



Common approaches for efficient method development with immobilised polysaccharide-derived chiral stationary phases[☆]

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ARTICLE INFO

Article history:

Received 13 April 2008

Accepted 17 June 2008

Available online 5 July 2008

This special volume is dedicated to Professor Lindner: the excellent Man, Scientist and Teacher.

Keywords:

Chemically immobilised polysaccharide-based chiral stationary phases (CSPs)
Enantiomer resolution
HPLC
Organic mobile phases
RP
SFC
Method development

ABSTRACT

Immobilised polysaccharide-based chiral stationary phases (CSPs) are a new generation of chromatographic materials combining the remarkable enantioselective performance of the polysaccharide derivatives and solvent versatility for enantiomeric resolution. Based on extensive experimental work, this article will focus on the approach to efficient method development with these immobilised CSPs (CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC) by applying a limited number of mobile phases. The manuscript will review the development of screening strategies, either by liquid and supercritical chromatography (LC and SFC), for the separation of enantiomers. The rational combination of both modes leads to efficient approaches to get enantiomeric resolutions in reasonable time frames and with high success rates.

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1. Introduction

Polysaccharide derivatives, being coated or immobilised on a silica matrix, have become the first and broadest choice of selectors to be used as chiral stationary phases (CSPs) for liquid and supercritical fluid chromatography (LC and SFC). Polysaccharide-derived CSPs are currently playing an essential role in the analytical and preparative separation of enantiomers. A number of publications in recent years have reviewed the multiple scientific achievements in their field and their applications in the separation of enantiomers [1–12]. Over two decades the coated polysaccharide-derived supports were becoming of routine use for most of the scientific and industrial groups working in the field. 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 [12–16]. 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, publications are collecting new separations [12–32].

These immobilised polysaccharide-derived CSPs have evident benefits that should be considered in the screening process. However, at first sight, the larger choice in solvents could be seen as implying more screening work and longer time to find the right mobile phase system(s) for a given separation. This should not be the real situation if a preliminary choice of solvent systems is performed to design the screening. 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 aims to overview a guideline for mobile phase selection in LC and SFC with the immobilised polysaccharide-derived CSPs. It will mainly focus on the practical aspects for their use.

[☆] This paper is part of the Special Issue 'Enantioseparations', dedicated to W. Lindner, edited by B. Chankvetadze and E. Francotte.

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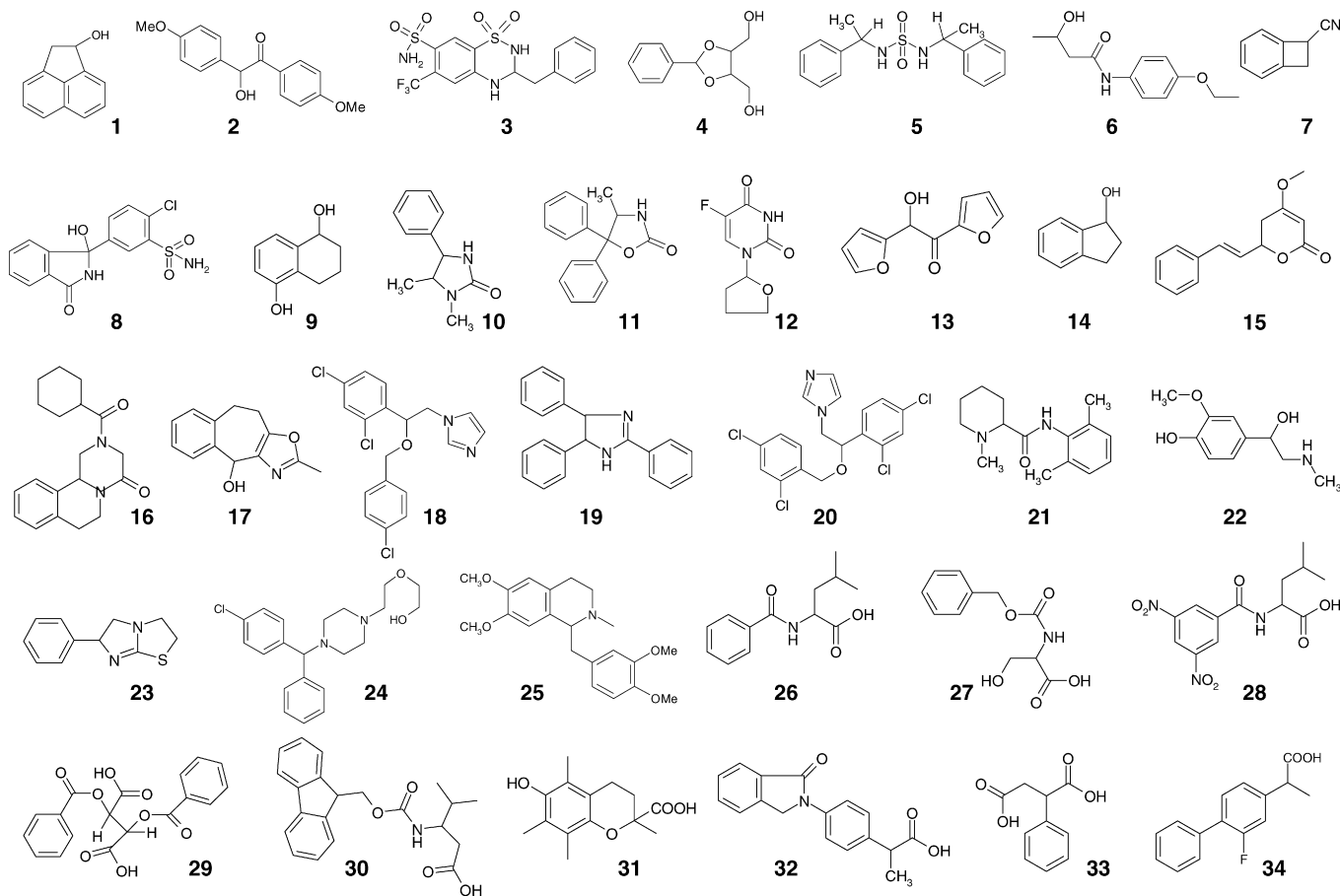


Fig. 1. Structures of the test compounds.

