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Cellulose tris(3,5-dichlorophenylcarbamate) immobilised on silica: A novel chiral stationary phase for resolution of enantiomers

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Abstract

CHIRALPAK IC is a new chiral stationary phase (CSP) made by immobilising cellulose tris(3,5-dichlorophenylcarbamate) on silica gel. The chiral selector is distinct from any other commercially available polysaccharide-based CSPs. Apart from its compatibility with the whole series of solvents; this CSP is able to operate under various chromatographic conditions and bring about new characteristics in enantiomeric recognition. It can afford many large and specific enantiomeric separations. It exhibits complementary properties with regard to the existing immobilised chiral packing materials of the same category.

Keywords: CHIRALPAK IC; Cellulose tris(3,5-dichlorophenylcarbamate); Immobilised polysaccharide-based chiral stationary phases; Enantiomeric resolution by liquid chromatography

1. Introduction

The polysaccharide-based chiral stationary phases (CSPs) in the CHIRALPAK and CHIRALCEL series have been the most widely utilised materials for enantiomeric resolution by liquid chromatography and SFC [1–12]. The first generation of CSPs based on coated polysaccharide derivatives on silica gels appeared in the early 1980s. Since then, considerable R&D efforts have been made in improving the applicability of these CSPs especially in terms of solvent compatibility and stability [13].

The recent advent of immobilised polysaccharide-based CSPs, demonstrated by the introduction of CHIRALPAK IA and CHIRALPAK IB in 2004, is the dawn of advanced chiral packing materials for chromatography. The chiral selectors are tris(3,5-dimethylphenylcarbamate) of amylose for CHIRALPAK IA and of cellulose for CHIRALPAK IB. The main innovative characteristic of these CSPs is the immobilisation of the selectors on the silica matrix, making these adsorbents robust and compatible to various solvent systems. The CSPs of this new generation certainly overcome the limitations of the coated polysaccharide-derived CSPs with a number of advantages such as new selectivity profile, enhancement of sample solubility in the mobile phase, possibility of injection automation for samples directly issuing various synthesis media, inhibition or minimisation of chromatographic on-line racemisation, etc. [14]. A series of successful applications on CHIRALPAK IA and CHIRALPAK IB at both analytical and preparative levels have been reported [14–23]. However, no CSP has universal enantiorecognition. The need for development of new and advanced materials therefore remains.

CHIRALPAK IC has been designed in such a context to enhance the success rate of enantiomeric resolution. The chiral selector contained in CHIRALPAK IC is cellulose tris(3,5-dichlorophenylcarbamate) (Fig. 1). The chiral polymer is immobilised onto silica gel matrix.

The high enantiorecognition ability of cellulose tris(3,5-dichlorophenylcarbamate) has been known for almost two decades. In a non-immobilised form, this cellulose derivative physically coated on a silica gel was reported as very

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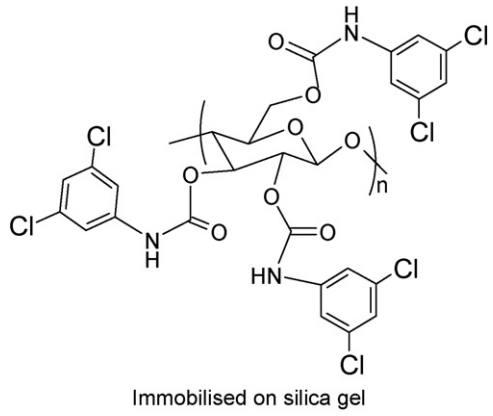

Fig. 1. Structure of the chiral selector in CHIRALPAK IC.

useful chiral packing material [24,25]. The same chiral selector had been the subject of a large number of investigations in enantiomeric resolution by HPLC, capillary chromatography and capillary electrochromatography [26–33]. Even though it has remarkable enantioselectivity for many compounds, it had not been widely applied because the polymer cellulose tris(3,5-dichlorophenylcarbamate) is soluble even in very commonly used normal-phase eluents. For instance, it is soluble in hexane–isopropanol in almost any ratios and the column packed with the coated cellulose tris(3,5-dichlorophenylcarbamate) could be destroyed after a few chromatographic runs [27–29].

Immobilisation has been the obvious approach to develop the high enantioselective power of this chiral selector. For years, chemists have been looking for the best combination between the efficient immobilisation of the polymer and optimum performance of the CSP. Different routes to achieve the immobilisation of cellulose tris(3,5-dichlorophenylcarbamate) have been tried and some chromatographic results obtained on the resulting CSPs were reported [34–36].

CHIRALPAK IC is made by using an improved proprietary immobilisation technology from Daicel Chemical Industries Ltd. to achieve high enantioselectivity, high column efficiency and universal solvent resistance. Nowadays, it is the single commercially available CSP carrying the chiral selector of cellulose tris(3,5-dichlorophenylcarbamate). In the current study, we will discuss the general aspects of CHIRALPAK IC and demonstrate its versatility in chiral separation under various chromatographic conditions. Its complementary characteristics with regard to CHIRALPAK IA and CHIRALPAK IB in enantiomeric resolution will also be addressed.

2. Experimental

2.1. Chemicals

The analytical CHIRALPAK IC columns (250 mm × 4.6 mm i.d.) were supplied by Daicel Chemical Industries Ltd. (Japan). Mobile phases for liquid chromatography were prepared from HPLC grade solvents. Hexane (*n*-), methanol (MeOH), *iso*-propanol (IPA), acetonitrile (ACN), methyl *t*-butyl ether (MtBE), ethyl acetate were purchased from Carlo-Erba (Val de Reuil, France). Ethanol (EtOH), tetrahydrofuran (THF), chloroform ($CHCl_3$), dichloromethane (CH_2Cl_2) and water were supplied by Fisher Scientific (France). Diethylamine (DEA), 2-aminoethanol (AE), trifluoroacetic acid (TFA), 1,3,5-tri-*tert*-butylbenzene (the void time marker) and the majority of the test racemic samples or enantiomers were obtained from Sigma–Aldrich (Saint Quentin Fallavier, France). The structures of compounds in test are presented in Fig. 2.

