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Reversed-phase screening strategies for liquid chromatography on polysaccharide-derived chiral stationary phases

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ABSTRACT

Immobilised polysaccharide-based chiral stationary phases (CSPs) are chromatographic materials that combine the remarkable enantioselective performance of the polysaccharide derivatives in addition to solvent versatility for enantiomeric resolution. Their behaviour under normal phase conditions and polar organic mode has been quite extensively discussed in several scientific communications. This article will focus on an approach to developing efficient chiral analytical methods with these immobilised CSPs (CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC) by applying a limited number of mobile phases under reversed-phase conditions. The manuscript will review the development of screening strategies by liquid chromatography for the separation of enantiomers in combination with applications compatible with LC–MS. The rational combination of this technique and the different supports will allow the identification of enantiomeric resolutions in reasonable time frames and with high success rates.

1. Introduction

In the light of published articles in scientific journals, as well as the multitude of industrial applications, coated and immobilised polysaccharide derivatives are considered as the first and broadest choice of selectors to be used as chiral stationary phases (CSPs) for liquid and supercritical fluid chromatography (LC and SFC). Their application domain extensively covers the chiral analytical scale. In addition they have been cited for the majority of the preparative examples described for the separation of enantiomers. A number of publications in recent years have reviewed their use in most of the scientific and industrial groups working in the field [1–14].

Over two decades the coated polysaccharide-derived supports have entered routine use. Since 2004 three immobilised polysaccharide-derived CSPs have become commercially available: CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC. They are based on tris-(3,5-dimethylphenylcarbamate) of amylose, tris-(3,5-dimethylphenylcarbamate) of cellulose and tris-(3,5-dichlorophenylcarbamate) of cellulose, respectively [13–18]. They combine the benefits of polysaccharides – namely their broad application scope and their preparative potential – with those of the immobilisation process, such as CSP robustness and extended range of solvents and applications. Since their introduction in the market several publications have described their use in new separations [13–37].

A previous article of our research team reviewed the guidelines for mobile phase selection in LC and SFC with the immobilised polysaccharide-derived CSPs combined with organic solvent compositions [14]. However, these CSPs can also be very successful in the reversed-phase mode (RP-mode).

A comprehensive report by Tachibana et al. published in 2001 [8] reviewed a broad range of applications on polysaccharide-type CSPs with water-compatible mobile phases. More recently, Huybrechts et al. reported a screening module for resolution of chiral drugs on polysaccharide CSPs under RP conditions [38]. In both articles, however, only coated supports were considered for RP applications. Moreover, LC–MS compatibility of the methods was not or only partially covered.

Although some RP applications of the immobilised CSPs have recently been reported [17,30,34–37], no publication until the moment has covered the whole aspects of a potential screening strategy.

The previous extensive investigations published by our research group were covering the screening with organic solvents mainly in HPLC [14,18], but also introduced some clues for SFC [14]. As a logical sequence, an intensive study on the chromatographic behaviour of the immobilised CSPs towards various mobile phase systems was undertaken in our labs and allowed us to develop simple and straightforward strategies for efficient method development. The present article will focus on a general screening methodology to be applied to the three immobilised polysaccharide-derived phases, but also to be possibly extended in the main trends to the coated supports. The compatibility of the methods with samples in aqueous media, as well as with LC–MS detection

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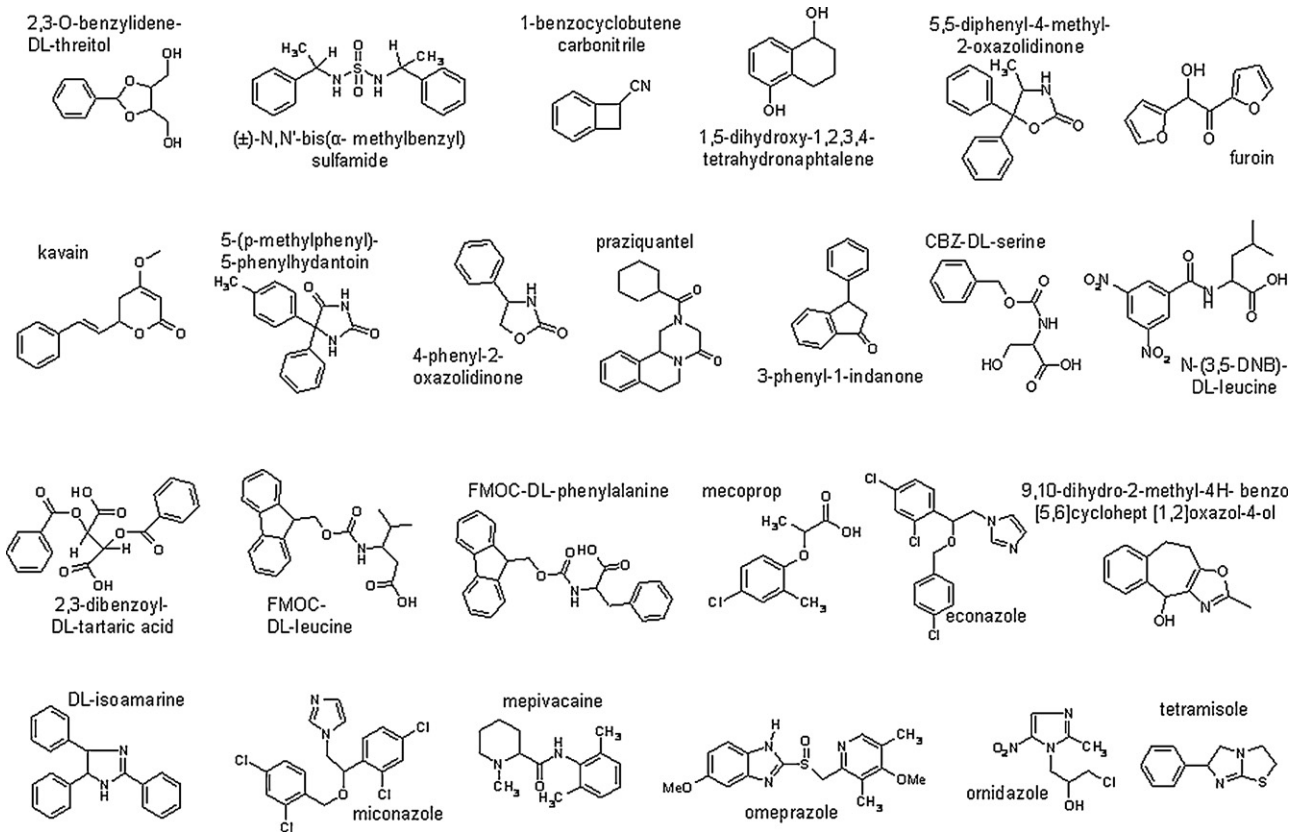

