pair, while w_1 and w_2 are the baseline widths in minutes of these peaks.

3. Results and discussion

The screening conditions of the used strategy have earlier been defined using a set of 28 chiral compounds and were then evaluated with a set of 38 compounds [17,18]. In our study the test set consisted of 62 compounds, with different pharmacological activities and various molecular structures. These compounds were analyzed following the screening steps of the strategy, which means a sequential testing of two mobile phases on the four non-chlorinated columns as well as on the two new, chlorinated columns. The executed experiments are from the screening part of the strategy and the separation results can be improved later in the optimization steps. It is important to note that for this study every compound with a Rs > 0, i.e. for which enantioselectivity was observed, was considered as 'separated' in the further discussion. Almost all analytes eluted in an analysis time considered acceptable, which was less than 20 min.

The results of this screening are shown in Tables 1 and 2 and in Fig. 1. The ACN-based mobile phase results in a broader enantioselectivity range than the MeOH-based on four of the six phases. Sepapak®-2 has the broadest enantioselectivity for the used test set, followed by the Chiralcel® OD-RH and the Chiralpak® AS-RH columns. The enantioselectivity of Sepapak®-3 is somewhat lower but comparable in number of separated compounds to Chiralcel® OJ-R and Chiralpak® AD-RH. These results make it worthwhile to examine them more closely.

3.1. CSPs without chlorine substituents

After screening the test set with the prescribed mobile phases on the four CSPs, the preferred column sequence for this dataset was Chiralcel® OD-RH > Chiralpak® AS-RH > Chiralcel® OJ-

Table 1
The resolutions (Rs) on the chlorine-containing polysaccharide-based columns obtained with three different mobile phases being ACN/DEA/TFA, MeOH/DEA/TFA and EtOH/DEA/TFA (100/0.1/0.1) (v/v/v)

	Sepapak-2 (Rs)			Sepapak-3 (Rs)		
	ACN	MeOH	EtOH	ACN	MeOH	EtOH
1. Acebutolol	0.61	0.00	0.00	5.03	0.00	0.00
2. Acenocoumarol	0.43	0.00	0.29	0.52	0.00	3.01
3. Alprenolol	0.00	0.00	0.00	0.00	0.00	0.00
4. Ambucetamide	0.38	0.00	0.00	0.00	0.00	0.00
5. Atenolol	**7.41**[a]	0.00	0.00	0.00	0.00	0.00
6. Atropine	0.40	0.00	0.00	0.00	0.00	0.00
7. Betaxolol	2.45	0.00	0.00	0.00	0.00	0.00
8. Bisoprolol	0.00	1.17	0.00	0.00	0.00	0.00
9. Bopindolol	0.00	5.31	0.00	0.00	4.27	0.00