The chemicals used to prepare the buffers or salt solutions included potassium dihydrogenophosphate (KH_2PO_4), phosphoric acid 85 wt.% (H_3PO_4), boric acid (H_3BO_3), sodium tetraborate decahydrate ($Na_2B_4O_7{\cdot}10H_2O$), and potassium hexafluorophosphate anhydrous (KPF6). All of them were Fluka products supplied by Sigma–Aldrich (Saint Quentin Fallavier, France).

Preparation of the phosphate buffer (pH 2): an amount of 50 mM KH_2PO_4 was dissolved in 500 ml water; the pH value of such solution was then adjusted to 2.0 by adding H_3PO_4. Such a buffer was then used to compose the RP mobile phase in combination with the polar organic modifiers.

Preparation of the borate buffer (pH 9): an amount of 20 mM $Na_2B_4O_7$ was dissolved in 500 ml water; the pH value of such solution was then adjusted to 9.0 by adding a solution of 20 mM H_3BO_3 dissolved in 500 ml water. Such a buffer was then used to compose the RP (reverse phase) mobile phase in combination with the polar organic modifiers.

2.2. Instrumentation

Two chromatographic instruments were used in this study. Both were Agilent 1100 series apparatus equipped with a quaternary pump, a vacuum degasser, a column oven, a multiple wavelength UV detector and a HP Chemstation software. An Evaporative Light Scattering Detector (ELSD 2000 from Alltech, France) was attached to one of the HPLC units via an interface 35900E for detection when ethyl acetate or $CHCl_3$ or CH_2Cl_2 was in use in the mobile phase.

3. Results and discussion

3.1. Organic mobile phases

The mixtures of alkanes (mainly hexane or heptane) and alcohols are the most commonly used mobile-phase systems owing to their versatility in generating enantiomeric resolutions on polysaccharide-based CSPs. Although these mixtures are typically used with the coated CSPs, they represent one of the most valuable mobile phases on the immobilised CSPs as well. CHIRALPAK IC follows such a rule with no exception.

During our study, about 70 chiral molecules of various natures are examined. Fig. 2 shows a part of them. By using hexane–IPA or hexane–EtOH in appropriate proportions, CHIRALPAK IC was found to be enantioselective for more than 85% of the whole set of compounds. Furthermore, more than half of the racemic compounds were fully resolved under such mobile-phase conditions with no extensive method optimisation. Three separation examples are depicted in Fig. 3 and a list in short

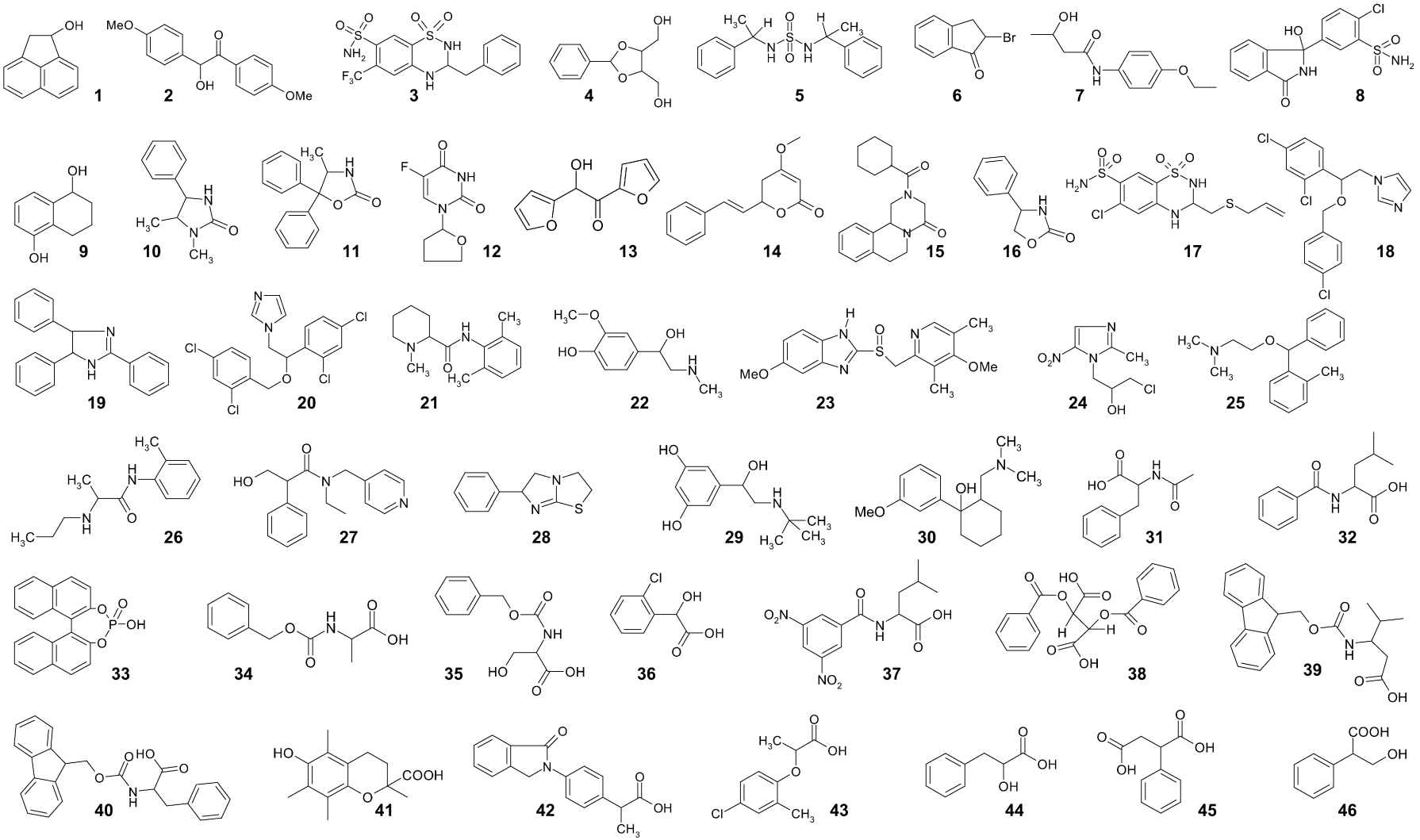

Fig. 2. Structures of the chiral compounds in test. (All the structures stand for the racemic forms. For compounds containing two stereogenic centers, please refer to their chemical names as indicated in the tables.)