2. Experimental

2.1. Chemicals

The analytical columns, CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC sized 250 mm × 4.6 mm (I.D.), were supplied by Daicel Chemical Industries, Ltd. (Tokyo, Japan). All these amylose or cellulose derivatives are chemically immobilised on 5 μm silica particles.

The mobile phases for liquid chromatography were prepared from HPLC grade solvents. *n*-Hexane (Hexane), methanol (MeOH), isopropanol (2-PrOH), methyl-*tert*-butyl ether (MtBE) were purchased from Fisher Scientific (Strasbourg, France). Ethanol (EtOH), tetrahydrofuran (THF) and dichloromethane (DCM or CH₂Cl₂) were supplied by Carlo-Erba (Val de Reuil, France). Diethylamine (DEA), 2-aminoethanol (AE), trifluoroacetic acid (TFA), 1,3,5-tri-*tert*-butylbenzene (the void time marker) and 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

Two HPLC instruments were used in this study. Both are Agilent 1100 series apparatus. Each is equipped with a quaternary pump, a vacuum degasser, a column oven, a multiple wavelength UV detector and 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. The main systems described in this article are: (1) hex-

ane/EtOH mixtures; (2) hexane/2-PrOH mixtures; (3) MtBE-based mixtures (combining with either hexane or alcohol); (4) CH₂Cl₂-based mixtures (combining with either hexane or alcohol) and (5) hexane/THF mixtures. The proportion of each mobile phase component or mobile phase additive was always measured by volume. The chromatographic runs were performed at a flow rate of 1.0 ml/min and at a column temperature of 25 °C. The dead time was estimated by injecting 1,3,5-tri-*tert*-butylbenzene as a non-retained marker. The resolution (*R_s*) between two enantiomers was determined by the following equation:

$$R_s = \frac{1.18(t_2 - t_1)}{W_{50(1)} + W_{50(2)}}$$

where *t*₁ and *t*₂ are respectively the retention times of the first and second eluted peaks; *W*₅₀₍₁₎ and *W*₅₀₍₂₎ are the corresponding peak widths at the half-peak height. Typically, a *R_s* value approaching 2 was required to achieve a baseline resolution of a pair of enantiomers.

3. HPLC method development on immobilised polysaccharide-derived CSPs: method optimisation in practice

3.1. Analytical method development in HPLC

Facing the continuously increasing number of chiral compounds in drug development and other domains, efficient and automated HPLC screening using columns packed with various CSPs have been a challenging subject and discussed by several authors [33–42].

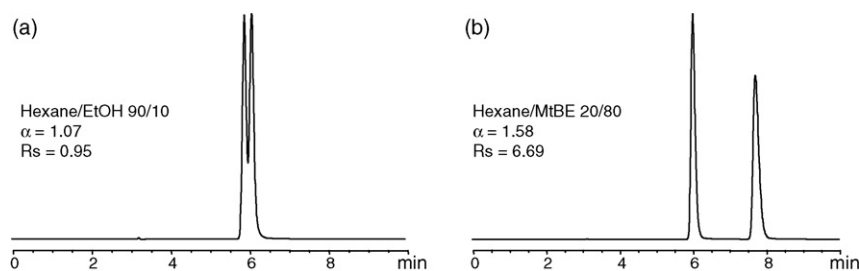


Fig. 2. Effect of mobile phase on enantiomer separation. Column: CHIRALPAK IC; Compound: 1-acenaphthenol (**1**).

The strategies to achieve enantiomer separations are quite variable from one laboratory to another depending on the type of substances in screening, the equipment available and the R&D background, but the motivation for the development of screening approaches remains common. We all search the easiest and the most reliable way to analyse a series of molecules with diverse structures and chemical properties in a reasonable time frame. This is also the standpoint for chiral compound screening based on the three columns packed with the immobilised CSPs.

If a successful analytical method has to be found on CHIRALPAK IA, CHIRALPAK IB and/or CHIRALPAK IC for enantiomer resolution of a given racemic compound, the main target would be achieving a good resolution degree and a short analysis time. At analytical level we can consider that the approach of method development on the immobilised CSPs can be based on a similar strategy to the one used for the coated-type phases. However, we are adding in this case a broader choice of solvents so that they can provide different selectivity profiles. The palette of mobile phase that can be applied on these columns can be extremely large. It may be expended over the whole series of HPLC solvents and their miscible combinations.

It is a very well known fact that the mobile phase may have major effects on the enantioselective performance of a CSP. One example can be given with the enantiomer separation of 1-acenaphthenol (compound **1**) on CHIRALPAK IC. As shown in Fig. 2(a), the enantiomers could merely be partially resolved with a very limited selectivity using a mixture of hexane/EtOH 90/10. When the mobile phase was changed to hexane/MtBE20/80, the first peak was detected at very similar time as in the former mobile phase condition but the second peak was eluted almost 2 min later (Fig. 2b). Both mobile phases were containing hexane, but the replacement of 10% EtOH with 80% MtBE led to a drastic enhancement in enantioselectivity (from $\alpha = 1.07$ to 1.58) and in resolution degree ($R_s = 0.95$ –6.69). The impact of the mobile phase on enantiomer resolution with the immobilised CSPs was also demonstrated in several previous studies [13–15,22,28,32].