10. Bupranolol	0.00	0.00	0.00	0.00	0.00	0.00
11. Carazolol	0.62	0.00	0.00	0.00	0.00	0.00
12 Carbinoxamine	**4.50**[a]	8.85	7.57	3.36	0.99	3.46
13. Carvedilol	0.00	0.00	0.00	0.00	0.00	0.00
14. Chlorphenamine	**10.90**[a]	9.64	11.59	0.00	0.00	1.98
15. Chlorthalidone	1.98	0.59	1.21	0.00	0.00	0.00
16. Cocaine	0.00	0.00	0.00	0.00	0.00	0.00
17. Diltiazem	0.00	0.00	0.00	0.00	0.00	0.00
18. Dimethindene	**18.94**[a]	8.49	8.36	2.03	1.84	3.10
19. Ephedrine	0.00	0.00	0.00	0.00	0.00	0.00
20. Esmolol	1.58	0.00	0.00	0.00	0.00	0.00
21. Fenoprofen	0.00	0.00	0.00	0.00	0.00	1.05
22. Flurbiprofen	0.00	0.00	0.00	0.00	0.00	0.00
23. Hexobarbital	2.68	4.58	8.02	0.32	0.00	12.06
24. Ibuprofen	0.00	0.00	0.00	0.00	0.00	0.00
25. Isothipendyl	0.00	0.00	0.00	0.00	0.00	0.00
26. Ketoprofen	0.00	0.00	0.00	0.00	0.00	1.28
27. Labetalol	0.00	0.00	0.00	0.00	0.00	0.00
28. Leucovorin	0.00	2.75	0.00	0.00	0.20	0.16
29. Mandelic acid	0.00	0.00	0.00	0.00	0.00	0.00
30. Mebeverine	0.00	0.51	0.62	0.00	0.00	0.00
31. Mepindolol	1.20	0.00	0.00	0.00	0.00	0.00
32. Meptazinol	0.00	0.00	0.00	0.00	0.00	0.00
33. Methadone	0.52	0.00	0.00	0.00	0.00	0.00
34. Metoprolol	0.00	0.00	0.00	0.00	0.00	0.00
35. Mianserin	2.18	0.00	0.00	0.45	0.00	0.00
36. Morphine	0.68	0.00	0.00	0.00	0.00	0.00
37. Nadolol	0.00	0.00	0.00	0.00	0.00	0.00
38. Naproxen	0.00	0.00	0.00	0.00	0.00	0.00
39. Naringenin	0.00	0.00	0.00	0.00	0.00	0.00
40. Nicardipine	0.41	0.00	0.00	0.00	0.00	0.00
41. Nimodipine	1.01	0.32	0.00	0.00	0.00	0.00
42. Nisoldipine	2.24	0.86	0.38	0.00	0.00	0.00
43. Nitrendipine	0.00	0.00	0.00	0.00	0.00	0.00
44. Oxazepam	2.11	1.21	0.00	1.07	0.00	0.66
45. Oxprenolol	0.93	0.00	0.00	0.00	0.00	0.00
46. Pindolol	0.83	0.00	0.00	0.00	0.00	0.00
47. Praziquantel	**0.00**[a]	**5.78**[a]	**6.18**[a]	1.05	0.65	**3.38**[a]
48. Procyclidine	0.00	0.00	0.00	0.00	0.00	0.00
49. Promethazine	0.00	0.00	0.00	0.00	0.00	0.00
50. Propiomazine	0.00	9.56	8.51	0.89	0.88	1.59
51. Propranolol	0.88	0.00	0.00	0.00	0.00	0.00
52. Salbutamol	11.17	0.00	0.00	0.00	0.00	0.00
53. Salmeterol	2.28	0.00	0.00	0.00	0.00	0.00
54. Sotalol	0.83	0.00	0.00	0.00	0.00	0.00
55. Sulpiride	1.08	0.00	0.72	0.00	0.00	0.00
56. Suprofen	2.56	0.00	0.00	2.03	0.00	3.55
57. Terbutaline	0.00	0.00	0.00	0.00	0.61	0.37
58. Tertatolol	3.30	0.00	0.00	0.00	0.00	0.00
59. Tetramisole	**5.67**[a]	0.00	0.00	0.00	0.00	0.00
60. Timolol	19.01	10.84	11.18	2.09	0.00	0.00
61. Verapamil	0.00	0.00	0.00	0.00	0.00	0.00
62. Warfarin	0.00	0.95	1.09	2.53	0.00	0.00