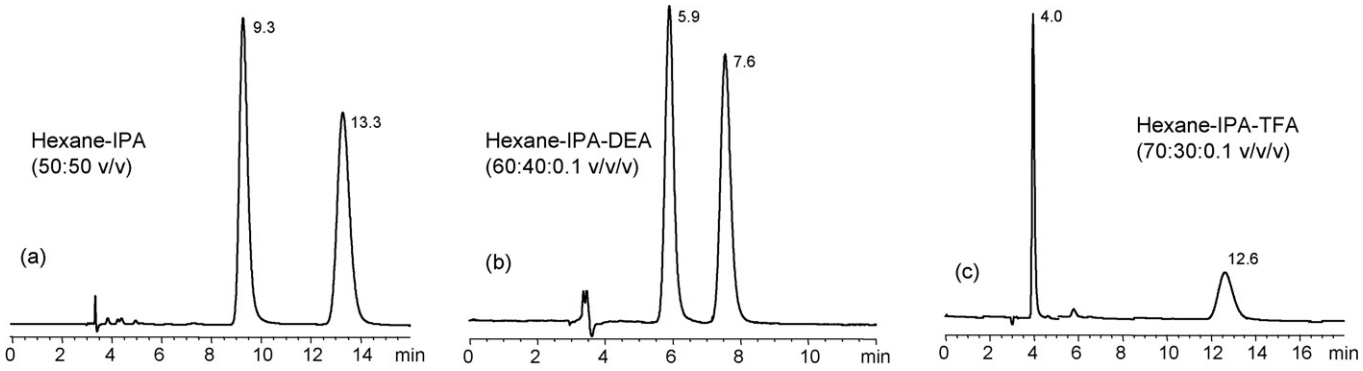

Fig. 3. Separation examples obtained on CHIRALPAK IC with mixtures of hexane/alcohol as mobile phase. Flow rate: 1.0 ml/min; temperature: 25 °C; compound: (a) chlorthalidone (**8**), (b) terbutaline (**29**), and (c) 2,3-dibenzoyl-DL-tartaric acid (**38**).

of the successful chiral resolutions on CHIRALPAK IC with hexane–alcohol as mobile phases is given in Table 1. As for any other polysaccharide-based CSPs, resolution of acidic compounds on CHIRALPAK IC needs the addition of an acidic additive (most commonly TFA). A basic additive in the mobile phase is often beneficial for resolution of chiral compounds of basic nature. The most commonly used basic additive is DEA. Addition of such an additive to alkane–alcohol mobile phases generally leads to satisfactory results.

The screening applied for the present study included a long series of solvent mixtures. The standard mobile phases applicable to the coated polysaccharide-based CSPs were investigated (mainly alkane–alcohol mixtures, acetonitrile, methanol and ethanol), together with an extensive list of the solvents classified as forbidden on the coated CSPs (subsequently called "non-standard" solvents).

The most common non-standard solvents include dichloromethane, MtBE, THF and ethyl acetate. As demonstrated in our previous studies [14,16], the benefits from the non-standard mobile-phase systems are multiple. For instance, different selectivity profile may be found owing to the presence of solvents of diverse nature and properties. This helps for the choice of the best method for resolution of a given pair of enantiomers. One example is given in Fig. 4 for the resolution of racemic *p*-anisoin (**2**). The enantioselectivity of *p*-anisoin varied in a drastic way in terms of the mobile-phase nature. While 40% ethyl acetate led to no resolution or medium degrees of separation were found with hexane–EtOH or hexane–IPA or hexane–THF or hexane–CH_2Cl_2, MtBE modified by 2% MeOH afforded exceptionally large resolution within the same time range. The resolution of racemic indoprofen (**42**) can be another illustrative instance of significant mobile-phase effect (Fig. 5). In this case, only partial resolution was observed with most kinds of mobile phases (chromatograms a–c). When dichloromethane was employed as the major component of the mobile phase, a full resolution could be easily achieved within 10 min (Fig. 5(d)). It should be noted that, in contrast to alkanes, all these non-standard solvents are miscible in the whole range with polar organic solvents. As a consequence, polar solvents such as methanol can be used as efficient modifier for retention adjustment, as demonstrated in Figs. 4(f) and 5(c) and (d).

In fact, the method improvements discussed above by either MtBE or CH_2Cl_2 in mobile phase were not isolated cases. Both MtBE and CH_2Cl_2 turned out to be especially versatile in affording efficient enantiomers separations. A number of successful resolutions by these two solvents are presented in Tables 2 and 3, respectively.