Fig. 1. Structures of the compounds.

would be taken into account on the basic design of the screening approach.

2. Experimental

2.1. Columns and chemicals

The analytical columns, CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC were supplied by Daicel Chemical Industries, Ltd. (Tokyo, Japan). The columns were sized 150 mm × 4.6 mm (I.D.) for RP runs and 250 mm × 4.6 mm (I.D.) for tests with polar eluents. All these amylose or cellulose derivatives are chemically immobilised on 5 μm silica particles. The chiral selectors contained in these CSPs are: tris(3,5-dimethylphenylcarbamate) of amylose for CHIRALPAK IA, tris(3,5-dimethylphenylcarbamate) of cellulose for CHIRALPAK IB and tris(3,5-chlorophenylcarbamate) of cellulose for CHIRALPAK IC.

The mobile phases for liquid chromatography were prepared from HPLC grade solvents: methanol (MeOH), acetonitrile (ACN), and tetrahydrofuran (THF) were purchased from Fisher Scientific (Strasbourg, France). Water was supplied by Carlo-Erba (Val de Reuil, France).

Diethylamine (DEA), trifluoroacetic acid (TFA), formic acid (HCOOH), phosphoric acid 85% by weight (H_3PO_4), ammonium hydroxide solution (28%, purity ≥99.99%), uracil, all the salts, as well as the racemic compounds (or enantiomers) were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France). The structures of the compounds in test are presented in Fig. 1.

2.2. Instrumentation and chromatographic conditions

The HPLC instrument used in this study was an Agilent 1100 series apparatus. It is equipped with a quaternary pump, a vacuum degasser, a column oven, a multiple wavelength UV detector, a HP Chemstation software and a column switching device.

Various mobile phase systems were investigated for HPLC study. All of them were composed of commonly used organic HPLC solvents and water or buffer systems.

For separation trails in polar organic mode, the pure polar solvent was used when the compounds in test were of neutral nature. However, 0.1% DEA was added into the polar solvent for enantiomer resolution of basic compounds whilst the mobile phase contained 0.1% TFA for acidic compounds.

In RP-mode, the type of analyte to be resolved determines the nature of the aqueous system. For neutral compounds, the aqueous component of the mobile phase would be simply water. For acidic compounds, the aqueous solution could be a phosphate buffer at pH 2.0 or a simple aqueous solutions adjusted at 2.0 using an acid (HCOOH, TFA or H_3PO_4). The solution of ammonium bicarbonate adjusted at pH 9.0 would be the most efficient aqueous mobile phase component for resolution of enantiomers of basic nature.

Preparation of the phosphate buffer pH 2: 25 mM KH_2PO_4 was dissolved in 1 L water; the pH value of such solution was then adjusted to 2.0 by adding 85% H_3PO_4.

Preparation of the ammonium bicarbonate pH 9 medium: 20 mM NH_4HCO_3 was dissolved in 1 L water; the pH value of such solution was then adjusted to 9.0 by adding diethylamine (DEA) or ammonium hydroxide solution (NH_4OH).

The proportion of each mobile phase component or mobile phase additive was always measured by volume. The mobile phase was always filtered through a membrane filter with pore size of 0.8 μm and then degassed before use. The chromatographic runs were performed at a flow rate of 1.0 mL/min or 0.7 mL/min (only for MeOH, with or without additives) and at a temperature of 25 °C. The dead time was determined by injecting uracil as a non-retained marker.