[a] The substances in bold have a retention time exceeding 30 min.

Table 2
The resolutions (Rs) on the no chlorine-containing columns obtained with the two prescribed mobile phases being ACN/DEA/TFA and MeOH/DEA/TFA, both (100/0.1/0.1) (v/v/v)

	Chiralpak AD-RH (Rs)		Chiralcel OD-RH (Rs)		Chiralpak AS-RH (Rs)		Chiralpak OJ-R (Rs)	
	ACN	MeOH	ACN	MeOH	ACN	MeOH	ACN	MeOH
1. Acebutolol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2. Acenocoumarol	0.91	1.40	0.00	1.98	0.65	0.40	0.00	0.40
3. Alprenolol	0.00	0.00	1.79	0.00	0.00	0.00	0.00	0.00
4. Ambucetamide	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5. Atenolol	0.00	0.00	1.79	0.00	6.84	0.00	0.00	0.00
6. Atropine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7. Betaxolol	0.00	0.00	6.00	0.33	0.00	0.00	0.00	0.00
8. Bisoprolol	0.00	0.91	0.00	0.00	0.00	0.00	0.00	0.00
9. Bopindolol	0.00	4.22	0.00	0.90	0.00	3.84	0.00	3.06
10. Bupranolol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
11. Carazolol	0.00	0.00	0.00	0.00	0.27	0.00	0.00	0.00
12 Carbinoxamine	2.54	0.00	2.78	0.00	0.49	0.00	8.14	0.00
13. Carvedilol	0.00	0.00	0.66	1.66	0.00	0.00	0.00	0.00
14. Chlorphenamine	0.00	0.00	0.64	0.00	0.00	0.00	4.67	0.00
15. Chlorthalidone	1.43	0.71	0.00	0.00	5.52	0.43	0.00	0.00
16. Cocaine	0.00	0.00	0.00	0.00	0.64	0.00	0.00	0.00
17. Diltiazem	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18. Dimethindene	1.34	0.00	1.66	3.47	0.00	0.00	6.20	0.00
19. Ephedrine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20. Esmolol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
21. Fenoprofen	0.00	0.00	0.00	0.00	0.00	0.00	1.60	0.00
22. Flurbiprofen	0.49	1.80	0.00	0.00	0.00	0.00	0.00	1.05
23. Hexobarbital	3.45	7.83	0.00	0.00	0.53	0.69	0.00	0.00
24. Ibuprofen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80
25. Isothipendyl	0.00	0.00	0.76	0.00	1.10	0.00	0.78	0.00
26. Ketoprofen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
27. Labetalol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
28. Leucovorin	0.00	0.22	0.00	0.00	0.00	0.52	0.00	0.00
29. Mandelic acid	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30. Mebeverine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
31. Mepindolol	0.00	0.00	5.42	1.78	0.88	0.00	1.28	0.00
32. Meptazinol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
33. Methadone	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
34. Metoprolol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
35. Mianserin	0.00	0.00	2.10	5.72	0.00	0.00	0.00	0.00
36. Morphine	0.00	0.00	0.00	0.00	0.00	0.00	4.14	0.00
37. Nadolol	0.00	0.00	1.05/0.88/1.02	0.00	0.00	0.00	0.00	0.00
38. Naproxen	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
39. Naringenin	0.21	3.00	0.00	0.00	0.00	0.36	0.00	0.00
40. Nicardipine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
41. Nimodipine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
42. Nisoldipine	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.00
43. Nitrendipine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
44. Oxazepam	0.00	2.25	0.00	5.94	3.88	3.53	0.00	0.26
45. Oxprenolol	0.00	0.00	3.17	0.00	0.00	0.00	0.00	0.00
46. Pindolol	0.00	0.00	6.05	1.18	0.81	0.00	1.22	0.00
47. Praziquantel	0.00	0.00	1.59	0.74	4.60	5.84	0.00	0.57
48. Procyclidine	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
49. Promethazine	0.00	0.00	0.00	0.00	1.40	1.92	0.00	1.59
50. Propiomazine	0.99	0.00	0.00	1.12	1.25	0.00	0.65	0.00
51. Propranolol	0.00	0.00	6.02	0.00	0.00	0.00	0.00	0.00
52. Salbutamol	0.00	0.00	0.96	0.00	0.00	0.00	0.00	0.00
53. Salmeterol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
54. Sotalol	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55. Sulpiride	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
56. Suprofen	0.00	1.83	0.00	0.00	0.00	0.00	0.00	2.36
57. Terbutaline	0.00	0.00	0.00	0.97	0.00	0.00	0.00	0.87
58. Tertatolol	0.00	0.00	3.18	0.00	0.76	0.00	0.00	0.00
59. Tetramisole	0.00	0.00	0.35	0.47	0.00	0.00	0.00	0.00
60. Timolol	0.00	0.00	1.43	0.00	0.00	0.00	0.00	0.00
61. Verapamil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
62. Warfarin	0.74	2.23	2.00	1.46	2.73	1.58	0.56	1.50

R > Chiralpak® AD-RH. The sequence proposed by Matthijs et al. [18] was Chiralpak® AD-RH > Chiralcel® OD-RH > Chiralpak® AS-RH > Chiralcel® OJ-R. The choice of the new sequence is based on the highest number of cumulatively separated compounds using the ACN-based mobile phase: Chiralcel® OD-RH gave 20 separations, Chiralpak® AS-RH was placed second with eight additional separations, while Chiralcel® OJ-R and Chiralpak® AD-RH each had two extra separated analytes. Chiralcel® OJ-R was placed third because of the highest absolute number of separated compounds. Fig. 2 shows the cumulative number of separated analytes, ranked in such a way that first the ACN-based results were considered followed by the MeOH-based because the first mobile phase showed the