These examples evidence the challenge of designing a screening set with a limited number of mobile phase combinations under the exigencies of fast method development. Having this goal in mind, a long list of solvents have been investigated in our laboratories either as major component or as modifiers of the mobile phases. A non-exhaustive list would include: hexane, propanols, ethanol, methanol, acetonitrile, water, THF, 1,4-dioxane, acetone, methyl- and ethyl acetate, toluene, MtBE, chloroform and dichloromethane.

After thorough investigations we could conclude that a reduced group of solvents and their mixtures could lead to better selectivity values for most of the compounds tested and could be considered as the primary set of mobile phases to be used with these three CSPs. In practice, it would be recommended to set the four first mobile phases on quaternary HPLC systems for screening. The selected solvents were: alkane/alcohol mixtures, MtBE mixtures with alcohol or alkane and THF mixtures. As an alternative to THF, chlorinated solvents such as DCM or chloroform can be used, if there are no environmental restrictions. Tested on a sample pool randomly composed of 70 chiral compounds, the combination of the three columns with four mobile phase systems could afford complete enantiomer resolution for above 90% of the racemics examined and enantiorecognition above 95%.

The key point would be setting the right conditions to start the screening, leading to appropriate retention times and successful chiral recognition. As an example, and based on a significant number of experimental data, the proportions indicated on Table 1 can be taken as starting point. The retentive behaviour of the compound will steer the adjustment of the mobile phase. With these first data one can decide which are the best solvent(s) or solvent ratios to perform the tests on CHIRALPAK IA, CHIRALPAK IB and CHIRALPAK IC.

The typical starting conditions represent the mobile phases of upper middle eluting strength. The indicated mobile phase compositions can consequently have most of the analytes eluted within a reasonable time range with a good probability of full resolution of enantiomers. However, there are particular families of compounds which are either extremely retained or quickly eluted in these conditions. If this observation is made, it would be necessary to adapt the starting mobile phase compositions to higher proportions of polar solvents or alkanes, respectively.

In order to demonstrate the effectiveness of the primary screening and the rationality for potential method optimisation, the chromatographic results from a screening sequence with hexane/2-PrOH 80/20 are detailed in Table 2.

The way to establish the criteria for successful method development can affect the number of experimental trials and the project timetable. Very often, the first aim of method development is not to achieve optimal separations but to find acceptable baseline resolutions and use them for qualitative or quantitative measurement of enantiomers. Of course, such a method can also be a starting point for further optimisation in view of preparative applications.

In our study, the stop time for data acquisition run was set at 20 min. For compounds with retention time superior to 20 min (or

Table 1
Recommended solvent systems for screening and optimisation on the three columns

Solvent mixtures	Alkane–2-PrOH	Alkane–EtOH	Alkane–MtBE–EtOH	Alkane–THF	Alkane–DCM–EtOH
Typical starting conditions	80:20	80:20	0:98:2	70:30	50:50:2
Advised optimisation range	99:1–50:50	99:1–50:50	80:20:0–0:40:60	95:5–0:100	85:15:0–0:80:20

Table 2

Screening results of a series of compounds using hexane/2-PrOH 80/20 as mobile phase

Compound		Chromatographic parameters				Column	Optimisation
No.	Name	t_2	$k'1$	α	R_s		
4	2,3-O-Benzylidene-DL-threitol	7.04	1.21	1.17	1.56	IA	–
		7.70	1.35	1.16	1.41	IB	–
		13.55	2.13	1.60	7.24	IC	–
6	Bucetin	11.08	2.02	1.39	4.63	IA	–
		6.05	1.01	1.00	0.00	IB	–
		8.81	1.79	1.04	0.55	IC	–
7	1-Benzocyclobutenecarbonitrile	4.50	0.54	1.00	0.00	IA	Yes
		5.12	0.70	1.31	4.21	IB	–
		7.72	1.48	1.02	0.44	IC	–
9	1,5-Dihydroxy-1,2,3,4-tetrahydronaphtalene	6.69	0.99	1.31	3.19	IA	–
		5.62	0.87	1.00	0.00	IB	–
		5.22	0.58	1.20	1.63	IC	–
10	1,5-Dimethyl-4-phenyl-2-imidazolidinone	8.25	1.22	1.50	5.17	IA	–
		8.19	1.63	1.05	0.80	IB	–
		>20				IC	Yes
11	5,5-Diphenyl-4-methyl-2-oxazolidinone	7.00	1.40	1.00	0.00	IA	–
		8.78	1.16	1.65	7.36	IB	–
		>20				IC	Yes
13	Furoin	11.5	2.31	1.28	4.34	IA	–
		9.09	1.78	1.14	1.98	IB	–
		>20				IC	Yes
14	1-Indanol	4.57	0.51	1.12	1.12	IA	Yes
		4.30	0.41	1.06	0.52	IB	Yes
		4.58	0.45	1.09	0.72	IC	Yes

Temperature: 25 °C, flow rate: 1.0 ml/min. Data in bold: complete resolution.