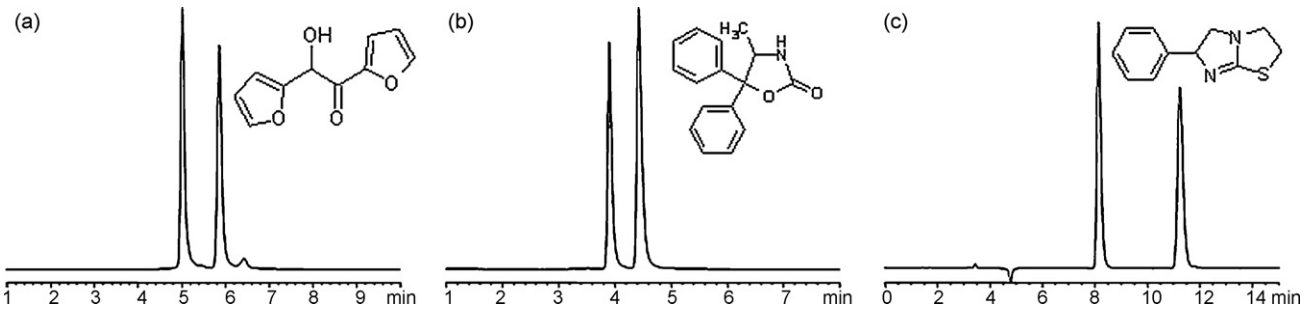

Fig. 2. Separation examples with polar eluent: (a) furoin on CHIRALPAK IA by 100% MeOH; (b) (S)-enriched 5,5-diphenyl-4-methyl-2-oxazolidinone on CHIRALPAK IB by 100% ACN; (c) tetramisole on CHIRALPAK IC by ACN/DEA 100/0.1.

3. Result and discussion: HPLC method development on immobilised polysaccharide-derived CSPs with water-compatible samples-method optimisation in practice

Biological samples and pharmaceutical formulations of drugs contain quite often water in their composition. When aiming to analyse their enantiomeric excess, the choice of the HPLC mobile phase composition becomes crucial in order to ensure the robustness of the method.

If a sample is soluble in aqueous medium and we look for a HPLC separation on polysaccharide-derived column, we have two possibilities:

(a) Developing a method in polar organic mode.
(b) Developing a method in reversed-phase (RP) conditions.

In this section, we will cover both options and give practical examples for them.

If a successful analytical method has to be found on at least one of the columns of CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC for enantiomer resolution of a given compound, the approach of method development can be based on a similar strategy to the one used for the coated-type phases. However, the palette of mobile phases applicable to these columns can be larger than on the coated ones. One can add, for example THF or any other water miscible solvent in the search of different selectivity profiles and higher success rate in method development.

3.1. Analytical method development in polar organic mode

For an efficient screening in the polar organic mode on CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC, the choice of the solvents used as mobile phases would be mainly concentrated on alcohols, acetonitrile and their combinations. Other water-miscible solvents, such as THF, could also be used in this case based on the column compatibility. However, THF is actually the strongest eluting solvent on the polysaccharide-derived CSPs. Apart from a few exceptions, the use of THF in its undiluted form would lead to very short retention times for most of the molecules resulting in no or compromised enantiomer resolution.

Therefore, screening sets in polar organic mode often include: 100% acetonitrile, 100% methanol or/and 100% ethanol (or their mixtures). These solvents would be used in pure form for neutral molecules. An acidic (i.e. TFA, formic acid or acetic acid) or a basic additive (i.e. DEA or TEA) would be included in the mobile phase for acidic and basic analytes, respectively. For molecules with a certain amphoteric nature, disproportioned combinations of TEA and TFA can also be used in order to improve peak shapes (ion-pairing strategy).

This preliminary screening allows to identify potential enantiorecognition with these columns. Some enantiomer resolutions with polar eluents are exemplified in Fig. 2. Of course, the method can also be subsequently optimised or fine-tuned by mixing these polar solvents between them and it is not rare finding successful examples with alcohol/acetonitrile mixtures.

However, for certain molecules this strategy will not be sufficient if the retention of the molecule or/and the enantioselectivity in the polar organic mode is very limited. If this is the case, it would be necessary switching to the RP-mode.

3.2. Analytical method development in RP-mode

The polysaccharide-type CSPs seem to be most frequently used with organic eluents (alkane mixtures or the polar solvents discussed above). However, resolution of enantiomers by aqueous eluent on the polysaccharide-based CSPs has a history almost as long as their applications with organic mobile phases [8,39,40]. Apart from the imposed character of the RP methods for direct injection of the samples issuing the biomedia, the choice of RP-mode was often related to scarce retentivity of the compounds, the low sample solubility of polar species in certain mobile phases and the lack of appropriate enantioselectivity in organic eluents. In the last decade an additional reason to prefer RP methods has been their suitability for LC–MS applications.

The chiral recognition mechanism of polysaccharides in the presence of water differs to the one observed in other solvent compositions. The strong solvation effect on the polymeric chiral selectors induced by water will certainly modify the interaction mechanisms of the polymer with the enantiomers. Therefore, RP chromatography is often complementary to normal phase (NP) mobile phases. The presence of water in the mobile phase has undoubtedly an impact on the enantiorecognition and offers the possibility of enhancing the chances of resolution success.

Several RP screening approaches were described with regard to the use of polysaccharide-based CSPs [8,38,41,42]. The effects of certain mobile phases were investigated and proved to be useful for given separations.

For the recent years, CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC have been successfully used in our laboratories as a column set for primary sample screening with organic mobile phases and in RP-mode. In the current investigation a number of enantiomeric mixtures were set as analytes and several goals were proposed for the study:

(a) Finding an efficient screening strategy in RP conditions on the three columns.
(b) Respecting mobile phases being MS-compatible, if possible as similar as possible to the ones used in routine achiral analysis.
(c) Getting a high success rate in this mode, not compromised by this choice of mobile phases.
(d) Extrapolating the same strategy to the polysaccharide-derived CSPs in general.