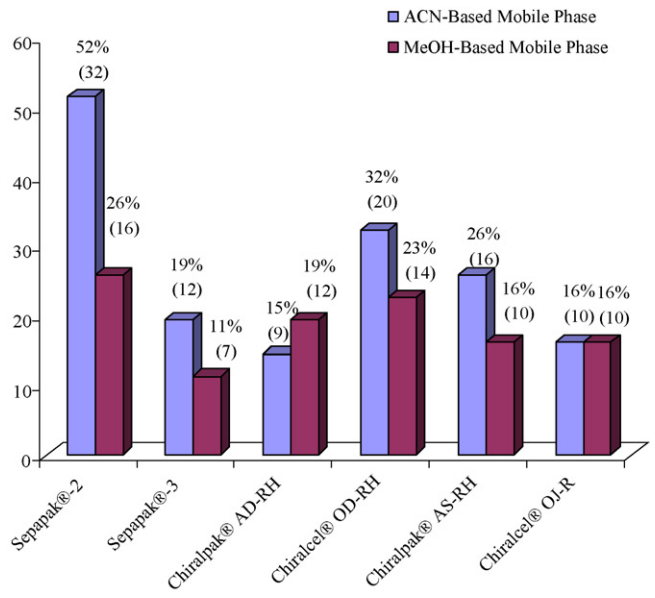


Fig. 1. Enantioselectivity obtained on the different systems, expressed in percentages and in absolute numbers.

broadest enantioselectivity. Screening with the ACN-based mobile phase resulted in a total of 32 separated compounds or 52% of the test set. It can be observed (Figs. 1 and 2) that the column sequences when considering either enantioselectivity (Chiralcel® OD-RH 20 compounds, Chiralpak® AS-RH 16, Chiralcel® OJ-R 10 and Chiralpak® AD-RH 9 substances) or cumulative increase is the same.

For the methanol-based mobile phase, Chiralcel® OD-RH showed again the broadest enantioselectivity with 14 separations, followed by Chiralpak® AD-RH (12), Chiralpak® AS-RH (10) and Chiralcel® OJ-R (10) as can be seen in Fig. 1. A total of 24 compounds (39%) were separated after the screening of the four CSPs with the methanol-based mobile phase. However, since the MeOH-based mobile phase is not the preferred, the identification of the column with broadest enantioselectivity is of limited interest. More interesting is to determine whether it allows increasing the cumulated number of separated compounds, i.e. to evaluate whether the MeOH-based mobile phase allows complementary separations to the ACN-based.

As suggested by Matthijs et al. [17] there is indeed complementarity between both mobile phases. The second mobile phase MeOH/DEA/TFA separated 5 extra compounds (bisoprolol, bopindolol, ibuprofen, leucovorin and terbutaline) on the not substituted CSPs as can be seen in Table 2 and Fig. 2. This results in a total of 37 separations (60%) for our data set with 62 compounds considering the eight chromatographic systems of the initial screening, i.e. the four phases combined with the two mobile phases.

The reason why the preferred column sequence is different in the published strategy compared to that in the strategy proposed here is probably to be found in the fact that a smaller set of test compounds was used in the development of Matthijs' strategy compared to the current study.

3.2. Chlorine-containing CSPs

The defined screening conditions were also executed on the two CSPs with chlorine-containing selectors to see whether or not they are applicable to these phases as well. If so, these new phases easily could be implemented in the battery of columns used in the screening strategy. For both the acetonitrile-based and the methanol-based mobile phases, Sepapak®-2 shows clearly more enantioselectivity than Sepapak®-3. With the acetonitrile-based mobile phase Sepapak®-2 could separate 32 compounds while the number of separated analytes for Sepapak®-3 was 12. Taking complementarity on both CSPs into account, 35 compounds of the test set (56%) could be separated with one mobile phase, ACN/DEA/TFA. For the second mobile phase, Sepapak®-2 gave sixteen and Sepapak®-3 gave seven separations, and jointly 17 (27%) analytes were separated. When considering the results of both mobile phases on both CSPs, 35 separations with the first and 5 additional with the second mobile phase were counted. This represents 40 out of 62 compounds or 65% of the test set that was separated successfully with only two columns and two mobile phases. The total number of separations on the four chlorine-containing polysaccharide systems (two CSPs with two mobile phases) already is higher than that obtained on the eight polysaccharide systems (four no chlorine-containing CSPs with two mobile phases) of the initial strategy, i.e. 40 versus 37.

A third mobile phase was also tested on these chlorine-containing CSPs to see whether additional enantioselectivity could be achieved. This mobile phase, consisting of EtOH/DEA/TFA