Table 3

Optimisation of separations from Table 1

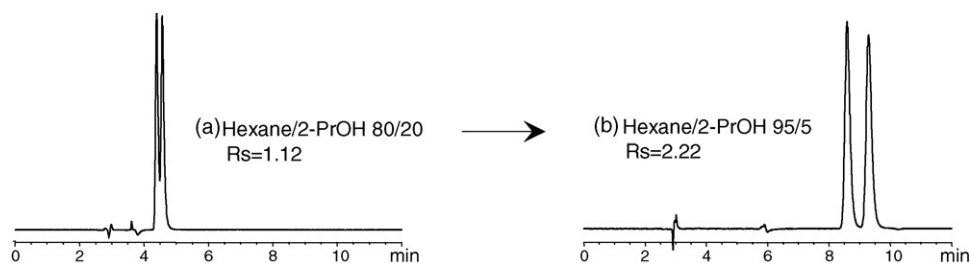
Compound		Chromatographic parameters				Column	Hexane/2-PrOH
No.	Name	t_2	$k'1$	α	R_s		
7	1-Benzocyclobutenecarbonitrile	5.98	1.05	1.00	0.00	IA	95/5
10	1,5-Dimethyl-4-phenyl-2-imidazolidinone	19.97	5.17	1.06	0.84	IC	60/40
11	5,5-Diphenyl-4-methyl-2-oxazolidinone	10.90	1.58	1.61	6.38	IC	60/40
13	Furoin	10.56	2.30	1.06	1.06	IC	60/40
14	1-Indanol	9.30	1.95	1.12	2.22	IA	95/5
		7.57	1.39	1.09	1.24	IB	95/5
		8.79	1.67	1.12	1.53	IC	95/5

Temperature: 25 °C, flow rate: 1.0 ml/min. Data in bold: successful optimisation.

$k'_2 > 6$, k'_2 being the capacity factor of the second eluting enantiomer), an adjustment of the mobile phase for stronger eluting strength should be considered. Oppositely, if the longest retention time is inferior to 6 min (or $k'_2 < 1$) and no complete enantiomer resolution is observed, it may be worth decreasing the eluting strength of the mobile phase.

As it can be observed in Table 2, seven of the eight racemic compounds were directly resolved with success using the mobile

phase hexane/2-PrOH 80/20. These “one-shot” enantiomer resolutions are marked by data in bold. Although much longer screening sequences were actually run in our laboratory and the large number of data can hardly be presented in this manuscript, this short sequence can be considered to be representative. Quite often, the successful resolution of a single pair of enantiomers was found on one of the three columns in screening. For instance, 2,3-O-benzylidene-DL-threitol (compound 4) was well resolved into its

**Fig. 3.** Separation optimisation of 1-indanol enantiomers (**14**) on CHIRALPAK IA. Mobile phase: (a) hexane/2-PrOH 80/20; (b) hexane/2-PrOH 95/5.

enantiomers on CHIRALPAK IC within 15 min with a resolution degree as high as 7.24, while only partial separations of the enantiomers were found on CHIRALPAK IA and CHIRALPAK IB. This confirms the wide-ranging complimentary properties of these three columns [16].

Some compounds proved to be too weakly or too strongly retained with the hexane/2-PrOH 80/20 mobile phase in certain columns. The optimisation step was then envisaged. In Table 3 are summarised the compounds, the mobile phases and the results from the optimisation step.

Due to their weak retention and narrow selectivity value, 1-indanol enantiomers (compound **14**) were just partially resolved on all the three columns with hexane/2-PrOH 80/20. By reducing the percentage of alcohol down to 5%, a baseline resolution could actually be achieved on CHIRALPAK IA (Fig. 3).

Among the strongly retained compounds ($t_2 > 20$ min), a second peak of racemic 5,5-diphenyl-4-methyl-2-oxazolidinone (compound **11**) was identified in the time window of the following injection on CHIRALPAK IC. To facilitate the discussion, the entire chromatogram was produced and is shown in Fig. 4(a) with accurate retention time for the second peak. The resolution of the two enantiomers was excellent in the initial conditions ($R_s = 8.60$), but a long time would be needed to complete the analysis (24 min). If the method is intended for routine product analysis, it would be advised to reduce the run time by increasing the percentage of 2-PrOH up to 40% (Fig. 4b) or even to 50%. Alternatively, as the resolution is still very good, it could be transferred to a shorter CHIRALPAK IC column (e.g. 4.6 mm \times 150 mm). Raising the column temperature up to 40 °C would also be helpful to gain analysis time and reduce the viscosity of the mobile phase mixture.

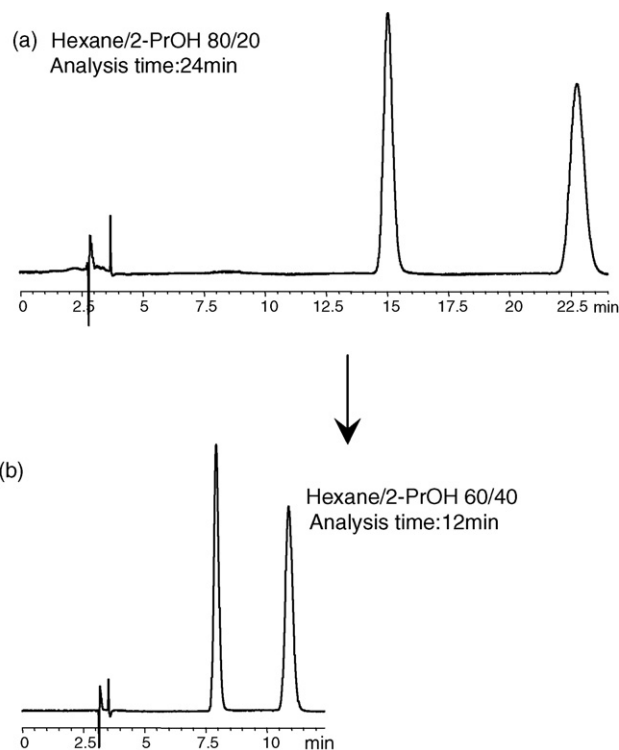


Fig. 4. Separation optimisation of 5,5-diphenyl-4-methyl-2-oxazolidinone enantiomers (**11**) on CHIRALPAK IC. Mobile phase: (a) hexane/2-PrOH 80/20; (b) hexane/2-PrOH 60/40.