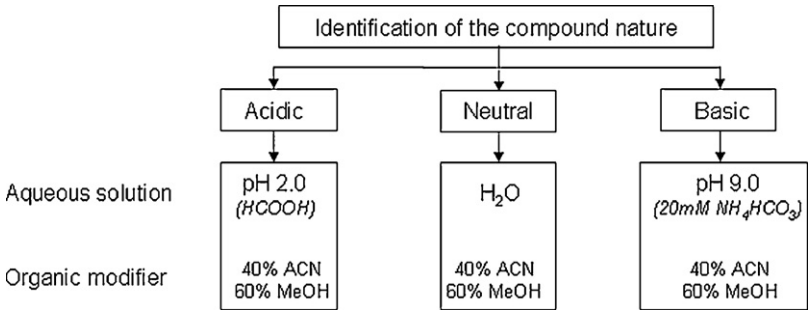

Fig. 3. "Decision tree" for method development in RP-mode.

Table 1
Enantiomer resolution of neutral compounds. Aqueous component: water.

Compound	ACN (modifier)				MeOH (modifier)				Column
	$k'1$	α	Rs	%	$k'1$	α	Rs	%	
2,3-O-Benzylidene-DL-threitol	1.23	2.32	4.31	15	0.54	4.34	4.88	55	CHIRALPAK IA
(±)-N,N′-bis(α-methylbenzyl) sulfamide	2.08	1.20	2.40	50	1.45	1.53	2.89	75	CHIRALPAK IA
1,5-Dihydroxy-1,2,3,4-tetrahydronaphtalene	2.42	1.88	4.89	15	1.45	1.78	4.18	45	CHIRALPAK IA
furoin	2.79	1.36	3.69	20	1.21	1.51	3.18	55	CHIRALPAK IA
5-(p-Methylphenyl)-5-phenylhydantoin	2.18	1.64	2.14	50	1.70	1.69	1.49	75	CHIRALPAK IA
4-Phenyl-2-oxazolidinone	3.30	1.22	2.43	20	2.90	1.09	0.64	45	CHIRALPAK IA
1-Benzocyclo butenecarbonitrile	3.04	1.11	1.92	35	2.03	1.19	2.55	55	CHIRALPAK IB
5,5-Diphenyl-4-methyl-2-oxazolidinone	3.36	1.36	5.67	40	0.81	1.58	3.23	75	CHIRALPAK IB
3-Phenyl-1-indanone	2.67	1.12	2.21	50	0.59	1.00	0.00	65	CHIRALPAK IB
5,5-Diphenyl-4-methyl-2-oxazolidinone	2.59	1.23	2.80	40	2.75	1.20	1.44	65	CHIRALPAK IC

The above four objectives were the basis of this research work and should be developed here.

It is practically proved and commonly admitted that, for resolution of enantiomers under RP conditions, the appropriate selection of the mobile phase is the first and critical step for method development. This selection should normally be done by considering the nature of the molecules to be analysed. Since the chiral selectors (the polysaccharide derivatives) are all neutral entities and do not afford any ionic interactions, it is essential to keep the analyte molecules non-ionised during the chromatographic process. Under this primary guideline, a "decision tree" can be defined for the kick-off of the method development (Fig. 3).

The selection of the mobile phase for neutral compounds is straightforward: plain water is suitable to be used as the aqueous component. The enantiomer resolutions of certain neutral compounds are summarised in Table 1. Some of the separations are presented in Fig. 4. It should be noted here that the enantiomer resolution of 5,5-diphenyl-4-methyl-2-oxazolidinone on CHIRALPAK IB with 100% ACN (in polar mode, Fig. 2(b)) is significantly improved by simple dilution of ACN with 60% water (in RP-mode, Fig. 4(b)). In this context, the RP-mode could be sometimes considered as an extension of the polar organic mode when retention is not sufficient in the absence of water.

The organic modifier plays an important role in regulating retention and modulating enantioselectivity. The first choice of modifier to be combined with water is ACN. This is owing to the multiple advantages that ACN can offer: low viscosity, low UV cut-off, adapted eluting strength on the polysaccharide-type CSPs and outstanding ability to induce good enantioselectivity. MeOH can also be a useful modifier, but with weaker eluting strength and leading to much more viscous mobile phases. In comparison with ACN and MeOH, THF seems to be a less versatile organic modifier. Although it may sometimes be a good alternative to ACN and MeOH (Fig. 5), it mostly does not contribute to enhancement in enantioselectivity, neither in mass transfer kinetics of the chromatographic process. It may be interesting to be investigated in certain cases with complicated separation, in combination with ACN and MeOH as major components, in order to modulate recognition. The relative eluting strength of these three organic modifiers can be ranked as follows: THF > ACN > MeOH.

The prerequisite for enantiomer resolution of acidic compounds is to use acidic mobile phases in order to suppress any dissoci-

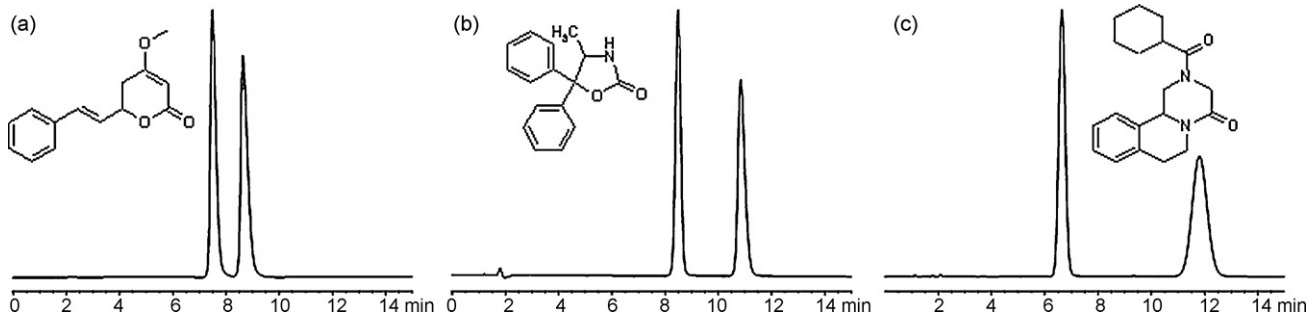

Fig. 4. Examples of enantiomer resolution of neutral compounds with ACN/H_2O mixtures: (a) kevain on CHIRALPAK IA with 50% ACN; (b) 5,5-diphenyl-4-methyl-2-oxazolidinone on CHIRALPAK IB with 40% ACN; (c) praziquantel on CHIRALPAK IC with 80% ACN.