Other solvents such as THF, chloroform and ethyl acetate could also be useful for enantiomeric resolutions as exemplified in Fig. 6. Among them, THF is definitely a good alternative

Table 1
Examples of enantiomeric separations on CHIRALPAK IC by mixtures of *n*-hexane/alcohol as mobile phases

Ref.	Name	k'_1	α	R_s	Mobile-phase composition (vol.%)
3	Bendroflumethiazide	2.36	1.33	3.09	Hexane–IPA (70:30)
4	2,3-*O*-Benzylidene-DL-threitol	2.12	1.65	5.98	Hexane–IPA (80:20)
6	2-Bromo-1-indanone	2.74	1.41	8.04	Hexane–IPA (85:15)
11	5,5-Diphenyl-4-methyl-2-oxazolidinone	1.70	1.53	7.62	Hexane–EtOH (80:20)
14	DL-Kavain	2.65	1.16	3.03	Hexane–EtOH (55:45)
16	4-Phenyl-2-oxazolidinone	2.36	1.43	6.00	Hexane–IPA (50:50)
17	Althiazide	1.92	1.35	3.44	Hexane–EtOH–DEA (75:25:0.1)
19	DL-Isoamarine	2.22	1.92	9.15	Hexane–IPA–DEA (90:10:0.1)
22	Metanephrine	1.98	1.63	6.17	Hexane–IPA–DEA (70:30:0.1)
23	Omeprazole	2.12	1.87	9.36	Hexane–EtOH–DEA (60:40:0.1)
31	*N*-Acetyl-DL-phenylalanine	2.86	1.49	4.04	Hexane–IPA–TFA (85:15:0.1)
33	1,1′-Binaphthyl-2,2′-diylhydrogenphosphate	0.86	1.70	5.06	Hexane–EtOH–TFA (75:25:0.1)
35	CBZ-DL-serine	2.34	1.76	6.91	Hexane–IPA–TFA (80:20:0.1)

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

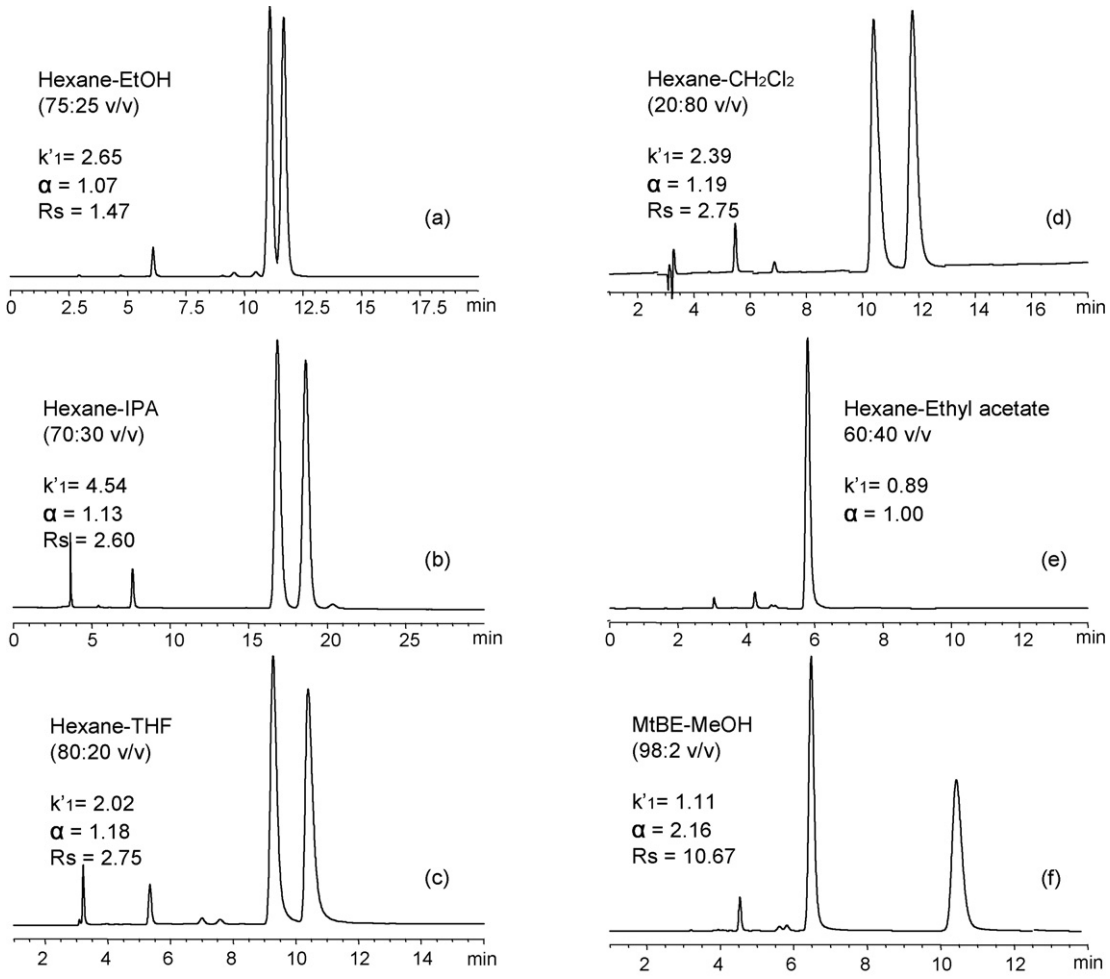

Fig. 4. Resolution of racemic *p*-anisoin (**2**) with various mobile-phase systems. Flow rate: 1.0 ml/min; temperature: 25 °C.