Table 4

Examples of separation from the screening using hexane/EtOH 80/20 as mobile phase

Compound		Chromatographic parameters				Additive (0.1%)	Column
No.	Name	t_2	$k'1$	α	R_s		
3	Bendroflumethiazide	10.2	1.82	1.30	3.75		IC
4	2,3-O-Benzylidene-DL-threitol	15.2	1.35	3.11	15.35		IA
5	N,N'-Bis(α -methylbenzyl) sulfamide	10.5	1.58	1.63	7.95		IA
11	5,5-Diphenyl-4-methyl-2-oxazolidinone	10.9	1.70	1.53	7.62		IC
16	Praziquantel	11.3	2.29	1.24	3.17		IB
22	Metanephrene	10.3	1.72	1.40	4.96	DEA	IC
24	Hydroxyzine	9.7	1.44	1.59	6.84	DEA	IA
27	CBZ-DL-serine	15.7	2.34	1.76	6.91	TFA	IC
28	N-(3,5-DNB)-DL-leucine	9.9	1.76	1.36	3.93	TFA	IA
32	Indoprofen	10.9	2.36	1.14	2.41	TFA	IB

Temperature: 25 °C, flow rate: 1.0 ml/min.

Table 5

Examples of separation from the screening using MtBE/MeOH 98/2 as mobile phase

Compound		Chromatographic parameters				Additive (0.1%)	Column
No.	Name	t_2	$k'1$	α	R_s		
11	5,5-Diphenyl-4-methyl-2-oxazolidinone	10.24	1.25	1.99	7.64		IB
13	Furoin	7.2	0.68	2.13	10.16		IA
		8.8	0.67	2.83	14.56		IC
15	Kavain	15.0	3.52	1.17	3.05		IA
20	Miconazole	8.8	1.39	1.35	3.99	AE	IC
21	Mepivacaine	8.6	1.47	1.33	3.03	AE	IA
		9.2	1.38	1.45	5.28	AE	IC
29	2,3-Dibenzoyl-DL-tartaric acid	11.3	1.26	2.14	5.41	TFA	IC
30	FMOC-DL-leucine	9.8	1.61	1.35	2.54	TFA	IC
33	Phenylsuccinic acid	10.0	1.59	1.42	4.06	TFA	IC

Temperature: 25 °C, flow rate: 1.0 ml/min.

Table 6Examples of separation from the screening using hexane/CH₂Cl₂/EtOH 50/50/2 as mobile phase

Compound		Chromatographic parameters				Additive (0.1%)	Column
No.	Name	<i>t</i> ₂	<i>k'</i> ₁	α	<i>R</i> _s		
5	<i>N,N'</i> -Bis(α -methylbenzyl)sulfamide	7.30	0.88	1.71	7.49	-	IA
6	Bucetin	15.49	3.65	1.11	2.16	-	IC
9	1,5-Dihydroxy-1,2,3,4-tetrahydronaphthalene	10.22	1.86	1.25	4.55	-	IC
17	9,10-Dihydro-2-methyl-4H-benzo[5,6]cyclohept[1,2]oxazol-4-ol	5.80	0.81	1.21	2.49	AE	IA
18	Econazole	5.18	0.56	1.40	3.75	AE	IA
21	Mepivacaine	8.15	1.19	1.50	4.16	AE	IA
23	Tetramisole	16.10	3.04	1.39	7.97	AE	IC
26	<i>N</i> -Benzoyl-DL-Leucine	6.36	0.80	1.39	4.43	TFA	IB
28	<i>N</i> -(3,5-DNB)-DL-leucine	18.7	2.92	1.74	9.20	TFA	IC
31	6-Hydrox-2,5,7,8-tetramethylchroman-2-carboxylic acid	7.28	1.03	1.45	5.36	TFA	IA

Temperature: 25 °C, flow rate: 1.0 ml/min.