Table 2
Effect of various acidic aqueous systems. Solute: N-(3,5-DNB)-DL-leucine, temperature: 25 °C, flow rate: 1.0 mL/min.

Aqueous solution (pH 2.0)	CHIRALPAK IA			CHIRALPAK IB			CHIRALPAK IC		
	$k'1$	α	Rs	$k'1$	α	Rs	$k'1$	α	Rs
a-1	3.10	1.60	3.24	1.84	2.01	10.15	1.17	1.38	2.97
a-2	3.28	1.62	3.64	2.46	1.70	7.56	1.22	1.38	3.01
a-3	4.00	1.53	3.21	1.69	2.07	10.07	1.20	1.38	3.01
a-4	3.43	1.57	3.41	1.85	2.02	9.72	0.96	1.37	2.76

a-1: HCOOH aq. pH 2.0; **a-2**: TFA aq. pH 2.0; **a-3**: H_3PO_4 aq. pH 2.0; **a-4**: phosphate buffer 25 mM pH 2.0.
Content of the organic modifier in the mobile phase: 40% ACN for CHIRALPAK IA; 50% ACN for CHIRALPAK IB; 45% ACN for CHIRALPAK IC.

Table 3
Enantiomer resolution of acidic compounds. Aqueous solution: HCOOH pH 2.0.

Compound	ACN (modifier)				MeOH (modifier)				Column
	$k'1$	α	Rs	%	$k'1$	α	Rs	%	
CBZ-DL-serine	3.51	1.33	1.55	10	1.31	1.83	3.07	45	CHIRALPAK IA
N-(3,5-DNB)-DL-leucine	3.10	1.60	3.24	40	2.62	1.38	1.37	75	CHIRALPAK IA
N-(3,5-DNB)-DL-leucine	1.84	2.01	10.15	50	3.21	1.37	2.88	75	CHIRALPAK IB
FMOC-DL-phenylalanine	2.79	1.07	1.05	60	2.45	1.42	3.22	85	CHIRALPAK IB
FMOC-DL-phenylalanine	1.54	1.30	2.68	50	2.17	1.46	2.41	75	CHIRALPAK IC
FMOC-DL-leucine	2.13	1.16	1.61	45	1.39	1.58	2.45	75	CHIRALPAK IC
2,3-dibenzoyl-DL-Tartaric acid	0.97	1.83	3.31	30	1.15	2.53	1.03	55	CHIRALPAK IC
Mecoprop	2.58	1.09	1.06	30	5.55	1.21	1.82	45	CHIRALPAK IC

ation of the acidic analytes. The most common and effective pH value of the aqueous solution would be around 2.0–2.5 at which most organic acids will be in a neutral state. Historically, the phosphate buffer or H_3PO_4 aqueous solution at pH 2.0 was recommended and frequently used to compose the acidic RP mobile phases. It is a good alternative in acidic screening of chiral analytes, as it is often the case in achiral analysis. Nevertheless, these inorganic species are incompatible with LC–MS detection in which all the additive to the mobile phase must be volatile. On the other hand, a low pH mobile phase without much buffering capacity is often sufficient. In this perspective, an alternative volatile acid has to be found both to adjust the mobile phase pH and to aid the performance of MS. In an attempt to identify the most suitable acid(s) and determine its/their potential effect in terms of retentive and enantioselective behaviour of the polysaccharide-based CSPs, a comparative study was carried out by injecting a series of 10 acidic racemates using four acidic solutions at pH 2. They are: HCOOH aq., TFA aq., H_3PO_4 aq. and 25 mM phosphate buffer. The typical chromatographic results obtained for the separation of N-(3,5-DNB)-DL-leucine enantiomers on the three columns are summarised in Table 2. It can be noticed that, depending on the column, the nature of the acidic solution may affect the capacity factor. The enantioselectivity, however, keeps more or less constant, except in the case of acidic solution (a-2) on CHIRALPAK IB. In this later case, the decrease in enantioselectivity from about 2.0 to 1.7 was induced by TFA. Although TFA is volatile and effective for low pH adjustment, it tends to suppress ionisation in the MS and is not necessarily the most suitable additive for this purpose. Therefore, HCOOH turned out to be the best choice for MS detection in that case. In Table 3 are listed some resolutions of enantiomers in the acidic category by using HCOOH pH 2 solutions. Two of these separations are illustrated in Fig. 6. Once again, the RP separation of N-(3,5-DNB)-DL-leucine on CHIRALPAK IB is clearly superior to that obtained with the polar organic mobile phase on the same column (Fig. 6(b and c)).