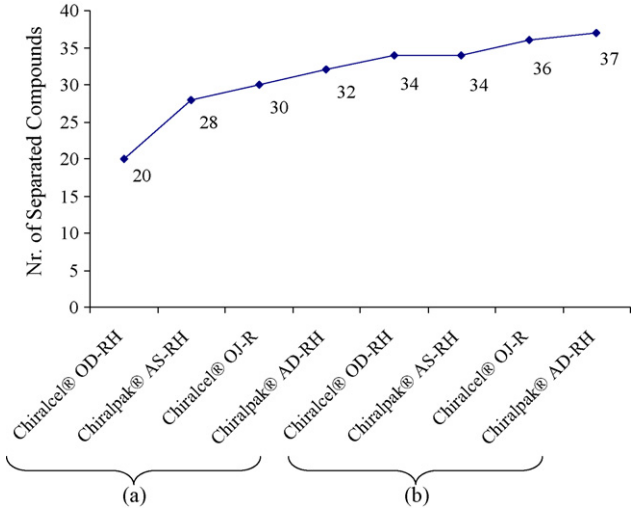


Fig. 2. The cumulative separation results on the no chlorine-containing CSPs with two mobile phases (a) ACN/DEA/TFA and (b) MeOH/DEA/TFA.

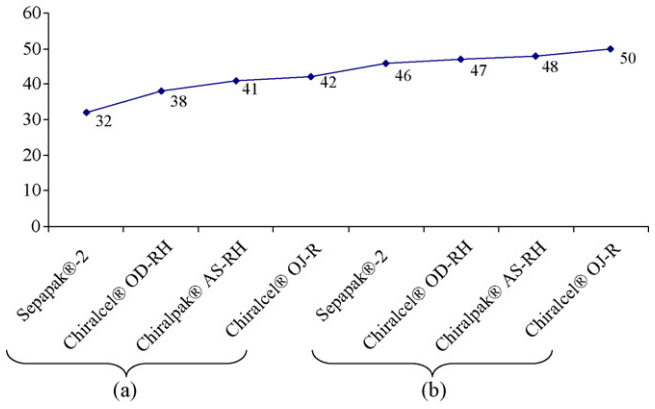
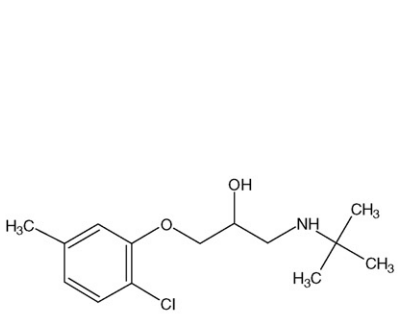
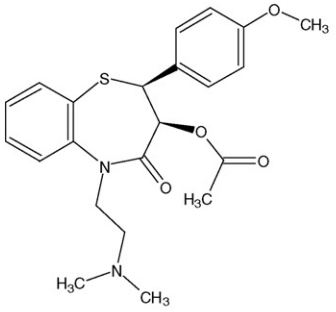


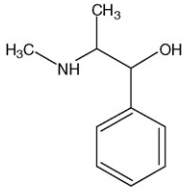
Fig. 3. The cumulative separation results on the CSPs resulting in the highest number of separations with (a) ACN/DEA/TFA and (b) MeOH/DEA/TFA as mobile phase.