Table 7

Examples of separation from the screening using hexane/THF 70/30 as mobile phase

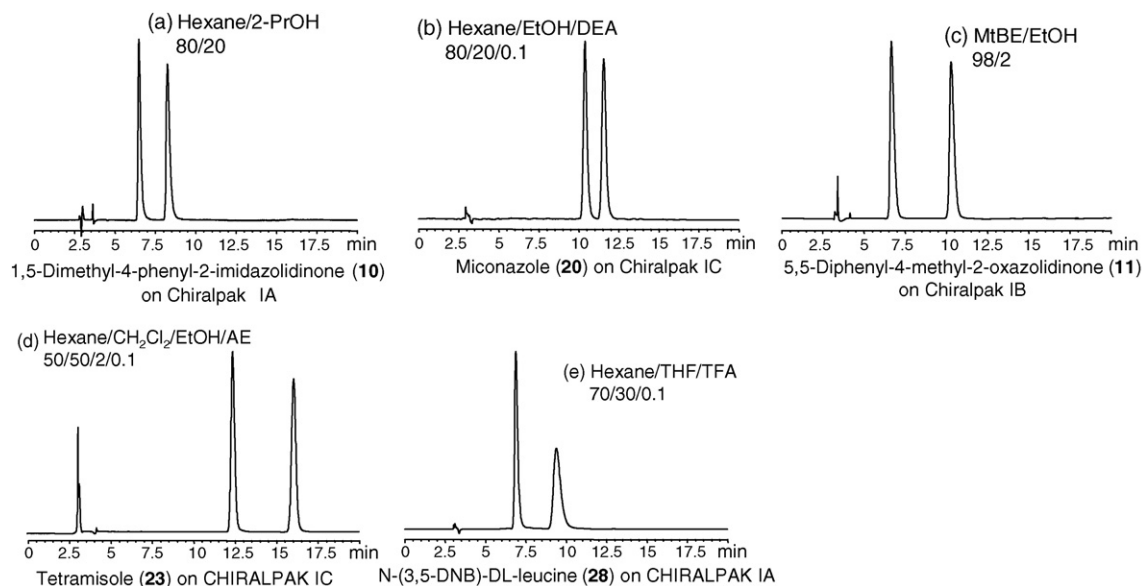
Compound		Chromatographic parameters				Additive (0.1%)	Column
No.	Name	<i>t</i> ₂	<i>k'</i> ₁	α	<i>R</i> _s		
2	<i>p</i> -Anisoil	11.6	2.25	1.33	5.74		IA
12	5-Fluoro-1-(tetrahydro-2-furyl)uracil	11.7	2.32	1.21	3.49		IC
13	Furoin	7.9	1.00	1.69	8.60		IA
15	Kavain	11.9	2.57	1.12	2.36		IC
19	DL-Isoamarine	7.5	0.97	1.61	5.34	DEA	IB
21	Mepivacaine	9.2	1.61	1.25	3.55	DEA	IC
23	Tetramisole	11.6	2.02	1.47	3.51	DEA	IA
		14.4	2.94	1.26	3.77	DEA	IC
26	<i>N</i> -Benzoyl-DL-Leucine	7.7	1.36	1.21	2.48	TFA	IA
28	<i>N</i> -(3,5-DNB)-DL-leucine	9.4	1.35	1.64	4.31	TFA	IA

Temperature: 25 °C, flow rate: 1.0 ml/min.

Contrary to the case discussed above, the strengthening of the mobile phase eluting power by adding more 2-PrOH into hexane proved to be unsuccessful for resolving compound **7** on CHIRALPAK IA and compounds **10** and **13** on CHIRALPAK IC (Table 3) by using only hexane/2-PrOH mixtures. The complete resolution of these enantiomers could be achieved on an alternative column CHIRALPAK IB or CHIRALPAK IA (Table 2).

The lack of complete separation with a single type of mobile phase does not mean the resolution defeat of a given racemate.

As previously mentioned, the mobile phase nature can play an essential role in enantiomer resolution. In Tables 4–7, a series of enantiomer resolutions are summarised using the rest of mixtures composing the primary set of mobile phase systems; that is, hexane/EtOH 80/20; MtBE/MeOH 98/2; hexane/CH₂Cl₂/EtOH 50/50/2; and hexane/THF 70/30. Statistically, suitable separations were achieved for more than 50% of the molecules examined without any further optimisation step. Fig. 5 gives some examples of enantiomer separations directly issued from the primary screen-