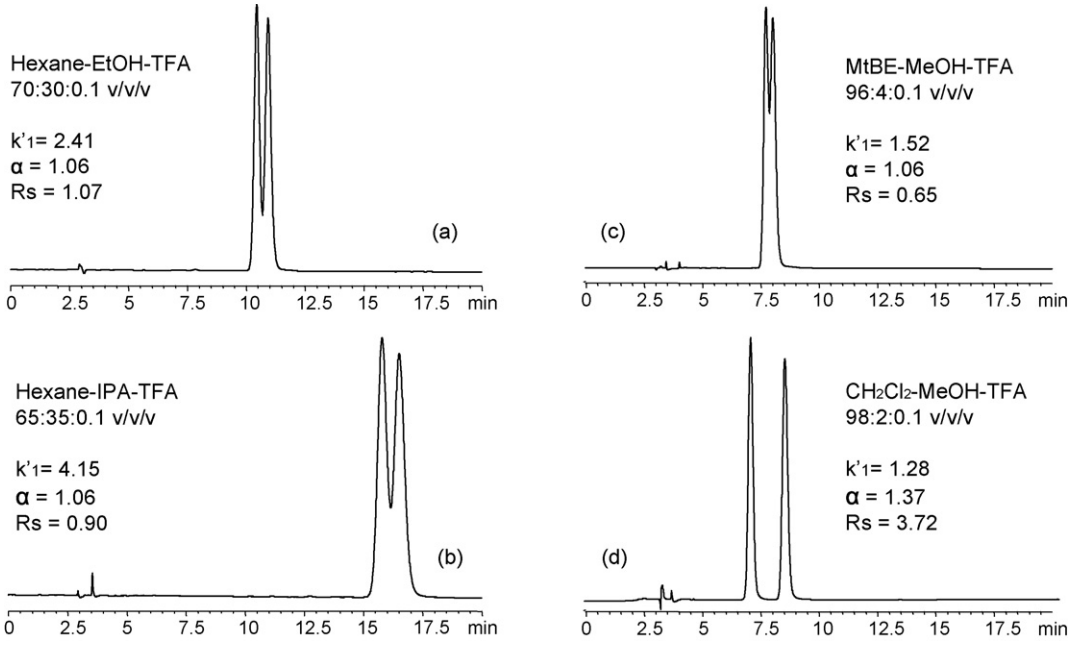

Fig. 5. Resolution of racemic indoprofen (**42**) with various mobile-phase systems. Flow rate: 1.0 ml/min; temperature: 25 °C.

Table 2
Examples of enantiomeric separations on CHIRALPAK IC by MtBE in mobile phase

Ref.	Name	k'_1	α	R_s	Mobile-phase composition (vol.%)
1	1-Acenaphthenol	0.96	1.58	6.69	MtBE–hexane (80:20)
3	2,3-*O*-Benzylidene-DL-threitol	2.28	1.54	6.08	MtBE (100)
5	*N*,*N*′-Bis(α-methylbenzyl)sulfamide	1.95	1.45	4.36	MtBE (100)
8	Chlorthalidone	1.65	1.64	4.19	MtBE–hexane (80:20)
11	5,5-Diphenyl-4-methyl-2-oxazolidinone	2.19	1.46	4.67	MtBE–MeOH (98:2)
13	Furoin	0.67	2.83	14.56	MtBE–MeOH (98:2)
18	Econazole	1.74	1.41	4.94	MtBE–MeOH–AE (94:6:0.1)
24	Ornidazole	1.05	1.44	4.96	MtBE–AE (100:0.1)
34	*N*-CBZ-DL-alanine	1.34	1.63	5.55	MtBE–TFA (100:0.1)
41	6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid	1.16	1.77	6.15	MtBE–hexane–TFA (50:50:0.1)
45	Phenylsuccinic acid	1.59	1.42	4.06	MtBE–hexane–TFA (80:20:0.1)
46	Tropic acid	1.45	1.46	5.09	MtBE–TFA (100:0.1)

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

Table 3
Examples of enantiomeric separations on CHIRALPAK IC by CH_2Cl_2 in mobile phase

Ref.	Name	k'_1	α	R_s	Mobile-phase composition (vol.%)
7	Bucetin	4.66	1.37	5.59	CH_2Cl_2 (100)
9	1,5-Dihydroxy-1,2,3,4-tetrahydronaphthalene	3.18	2.35	15.73	CH_2Cl_2–hexane (80:20)
10	1,5-Dimethyl-4-phenyl-2-imidazolidinone ((4S, 5R)- and (4R, 5S)-)	2.24	1.20	3.31	CH_2Cl_2–MeOH (98:2)
12	5-Fluoro-1-(tetrahydro-2-furyl)uracil	0.96	1.21	2.90	CH_2Cl_2–MeOH (95:5)
16	4-Phenyl-2-oxazolidinone	1.28	1.43	6.40	CH_2Cl_2–MeOH (98:2)
26	Prilocaine	0.59	1.27	2.78	CH_2Cl_2–hexane–AE (50:50:0.1)
30	Tramadol	0.36	1.47	2.53	CH_2Cl_2–hexane–EtOH–AE (34.5:64.5:1:0.1)
32	*N*-Benzoyl-DL-leucine	0.76	1.23	2.59	CH_2Cl_2–MeOH–TFA (98:2:0.1)
36	2-Chloromandelic acid	4.13	1.36	6.08	CH_2Cl_2–TFA (100:0.1)
38	2,3-Dibenzoyl-DL-tartaric acid	0.39	5.46	14.49	CH_2Cl_2–MeOH–TFA (98:2:0.1)
43	Mecoprop	5.31	1.55	8.11	CH_2Cl_2–hexane–TFA (35:65:0.1)
44	3-Phenyllactic acid	3.20	1.28	4.76	CH_2Cl_2–TFA (100:0.1)

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

for mobile-phase composition owing to the enantioselectivity it could frequently induce and its low UV background. In addition, it is friendlier than CH_2Cl_2 while the use of this later is sometimes limited due to the environmentally driven legal restrictions.

As previously mentioned, addition of a basic additive into the mobile phase may be essential for good resolution of basic compounds. For mobile phases composed of hexane and solvents of mid-polarity, 2-aminoethanol (AE) is often more efficient than DEA. However, as AE is a highly polar chemical species, its miscibility in these mobile phases should be carefully considered. For instance, in combination with hexane, the percentage of the component of mid-polarity should exceed the threshold (by volume), that is ≥60% MtBE; ≥45% CH_2Cl_2; ≥40% THF or ethyl acetate. A more direct approach to render AE miscible with this kind of solvent systems is to add a small amount of ethanol (e.g. 1 vol.%) in the mobile phase.