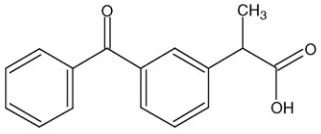
Bupranolol



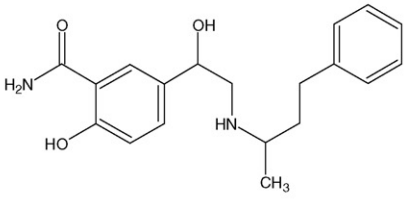
Diltiazem



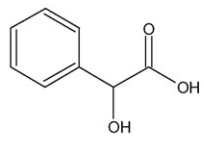
Ephedrine



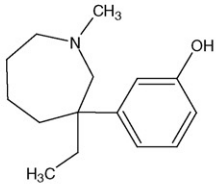
Ketoprofen



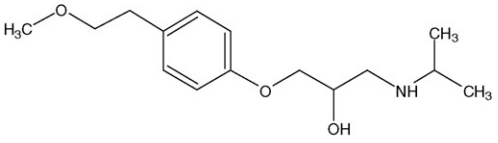
Labetalol



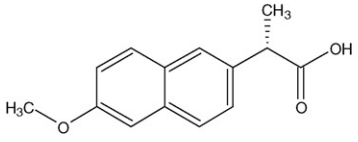
Mandelic Acid



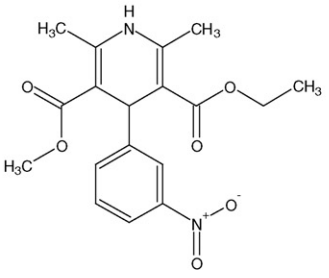
Meptazinol



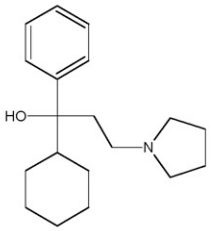
Metoprolol



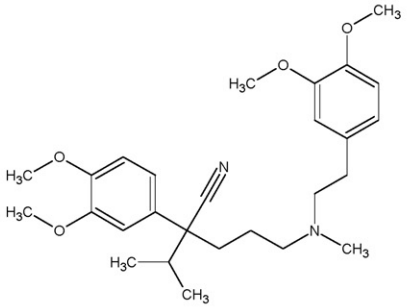
Naproxen



Nitrendipine



Procyclidine



Verapamil

Fig. 4. The compounds that were not separated at any screening condition.

(100/0.1/0.1) (v/v/v), showed enantioselectivity for 13 compounds on Sepapak®-2 and for 13 on Sepapak®-3. The cumulative number of separations with both phases was 19. This is higher than the 17 compounds that were separated on both CSPs with MeOH/DEA/TFA. The benefit of the ethanol-based mobile phase is that two additional compounds (fenoprofen and ketoprofen) are separated compared to the results achieved with the other two mobile phases on the chlorine-containing phases. The above means that when EtOH is used as second mobile phase in total also 40 compounds are separated. When considering the six chromatographic systems 42 compounds (68%) are separated after the screening step. This situation is still more economic in number of chromatographic systems tested than the initial strategy (six versus eight) and it provides five extra separations.

The analysis times on the chlorine-containing CPSs considered in combination with the three mobile phases, are also advantageous since most compounds eluted within 20 min. However, a limited number of compounds eluted in more than 30 min. These substances are marked in bold Table 1. Most of them are baseline resolved.

Another observation for the chlorine-containing CSPs is that they show enantioselectivity for 11 compounds which could not be resolved by the selectors without chlorine. These compounds are acebutolol, ambucetamide, atropine, esmolol, mebeverine, methadone, nicardipine, nimodipine, salmeterol, sotalol and sulpiride. It was Sepapak®-2 that separated all these compounds. Sepapak®-3 showed only enantioselectivity for acebutolol. Except for mebeverine, these separations were achieved with ACN/DEA/TFA as mobile phase. In summary, 11/50 (or 22%) of the test compounds could only be separated in the screening step using a chlorine-containing CSP.

3.3. Strategy updates

In order to improve the success rate of the current strategy, several options become apparent. A first possibility is to abandon the eight non-chlorine-substituted systems and replace them with four or possibly six chromatographic systems involving the two chlorine-containing CSPs. A second possibility is to select eight systems (same workload as initial strategy) which allow the maximum number of separations to be achieved for the test set investigated. In this approach, the sequence of using the mobile phases remains the same as in the initial strategy, which means that first ACN/DEA/TFA is tested before MeOH/DEA/TFA. Secondly the sequence of the stationary phases within both mobile phases is determined by the maximal cumulative increase in separations observed (see Fig. 3). According to this approach 50/62 (or 81%) of the substances were separated. This number is equal to the total number of compounds separated by screening the four no chlorine- and with the two chlorine-containing CSPs. In fact the selection of Fig. 3, where one chlorinated CSP (Sepapak®-2) replaces a no chlorine-containing (Chiralpak® AD-RH), provides 13 additional separations compared to the initial strategy of Fig. 2. For this test set Chiralpak® AD-RH and Sepapak®-3 did not show any complementary separations compared to those observed on the eight systems presented in Fig. 3. This result is in contrast with the findings of Matthijs et al. [18] who were able to separate most of their test compounds on Chiralpak® AD-RH.