**Fig. 5.** Enantiomer resolution methods directly issued from the primary screening conditions.

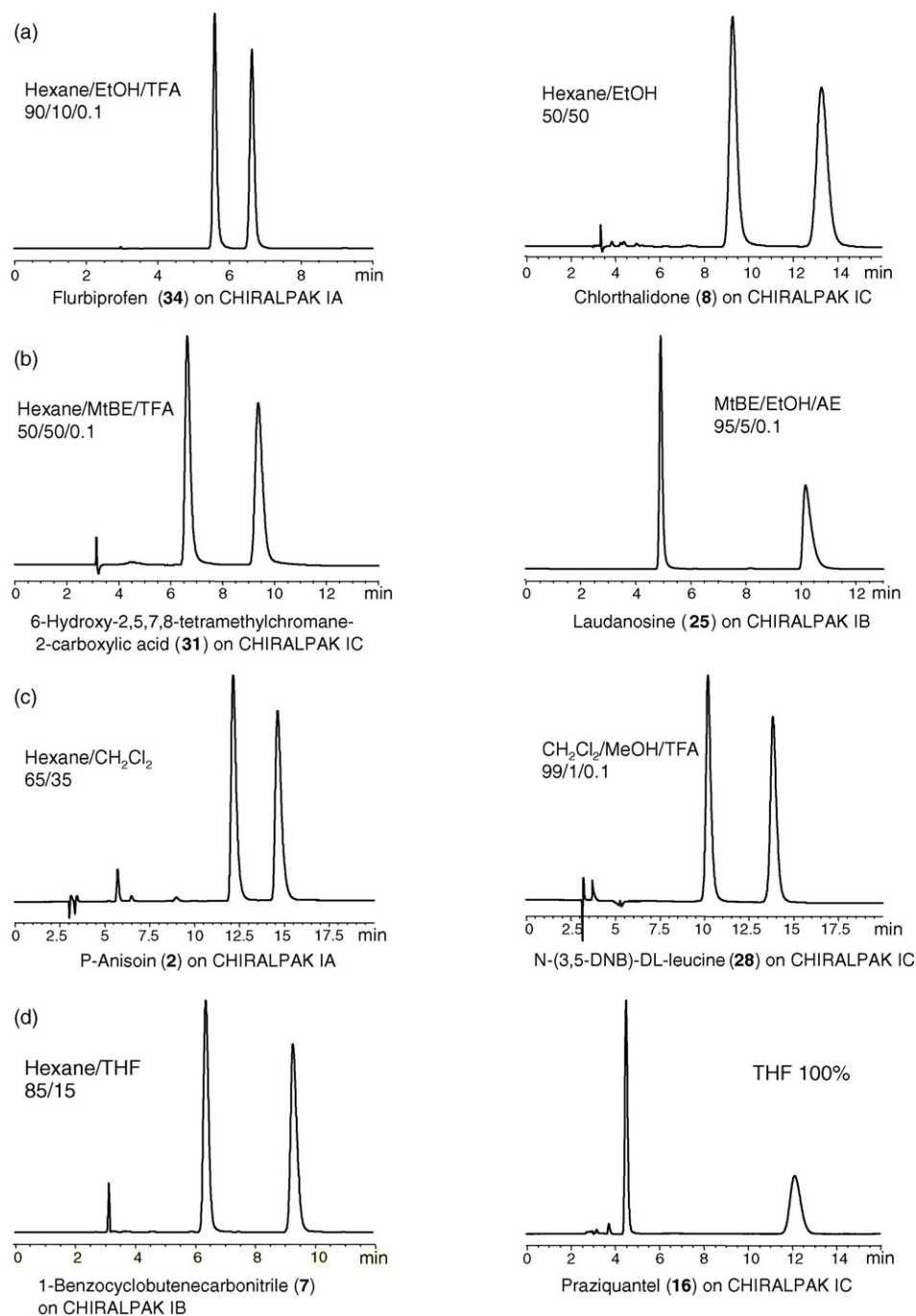


Fig. 6. Examples of optimised separations by modulating mobile phase strength. (a) Hexane/EtOH mobile phases; (b) MtBE-based mobile phases; (c) DCM-based mobile phases; (d) THF-based mobile phases.

ing using each of the recommended starting mobile phase systems. Fig. 6 displays certain enantiomer resolutions achieved after some optimisation efforts.

For basic and acidic samples, it may be necessary to incorporate an additive in the mobile phase in order to optimise the separation, as it has been done usually with the coated polysaccharide-derived CSPs. Thus, basic samples may require a basic additive (DEA, butylamine, 2-aminoethanol, ethylenediamine, ...) and acidic compounds the addition of an acid (TFA, acetic or formic acid, ...). The percentage needed is typically 0.1% and should normally not exceed 0.5% [12–15].

It has been found that certain amines, such as EDA (ethylenediamine) and 2-aminoethanol (AE) induce much better behaviour

for certain basic compounds on CHIRALPAK IB than the more commonly used DEA. This may also be true on the other columns. Resolution degree and peak symmetry can be dramatically improved with this type of additives. For practical purposes, it is possible and recommended to run the general screening with DEA, as the miscibility of AE and EDA is limited in hexane-based mobile phases in the absence of alcohols. However, it may be important in the optimisation step to consider this fact and apply the change if there is no solubility limitation. For instance, if AE is mixed with hexane, the proportion of MtBE or DCM should be at least 60% or 45%, respectively, by volume. Addition of a small percentage of ethanol (e.g. 2%) into the mobile phase may be a kind of assurance for the additive

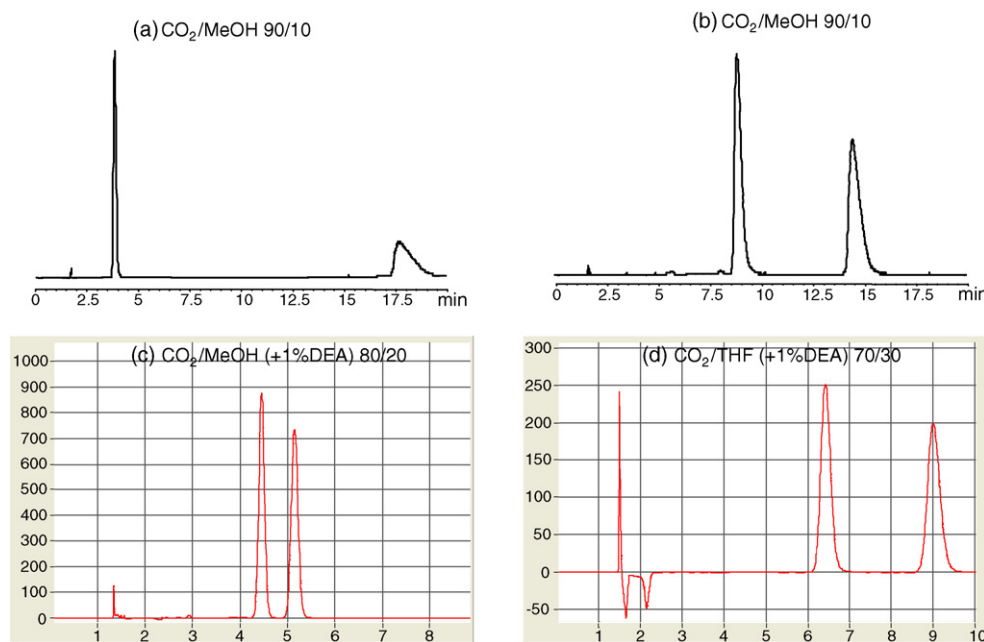


Fig. 7. Examples of enantiomer separation of SFC. (a) Mephobarbital; (b) Warfarin on CHIRALPAK IA; (c) Nicardipine; (d) Omeprazole on CHIRALPAK IC.

miscibility even with quite low percentages of MtBE or DCM in hexane.