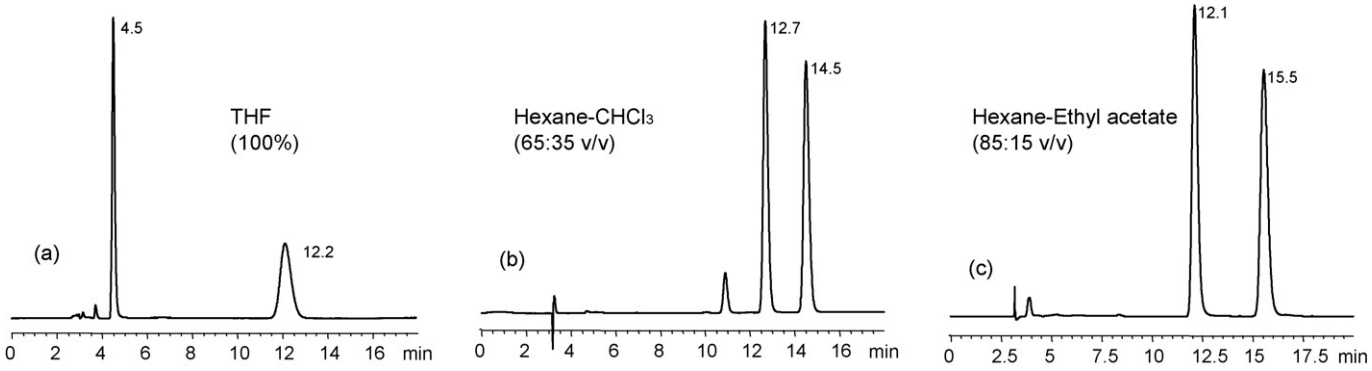

Fig. 6. Separation examples by THF, chloroform and ethyl acetate in mobile phase. Flow rate: 1.0 ml/min; temperature: 25 °C; compound: (a) praziquantel (**15**), (b) 2-bromo-1-indanone (**6**), and (c) 1,5-dihydroxy-1,2,3,4-tetrahydronaphthalene (**9**).

Table 4
Examples of enantiomeric separations with ACN or MeOH as mobile phase

Solute		ACN			MeOH		
Ref.	Name	k'_1	α	R_s	k'_1	α	R_s
15	Praziquantel	8.26	2.70	>10	3.13	1.99	9.04
18	Econazole	3.84	1.17	2.45	0.73	1.06	0.36
19	DL-Isoamarine	0.37	1.37	2.95	0.17	1.00	0.00
20	Miconazole	5.59	1.20	3.79	1.26	1.00	0.00
21	Mepivacaine	0.77	1.25	2.48	0.24	1.08	0.48
27	Tropicamide	3.37	3.40	6.08	0.62	1.06	0.49
28	Tetramisole	1.68	1.61	9.51	0.48	1.10	0.55

Temperature: 25 °C; flow rate: 1.0 ml/min for ACN; 0.7 ml/min for MeOH; additive: DEA 0.1 vol.% except for Praziquantel.

Polar organic solvents, typically ACN and MeOH, are also widely used as HPLC mobile phases for chiral separation on the polysaccharide-derived CSPs [8,12,18,27–29,37–40]. The use of solvents of this kind has several advantages such as the simplicity in mobile-phase preparation, the possibility to enhance the sample solubility in the mobile phase and get the compounds quickly eluted while no elution or too long retention is observed with hexane–alcohol mixtures. As shown in Table 4, ACN could lead to promising separations on CHIRALPAK IC, especially for compounds of basic nature. In contrast, complete resolutions could be observed with MeOH in a relatively attenuated frequency, often due to the low retention that MeOH induced for many compounds examined. The high capacity factors ($k'_1 = 3.13$, $k'_2 = 6.23$) of praziquantel (**15**) were one of the few exceptions among the compounds investigated. Although MeOH seemed to be a too strong eluent on CHIRALPAK IC, it was proved to be a versatile modifier when added into aqueous mobile phase for reverse-phase operations or into certain non-standard solvents for efficient sample elution.

3.2. Aqueous mobile phase (RP mode)

Chromatography under reverse-phase (RP) mode is 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 [4].

As previously mentioned, many compounds were eluted quickly from CHIRALPAK IC columns by MeOH. The low retention under such conditions might be the main cause for unsatisfactory resolutions in such conditions. However, the addition of the aqueous component into the polar solvents could have drastic effects in adjusting capacity factors and contributing to the improved peaks resolution. As demonstrated in Fig. 7(a), CHIRALPAK IC afforded quite a high enantioselectivity value ($\alpha = 2.42$) to 2,3-dibenzoyl-DL-tartaric acid (**38**). MeOH–TFA (100:0.1, v/v) was a so strong eluent that the peaks eluted within 5 min at 0.7 ml/min ($k'_2 = 0.05$). As a result, the high enantioselectivity only generated a very marginal resolution. A simple addition of 25% water into MeOH led to a full resolution of the two enantiomers.