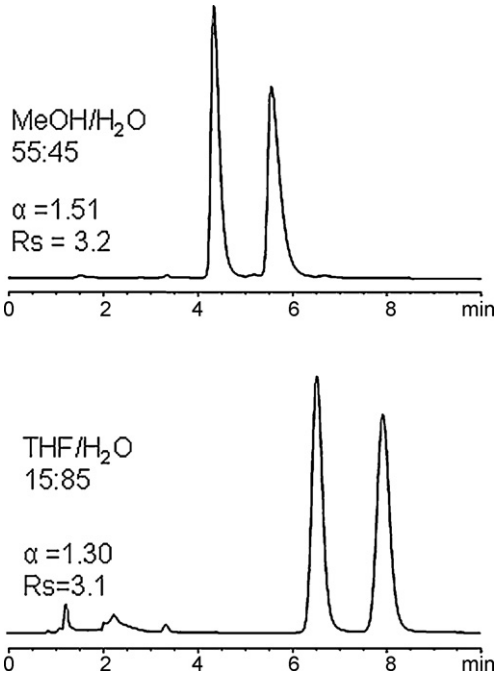

Fig. 5. Enantiomer resolution of furoin on CHIRALPAK IA.

The effect of the organic modifier can be quite remarkable. As indicated by the enantioselectivity and resolution values in Table 3, ACN is the best choice for resolution of around 50% of the compounds. For the second half, however, MeOH turns out to be more effective. As shown in Fig. 7, the partial resolution of FMOC-DL-leucine enantiomers could be transformed into a complete separation by switching the organic modifier from 45% ACN to 75% MeOH. Therefore, it is worth including MeOH in the sample screening for the search of the best method, as suggested by the scheme in Fig. 3. Other alcohols such as ethanol or 2-propanol may be useful in certain cases.

Based on the same reasoning, basic aqueous solutions should normally be in use to suppress the ionisation of basic solutes. In this case, the aqueous solution can be regulated at pH 8, or preferably at pH 9.

In the same effort to develop the RP methods suitable to MS detection for basic compounds, a series of basic aqueous media were investigated. It included: phosphate buffer pH 8, borate buffer pH 9, 0.1 M KPF_6 aqueous solution, NH_4HCO_3 aq. pH 9 and NH_4OAc

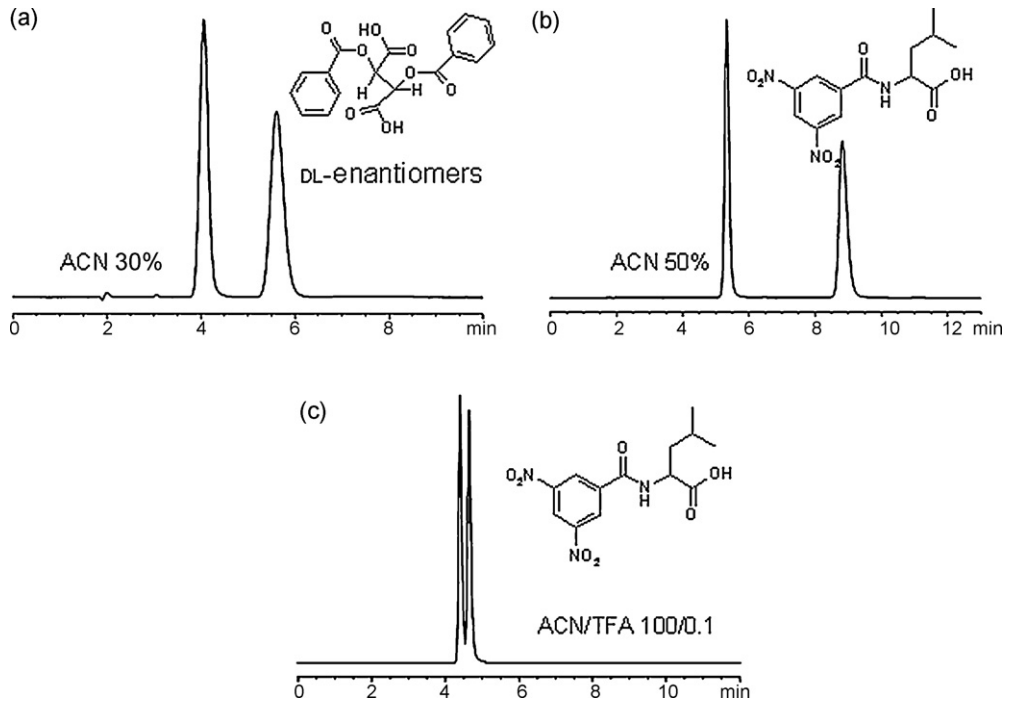

Fig. 6. Examples of enantiomer resolution of acidic compounds with ACN in HCOOH aq. pH 2.0 solution (a) on CHIRALPAK IC and (b) on CHIRALPAK IB in comparison with (c) the polar mobile phase ACN/TFA 100/0.1 on CHIRALPAK IB.

aq. pH 9. As a matter of fact, the enantioselectivity is not influenced very much by the nature of the basic aqueous solutions at a given concentration of a given organic modifier, as demonstrated by the results in Table 4. It should be noted that phosphate, borate and KPF6 had been frequently used in many laboratories on the polysaccharide-type columns. Although the separations based on these aqueous systems could be very effective in terms of recognition, they would obviously be troublesome in method conversion to LC–MS due to the lack of compatibility between the mobile phase and the MS detection. On the other side, both ammonium bicarbonate and ammonium acetate are volatile salts. They could not only fulfil the requirements of MS conditions but can also meet the expectations in terms of the enantioselective and resolution performance of the chromatographic methods. The detailed results of such a complex comparative study will be reported in a separated communication.