The conditions summarised until here allow an efficient method development strategy. However, if a more exhaustive investigation is to be carried out with a given compound, or the results from the screening with the first set of mobile phases are not satisfactory, it is possible considering expanding the solvent range. For example, ethyl acetate, acetonitrile, pure alcohols, acetone, 1,4-dioxane, toluene, methylal or ethylal have demonstrated to be useful as mobile phase components for successful chiral separations. Interesting results have already been reported in previous publications at analytical and preparative levels [12–16].

No particular advice needs to be given for the transition between different mobile phases, as long as miscibility issues are considered. However, when switching from very different mobile phase conditions, equilibration times have to be adapted (ca. 40 min for analytical columns at 1 ml/min) in order to ensure reproducibility as a result of complete equilibration of the column.

Taking into account the different nature and proportion of the solvents described earlier the use of a DAD detector may be highly recommended. Certain mobile phases containing toluene, acetone and high proportions of DCM or EtOAc may cause UV detection problems. Other type of detectors may be necessary in line, such as ELSD, polarimeter, CD or RI detector [12,24].

Reversed-phase (RP) applications are also possible with this type of polysaccharide-derived supports and may be particularly convenient for resolution of compound mixtures from biological media. It is also an alternative to offer efficient separation methods while the trails with organic mobile phases are deficient. The feasibility and method development in RP mode on polysaccharide-derived CSPs were discussed in an exhaustive review article [8], but new applications have been developed since then [15,43], also fully compatible with LC–MS conditions, and will be the object of an article in the near future.

3.2. Preparative method development in HPLC

Since their introduction in early 1980s, the polysaccharide-based coated CSPs have been widely used not only for analytical

purpose, but for a large number of preparative applications as well. This is due to their high enantioselectivity and their outstanding loading capacity. Endowed with the solvent versatility, the immobilised CSPs are able to enhance the applicability of polysaccharide-based packing materials in the production of pure enantiomers by liquid chromatography [22].

For the development of separation methods with preparative perspective, a compromise of sample solubility, enantioselectivity, resolution and retention time is often needed [12,22]. One of the typical restrictions for preparative chromatography is the sample solubility in the mobile phase which has been selected to achieve the separation. With the immobilised CSPs, the solvents having the highest dissolution power for the solute can be considered as mobile phase or mobile phase components regardless of their nature. Once two or three solvents which lead to the higher solubility of the product are identified, the strategies regarding enantioselectivity properties that have been discussed for analytical method development can be applied to make the final decision. A recent article reported the impact of the sample solubility in the mobile phase on the productivity for process chromatography [22]. This piece of work also demonstrated how the chromatographic method optimisation could be guided directly by the sample solubility on the immobilised CSPs with the possibility to improve the enantioselectivity, to optimise process parameters and to increase productivity. Though this study was dealing with a specific chiral separation, it offers a general approach for method development when the solubility of the compound to be separated is critical for preparative separations.

4. Method development in SFC

SFC applications in the separation of enantiomers are a growing field and deserve a dedicated article for themselves. In the present article, we will simply give a short introduction about useful mobile phases and few application examples, but a complete overview will be given in a separated manuscript under preparation.

The tests developed until present with the immobilised polysaccharide-derived CSPs show that excellent success rates are achieved by using alcohol co-solvents, as it was already the case

with the coated phases [43–46]. In particular, ethanol, methanol, as well as 2-PrOH are very good choices to start with. Acetonitrile seems to have lower success rate in most of the cases, but it may be an option for a secondary screening.

Among the extended range of solvents, a full investigation is in course as it was made for LC separations. Initially we were using them specially to get enhanced solubility and also to solve difficult separations or to process unstable compounds in alcohols. However, it is clear that the new solvents are offering also exceptional enantioselectivity profiles. THF, MtBE, DCM or ethyl acetate, for example, can be used as the CO₂ modifier. It has been observed that pure DCM, MtBE and ethyl acetate may not be strong enough to elute certain compounds. This is the reason why the suitable screening system may most probably include a MtBE/alcohol, DCM/alcohol and/or ethyl acetate/alcohol combination.

For the time being, if we have to recommend a screening with 4 or 5 solvent combinations, it should include for example:

- 20% MeOH in CO₂.
- 20% EtOH in CO₂.
- 25% MtBE/MeOH (80/20) in CO₂.
- 25% THF in CO₂.
- 20% DCM/MeOH (90/10) in CO₂.

As already mentioned, success rate is already very high with alcohols modifiers. Therefore, it is already an option having the three immobilised columns (CHIRALPAK IA, IB and IC) being screened with them, as first strategy and later broaden the solvent range, if no satisfactory separation is achieved or there are solubility or stability issues. Some examples of enantiomer resolution by SFC are given in Fig. 7.

5. 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. Common method development approaches can be applied on them in every chromatographic mode. For liquid chromatography, a four mobile phase system on quaternary HPLC systems would lead to success rates higher than 90%. In preparative LC, the method development could be steered by sample solubility but could follow the same guideline if the solubility is not a real issue.

The columns can also be used in RP-conditions, with mobile phases being compatible with aqueous samples and in LC-MS configurations. Moreover, the positive results identified by the scientific community in SFC-mode allow the use of LC and SFC as a very efficient combination for the resolution of enantiomers, either at analytical or preparative level.

Acknowledgements

We are grateful to Mr. Atsushi Ohnishi and his colleagues at Dai-Cell Chemical Industries Ltd. for their outstanding R&D work and helpful discussion. Our particular thanks go to Mr. Dung Nguyen for his remarkable contribution to the experimental work.

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