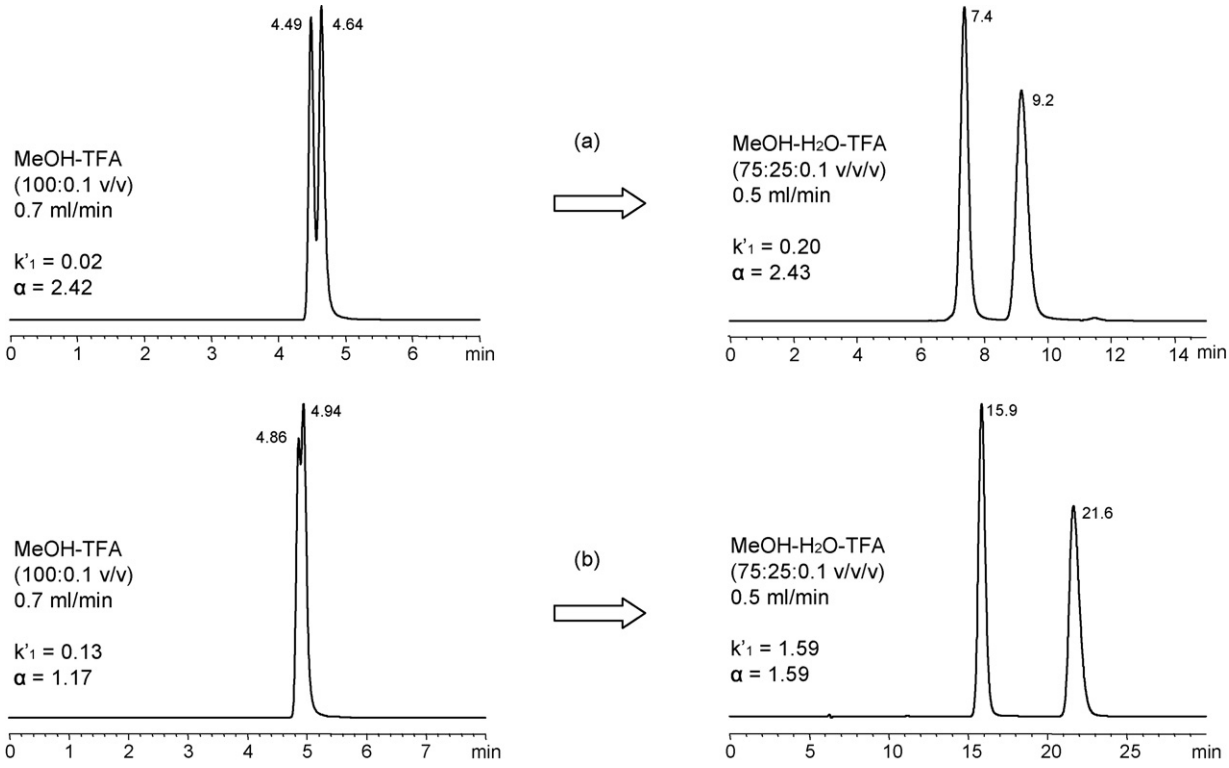

Fig. 7. Examples of method improvement in RP mode with regard to methanol. Temperature: 25 °C; compound: (a) 2,3-dibenzoyl-DL-tartaric acid (**38**) and (b) FMOC-DL-leucine (**39**).

Table 5
Examples of better results with aqueous mobile phase than with polar solvents

Ref.	Name	k'_1	α	R_s	Mobile-phase composition (vol.%)
5	5,5-Diphenyl-4-methyl-2-oxazolidinone	0.29	1.31	0.34	ACN (100%)
		1.15	**1.21**	**3.00**	**ACN–H_2O (50:50)**
17	Althiazide	0.12	1.00	0.00	ACN–DEA (100:0.1)
		2.34	**1.17**	**2.05**	**ACN–0.1 M KPF6 aq. (30:70)**
19	DL-Isoamarine	0.37	1.37	2.95	ACN–DEA (100:0.1)
		2.11	**1.30**	**5.36**	**ACN–0.02 M borate buffer (pH 9) (55:45)**
37	*N*-(3,5-DNB)-DL-leucine	0.29	1.27	1.75	ACN–TFA (100:0.1)
		0.91	**1.33**	**3.90**	**ACN–0.05 M phosphate buffer (pH 2) (50:50)**
40	FMOC-DL-phenylalanine	0.16	1.22	0.89	MeOH–TFA (100:0.1)
		2.60	**1.52**	**6.02**	**MeOH–H_2O–TFA (75:25:0.1)**

Temperature: 25 °C; flow rate: 0.5 ml/min for aqueous mobile phase; 0.7 ml/min for MeOH (+ or − additive); 1.0 ml/min for ACN (+ or − additive).

The aqueous mobile phase may also be beneficial for selectivity enhancement. This can be exemplified by the resolution of FMOC-DL-leucine (**39**) (Fig. 7(b)). As for 2,3-dibenzoyl-DL-tartaric acid, the eluent MeOH–TFA (100:0.1, v/v) resulted in minute capacity factors ($k'_1 = 0.13$, $k'_2 = 0.15$) for the given compound. A very large resolution occurred when combining 25% water with MeOH. In this later case, not only the retention was reasonably improved, but the enantioselectivity was increased too (from 1.17 to 1.59). Table 5 gives some additional examples of better resolutions owing to aqueous mobile phase (in bold). It should be noted that the retention times of a pair of enantiomers are not necessarily always increasing with the presence or increasing amount of water in the mobile phase. An opposite tendency was observed for compounds such as praziquantel (**15**), omeprazole (**23**) and miconazole (**20**). This phenomenon might be a characteristic of specific retention