The ammonium bicarbonate solution at pH 9 was once used as a RP screening module for chiral separation trails of a large number of drug substances and intermediates [38]. In our case, the screening of basic compounds on the three immobilised CSPs using the same aqueous system proved to be satisfactory. It was also found that, comparing to the ammonium acetate solution, the ammonium bicarbonate medium could sometimes afford better peak shapes, therefore higher resolution of the enantiomers. The successful separations of certain basic compounds are summarised in Table 5. Two of them are presented in Fig. 8.

The adjustment of pH of the ammonium bicarbonate solution was performed with DEA in many cases here reported, but it could be also performed with an ammonium hydroxide solution. As described on NP applications, the change of counter-ion can have an effect on peak sharpness and symmetry and should sometimes be considered for method optimisation.

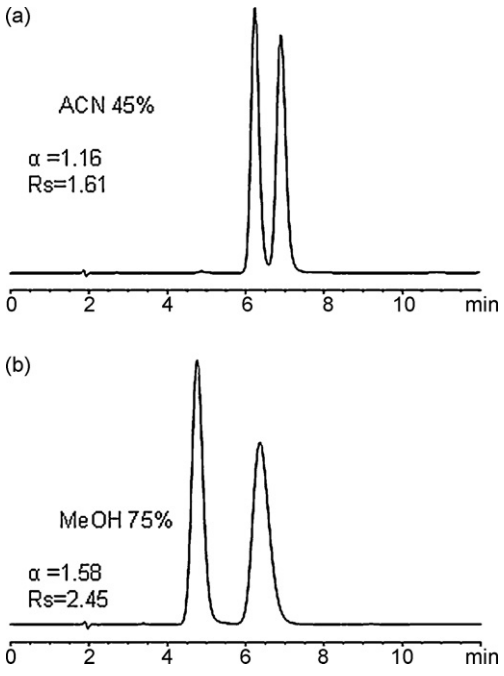

Fig. 7. Effect of the organic modifier. Solute: FMOC-DL-leucine; column: CHIRALPAK IC; aqueous component: HCOOH aq. pH 2.0.

Table 4
Effect of various basic aqueous systems. Solutes: 9,10-dihydro-2-methyl-4H-benzo [5,6] cyclohept[1,2]oxazol-4-ol on CHIRALPAK IA; DL-isoamarine on CHIRALPAK IB, Tropicamide on CHIRALPAK IC. Temperature: 25 °C, flow rate: 1.0 mL/min.

Aqueous solution	CHIRALPAK IA			CHIRALPAK IB			CHIRALPAK IC		
	$k'1$	α	Rs	$k'1$	α	Rs	$k'1$	α	Rs
b-1	2.37	1.33	3.00	2.73	1.25	3.76	2.46	1.49	2.10
b-2	2.37	1.33	3.00	2.77	1.22	2.58	2.55	1.47	2.06
b-3	2.52	1.29	2.66	1.65	1.31	3.51	2.60	1.46	2.09
b-4	2.16	1.30	2.52	2.65	1.26	2.89	2.34	1.49	2.09
b-5	2.28	1.30	2.43	1.50	1.27	2.00	2.45	1.49	2.03

b-1: 20 mM NH_4HCO_3 aq. pH 9.0.; **b-2**: 20 mM NH_4OAc aq. pH 9.0; **b-3**: 20 mM borate buffer pH 9.0; **b-4**: 20 mM phosphate buffer pH 8.0; **b-5**: 100 mM KPF_6 solution. Content of the organic modifier in the mobile phase: 30% ACN for CHIRALPAK IA; 60% ACN for CHIRALPAK IB; 35% ACN for CHIRALPAK IC

Table 5
Enantiomer resolution of basic compounds. Aqueous solution: 20 mM NH_4HCO_3 pH 9.0.

Compound	ACN (modifier)				MeOH (modifier)				Column
	$k'1$	α	Rs	%	$k'1$	α	Rs	%	
9,10-Dihydro-2-methyl-4H-benzo[5,6]cyclohept [1,2]oxazol-4-ol	2.37	1.33	3.00	30	3.21	1.15	0.60	55	CHIRALPAK IA
Ornidazole	0.26	5.48	21.0	70					CHIRALPAK IA
DL-Isoamarine	1.32	1.64	4.70	70	1.06	1.47	2.10	85	CHIRALPAK IA
DL-Isoamarine	2.73	1.25	3.76	60	1.33	1.48	4.04	85	CHIRALPAK IB
Econazole	3.76	1.15	1.98	70					CHIRALPAK IC
Omeprazole	3.72	1.20	1.86	30	1.74	1.41	2.54	85	CHIRALPAK IC
Miconazole	3.53	1.18	2.28	80					CHIRALPAK IC
Mepivacaine	3.16	1.16	1.94	35					CHIRALPAK IC
Tropicamide	2.46	1.49	2.10	35					CHIRALPAK IC
Tetramisole	3.66	1.38	5.07	40	2.17	1.14	1.29	75	CHIRALPAK IC

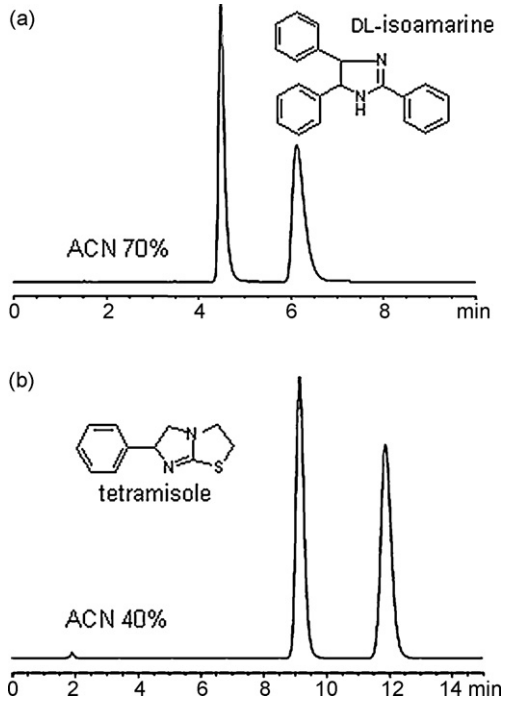

Fig. 8. Examples of enantiomer resolution of basic compounds with ACN in 20 mM NH_4HCO_3 aq. pH 9.0 (by DEA) solution on (a) CHIRALPAK IA and (b) CHIRALPAK IC.