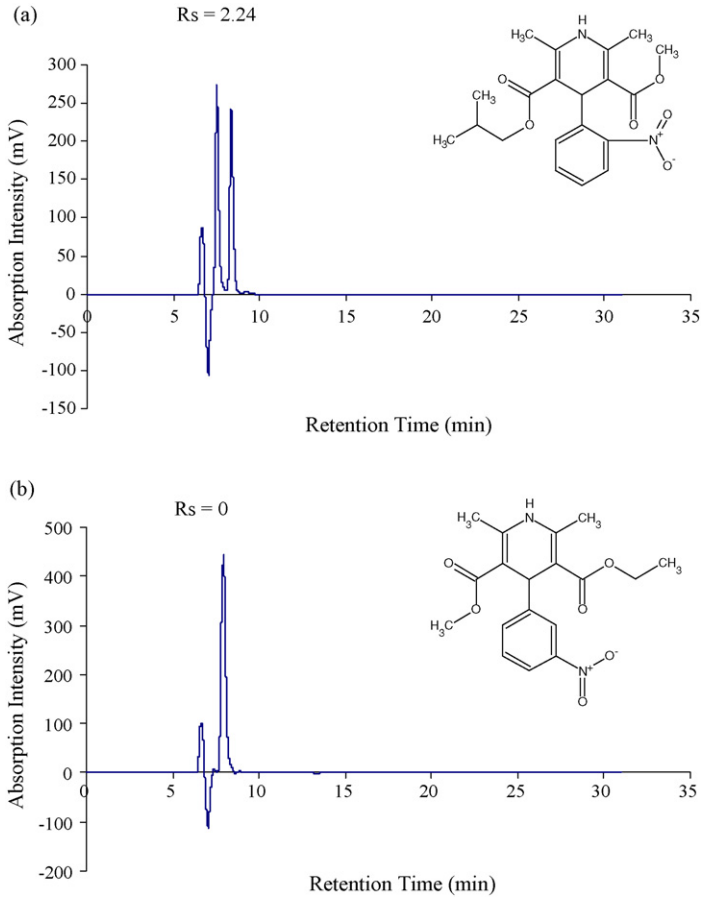


Fig. 5. Chromatograms of two structural analogues (a) nisoldipine and (b) nitrendipine analyzed at the same conditions (see text).

From the 50 compounds that are separated on these four selected phases, 35 showed a Rs ≥ 1.50 which means that a baseline separation was achieved. The analytes that did not show enantioseparation on any system during the screening are shown in Fig. 4. These compounds represent various structural features. A general rule concerning the separation of specific compounds cannot be derived since there are structural analogues of the analytes in Fig. 4 that do show enantioseparation. An example is given in Fig. 5 where the chromatograms of nisoldipine and nitrendipine on Sepapak®-2 with ACN/DEA/TFA as mobile phase are shown. While nisoldipine has a resolution of 2.24, nitrendipine is not separated at all (Rs = 0). This confirms the difficulty in elucidating chiral recognition mechanisms and predicting chiral resolution in any given system.

4. Conclusion

With the introduction of chlorine-containing CSPs on the market, research is needed to evaluate their utility for chiral analysis. The experimental conditions used in this study on two chlorine-containing CSPs were those of an earlier defined generic separation strategy. The two chlorine-containing and the four CSPs without chlorine (included in the original strategy) were tested with a set of 62 pharmaceutical compounds. The current results suggest to use ACN/DEA/TFA as the first choice mobile phase followed by MeOH/DEA/TFA as previously proposed by Matthijs et al. [18] in their screening strategy. However, the preferred sequence of testing the CSPs in the initially strategy changed probably due to the inclusion of a larger test set in the present study. For the initially applied four CSPs the preferred sequence becomes Chiralcel® OD-RH > Chiralpak® AS-RH > Chiralcel® OJ-R > Chiralpak® AD-RH. When considering only the chlorinated CSPs, Sepapak®-2 should be tested first. When choosing the eight chromatographic systems for screening that have the promises of broadest enantioselectivity, the preferred sequence of CSPs is Sepapak®2 > Chiralcel® OD-RH > Chiralpak® AS-RH > Chiralcel® OJ-R, each with the ACN and MeOH-based mobile phases. Based on the current results two phases Sepapak®-3 and Chiralpak® AD-RH, are eliminated since they gave no complementary enantioseparations compared to the first four CSPs. These latter two phases, when available in a lab, could occasionally be included in one of the optimization steps.

The screening on the best eight systems results in a separation of 50/62 compounds (81% of the total test set). A baseline resolution of Rs ≥ 1.5 was achieved for 35/62 (56%) compounds with short analysis times. In summary, the new chlorine-containing polysaccharide-based chiral stationary phases show additional enantiorecognition abilities compared to the polysaccharide-based selectors without chlorine. Including them in a screening strategy, either alone or in combination with no chlorine-containing phases, results in improved success rates compared to the initial protocol based on screening four no chlorine-containing CSPs.