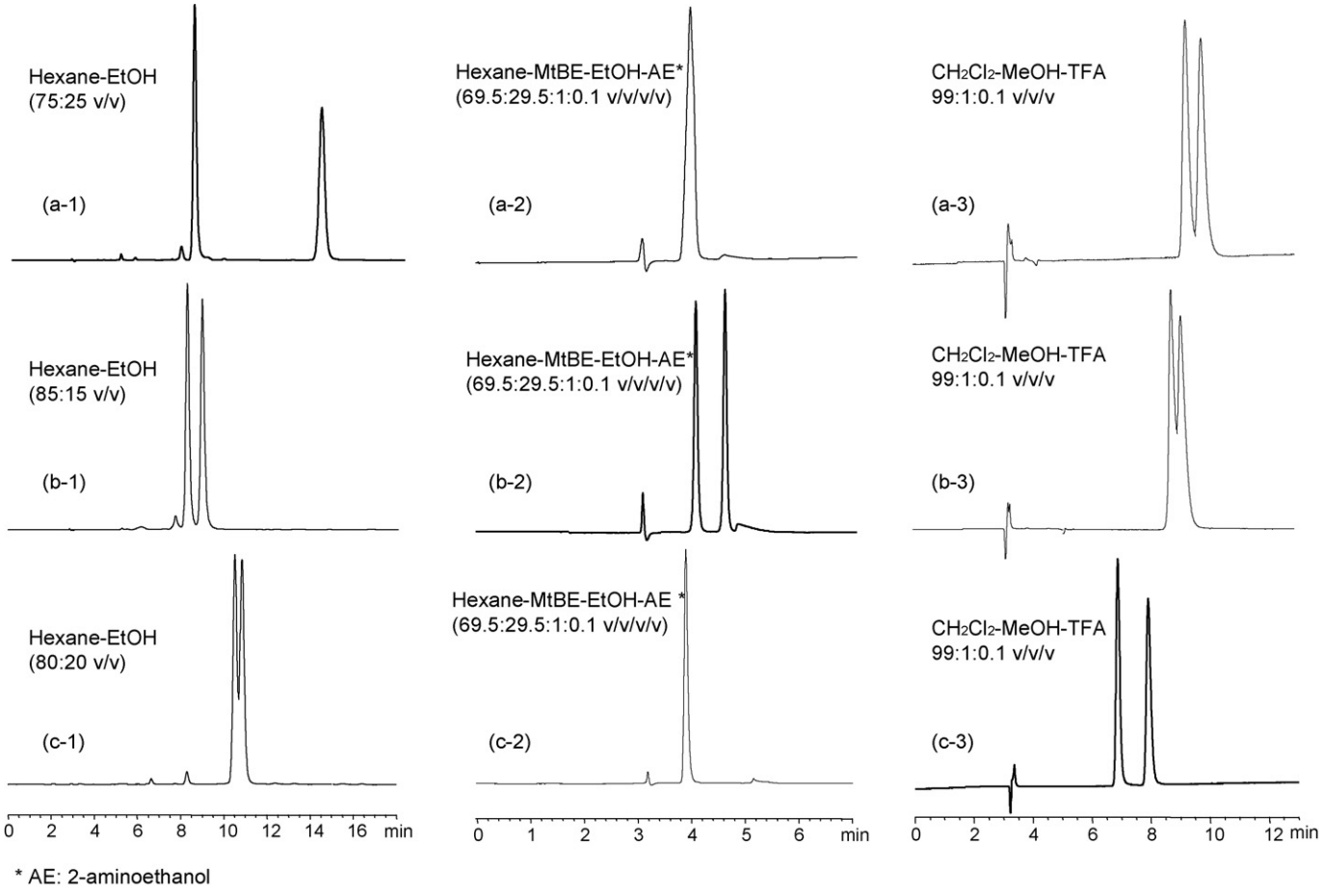

Fig. 8. Demonstration of the complementary properties of the three immobilised CSPs. Flow rate: 1.0 ml/min; temperature: 25 °C; CSP: (a) CHIRALPAK IA, (b) CHIRALPAK IB, and (c) CHIRALPAK IC; compound: (1) furoin (**13**), (2) orphenadrine (**25**), and (3) tropic acid (**46**).

mechanisms and related to the sample and the CSP structures, the potential ionic behaviour of the analyte in aqueous media and chaotropic properties of the mobile phase. Even though there is no clear rule as to the approach for method optimisation, the aqueous mobile-phase systems on CHIRALPAK IC may be considered as a kind of extension of the polar organic eluents for the search of full resolution of the enantiomers.

3.3. Complimentary properties of the immobilised CSPs

As shown in Fig. 1, the chiral selector in CHIRALPAK IC is distinct not only from CHIRALPAK IA and CHIRALPAK IB but from any other commercially available polysaccharide CSPs as well. Its chemical specificity brings about new characteristics in enantiomeric recognition for compounds which could not be properly resolved elsewhere. Moreover, CHIRALPAK IC exhibits complementary properties to CHIRALPAK IA and CHIRALPAK IB. Based on our extensive experimental results obtained, it becomes clear that a combination of the three CSPs can offer a very high success rate in chiral screening of a great variety of racemic compounds. Such complementariness among the three chiral supports has been the subject of a comprehensive investigation in our laboratories and can hardly be discussed in an exhaustive way in this current manuscript. However, some examples can be given here as a simple demonstration of the complementary feature. Using the same type of mobile phase, the best resolution of furoin (**13**) was found on CHIRALPAK IA (Fig. 8(a-1)). With a mobile phase composed of MtBE–hexane–EtOH with AE as basic additive, CHIRALPAK IB afforded very efficient resolution of orphenadrine (**25**) while neither CHIRALPAK IA nor CHIRALPAK IC could exhibit efficient enantiomeric recognition to this racemate (Fig. 8(b-2)). As far as the racemic tropic acid (**46**) is concerned, the most suitable CSP for its resolution was CHIRALPAK IC in combination with a CH_2Cl_2-based mobile phase (Fig. 8(c-3)).

4. Conclusion

CHIRALPAK IC is an outstanding chiral packing material for enantiomeric resolution. Apart from its robustness conferred by immobilisation, it exhibits enantioselectivity for a large number of enantiomers of various natures. In HPLC, the most versatile mobile phases to obtain efficient chiral separation on CHIRALPAK IC are hexane–IPA, hexane–EtOH, MtBE-based and CH_2Cl_2-based eluents. It can also operate under RP and SFC conditions. CHIRALPAK IC can be easily inserted in a screening system with columns packed with any other CSPs. The full exploitation of its enantioselective performance would be in combination with the CSPs of the same nature, that is, CHIRALPAK IA and CHIRALPAK IB. A high success rate can be attained owing to their complementary properties in chiral separation. The global strategy for method development on these three CSPs will be the topic of further publications.

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