The chromatographic separation of basic enantiomers in RP conditions is often working at pH 9, however, for certain primary amines it may be necessary to reach pH 10 for proper enantiomeric resolution. This can be achieved with the same type of buffered systems. As in any silica-based columns, it would be advised to rinse the column after use with neutral solutions, in order to enhance column durability.

As mentioned at the beginning of this section, the key point for RP method development would be setting the right conditions to start the sample screening. Gradient elution can certainly be used for sample and mobile phase screening, but isocratic elution mode seems to be more convenient and straightforward, unless we would be having a number of molecules of different polarity in our sample. Based on a significant number of experimental data, the mobile phase compositions indicated in Fig. 3 can be recommended as starting point. The typical starting conditions represent actually the mobile phases of upper middle eluting strength (containing enough organic modifier and enabling elution of most of the compounds in a reasonable analysis time frame). In many cases, successful separations can be directly obtained issuing the screening procedure. However, certain compounds or families of compounds are either extremely retained or too fast eluted in these conditions. In this case, an optimisation step should be considered for the individual samples, by playing the nature and the percentage of the organic modifiers. The scheme for method optimisation is proposed in Fig. 9. The use of other organic modifiers, such as ethanol, 1-propanol, 2-propanol and even THF can also be considered on CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC. In our practice, however, these modifiers would be tried only if no hit is achieved with ACN and MeOH. With these first or optimised data one can

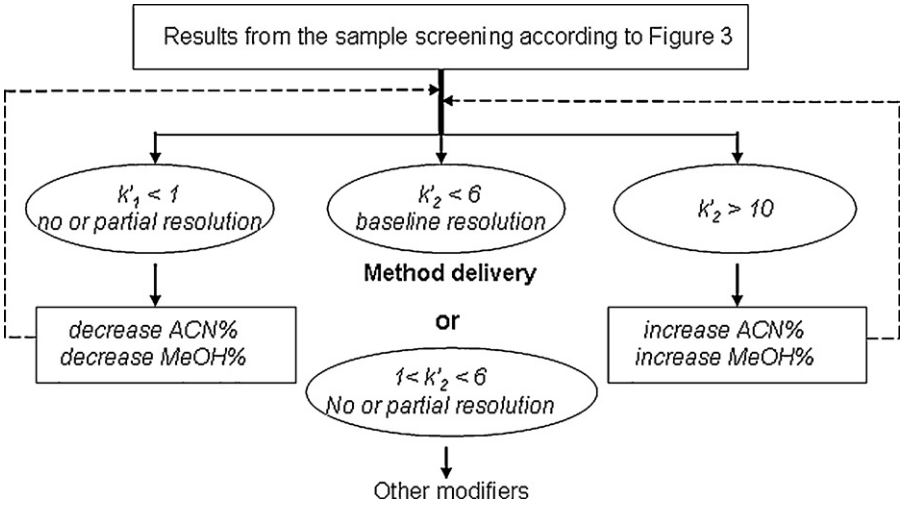

Fig. 9. Scheme for method optimisation.

decide which are the best mobile phase conditions to perform the tests or validate the methods.

In the case of ammonium-derived compounds with a permanent charge on the molecule, it may be better developing a method on the acidic mode. An aqueous buffer at pH around 2 or 3 would often yield good enantiorecognition. It may happen that peaks may have strong tailing. In such a case, the addition of 1% TEA to the aq. phase, before adjusting the pH at the acidic range would be a useful strategy to reach suitable peak shapes. This procedure is often used in achiral screenings as a way to reduce interactions with the silanol groups on the silica surface.

An interesting feature of immobilised polysaccharide-derived supports is that it would be possible to use the same column in organic and water containing solvents without compromising the reproducibility of results on both modes. This possibility does exist due to the fact that a washing protocol between RP and organic mode in strong solvents (such as THF and DMF) can be applied, in order to eliminate water traces adsorbed on the polymer. This protocol is described in detail on the instruction manual of the immobilised columns, but will not be compatible with the previously existing coated-versions.

4. Conclusion

Owing to their broad and complementary enantioselective properties as well as their significantly enhanced solvent compatibility, the combination of the three immobilised CSPs (CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC) constitutes a powerful column set for resolution enantiomers in various modes: organic mobile phases, aqueous mobile phases (RP-mode) and SFC.

For liquid RP chromatography, a simple screening with a reduced number of organic solvents and a selection of aqueous solutions adapted to the sample nature has demonstrated its effectiveness for enantiomer resolution. Although the current screening strategy does not aim to be the only possibility on this type of columns, it proposes mobile phase systems being compatible with aqueous samples and in LC–MS configurations, whilst getting high success rates in enantiorecognition. These systems have been developed on 5 μm columns, but are currently applied to the corresponding 3 μm supports, leading to very fast resolutions and improved efficiencies.

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