Acknowledgements

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References

[1] Investigation of Chiral Active Substances, Directive 75/318/EEC as amended http://www.emea.europe.eu/pdfs/human/qwp/3cc29aen.pdf (accessed on February 26, 2008).
[2] ICH Topic Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances http://www.eudora.org/emea.html (accessed on February 26, 2008).
[3] FDA Policy Statement for the Development of New Stereoisomeric Drugs http://www.fda.gov/cder/guidance/stereo.htm (accessed on February 26, 2008).
[4] E. Francotte, in: R. Mannhold, H. Kubinyi, G. Folkers (Eds.), Chirality in Drug Research, Whiley-VCH, Weinheim, Germany, 2006, p. 155.
[5] H. Ates, D. Mangelings, Y. Vander Heyden, J. Pharm. Biomed. Anal. 48 (2008) 288.
[6] G. Gübitz, M.G. Schmid, Biopharm. Drug Dispos. 22 (2001) 291.
[7] D. Mangelings, Y. Vander Heyden, in: E. Grushka, N. Grinberg (Eds.), Advances in Chromatography, CRC Press-Taylor and Francis Group, New-York, USA, 2008, p. 175.
[8] V. Schurig, J. Chromatogr. A 906 (2001) 275.
[9] G. Blaschke, B. Chankvetadze, J. Chromatogr. A 875 (2000) 3.
[10] A. Rizzi, Electrophoresis 22 (2001) 3079.
[11] Y. Zhang, D.-R. Wu, D.B. Wang-Iverson, A.A. Tymiak, Drug Discov. Today 10 (2005) 571.
[12] C. Perrin, V.A. Vu, N. Matthijs, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 947 (2002) 69.
[13] C. Perrin, N. Matthijs, D. Mangelings, C. Granier-Layoux, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 966 (2002) 119.
[14] N. Matthijs, Y. Vander Heyden, Biomed. Chromatogr. 20 (2006) 696.
[15] N. Matthijs, S. Van Hemelryck, M. Maftouh, D.L. Massart, Y. Vander Heyden, Anal. Chim. Acta 525 (2004) 247.
[16] N. Matthijs, C. Perrin, M. Maftouh, D.L. Massart, Y. Vander Heyden, J. Chromatogr. A 1041 (2004) 119.
[17] N. Matthijs, M. Maftouh, Y. Vander Heyden, J. Chromatogr. A 1111 (2006)48.
[18] N. Matthijs, M. Maftouh, Y. Vander Heyden, J. Sep. Sci. 29 (2006) 1353.
[19] T. Huybrechts, G. Török, T. Vennekens, R. Sneyers, S. Vrielynck, I. Somers, LCGC Eur. 20 (2007) 320.
[20] B. Chankvetadze, I. Kartozia, C. Yamamoto, Y. Okamoto, J. Pharm. Biomed. Anal. 27 (2002) 467.
[21] T. Zhang, D. Nguyen, P. Franco, Y. Isobe, T. Michishita, T. Murakami, J. Pharm. Biomed. Anal. 46 (2008) 882.
[22] T. Zhang, C. Kientzy, P. Franco, A. Ohnishi, Y. Kagamihara, H. Kurosawa, J. Chromatogr. A 1075 (2005) 65.
[23] Y.K. Ye, R.W. Stringham, Chirality 18 (2006) 519.
[24] Y.K. Ye, R. Stringham, J. Chromatogr. A 927 (2001) 53.
[25] P. Borman, B. Boughtflower, K. Cattanach, K. Crane, K. Freebairn, G. Jonas, I. Mutton, A. Patel, M. Sanders, D. Thompson, Chirality 15 (2003) S1.
[26] T. Ikai, C. Yamamoto, M. Kamigaito, Y. Okamoto, Chirality 17 (2005)299.
[27] I. Ali, L. Naim, A. Ghanem, H.Y. Aboul-Enein, Talanta 69 (2006) 1013.
[28] A. Ghanem, H. Hoenen, H.Y. Aboul-Enein, Talanta 68 (2006) 602.
[29] A. Ghanem, H.Y. Aboul-Enein, J. Liq. Chrom. Relat. Technol. 28 (2005) 2863.
[30] H.A. Wetli, E. Francotte, J. Sep. Sci. 30 (2007) 1255.
[31] E. Francotte, D. Huynh, J. Pharm. Biomed. Anal. 27 (2002) 421.
[32] B. Chankvetadze, E. Yashima, Y. Okamoto, J. Chromatogr. A 694 (1995) 101.
[33] B. Chankvetadze, L. Chankvetadze, Sh. Sidamonidze, E. Yashima, Y. Okamoto, J. Pharm. Biomed. Anal. 14 (1996) 1295.
[34] B. Chankvetadze, L. Chankvetadze, S. Sidamonidze, E. Kasashima, E. Yashima, Y. Okamoto, J. Chromatogr. A 787 (1997) 67.
[35] The United States Pharmacopeia, USP 25, NF 20, 2002, United States Pharmacopeial Convention, Rockville, MD, pp., 1991.