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Optimization of the chiral separation of a Ca-sensitizing drug on an immobilized polysaccharide-based chiral stationary phase Case study with a preparative perspective

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Abstract

Sample solubility in the mobile phase and enantioselectivity are key factors in chiral preparative chromatography. In the search for a high throughput process for production of pure enantiomers, the rational design of the mobile phase and the selection of a suitable chiral stationary phase (CSP) are essential. However, one may sometimes be faced with the incompatibility between the CSP and the preferential eluent for sample solubility. Such a limitation may be circumvented by using an immobilized CSP such as CHIRALPAK® IA. In this manuscript, the chiral separation of a Ca-sensitizing drug (EMD 53986) is optimized on CHIRALPAK® IA in terms of sample solubility, enantioselectivity and preparative productivity. The approaches for method optimization and the impact of sample solubility on productivity are discussed. The preparative potential of CHIRALPAK® IA is also demonstrated.

Keywords: CHIRALPAK® IA; Amylose 3,5-dimethylphenylcarbamate; Immobilized chiral stationary phase; Chiral drug; Chiral separation; Sample solubility; Method development; Productivity; SMB

1. Introduction

In the early 1990s, Jonas et al. reported the discovery of a new class of cardiotonic agents: the 5-(1-acyltetrahydroquinolyl)-thiadiazinones [1,2]. Among them, the 3,6-dihydro-5-[1,2,3,4-tetrahydro-1-(3,4-dimethoxybenzoyl)-6-quinolyl]-6-methyl-2H-1,3,4-thiadiazin-2-one, denoted by the inventors as EMD 53998 (Fig. 1), was found promising for its Ca-sensitizing activity. During the in vitro studies, it was found that the Ca-sensitizing activity of this compound was highly stereospecific and resided only in the (+)-enantiomer. In contrast, the (−)-enantiomer was a pure phosphodiesterase (PDE) III-inhibitor totally devoid of Ca-sensitizing activity [3,4]. Therefore, there was a real need for the isolation of the two pure enantiomers to evaluate individually these two distinct therapeutic actions.

In an attempt to develop a separation route for the two enantiomers, Schulte et al. found that EMD 53986 (Fig. 1), the precursor of EMD 53998, was more easily resolved by liquid chromatography using chiral stationary phases (CSPs) [5–10]. The range of CSPs investigated included those commercially available at that time, such as the ones made by coating the amylose or cellulose derivatives on silica gel (e.g. CHIRALPAK® AD, CHIRALPAK® AS, CHIRALCEL® OD and CHIRALCEL® OJ), the pure polysaccharide derivatives in beads, as well as ChiraSpher (poly[(*S*)-*N*-acryloylphenylalanine ethyl ester] bonded on silica). From this list, CHIRALPAK® AD afforded the best chiral separation for EMD 53986 when combined with 100% ethanol as eluent. However, the specific productivity was calculated to be relatively low (430 g enantiomer/kg CSP/day) for production of the enantiomers in a large scale. This was directly attributable to the very poor solubility of EMD 53986 in ethanol. In spite of the high enantioselectivity value ($\alpha = 3.10$), the low solubility of the compound in the mobile phase was strongly limiting

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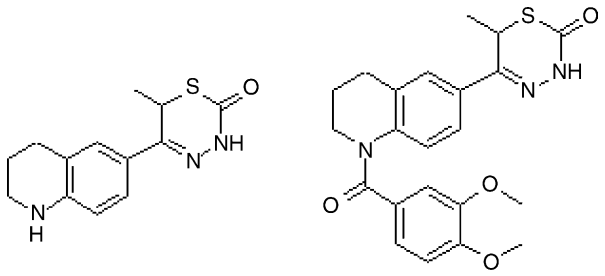

Fig. 1. Structures of EMD 53986 and EMD 53998.

the output of the preparative separation of EMD 53986 [10].

The solubility of a sample in the mobile phase is considered as one of the most critical factors for preparative chromatography. In order to render a preparative separation practically feasible and/or economically attractive, the racemic compound must be soluble enough in the solvent eluent. It would be helpful to incorporate in the mobile phase the solvents that can readily dissolve the solute. However, this requirement cannot always be fulfilled in practice, either due to the unsatisfactory separation induced by the solvents contributing to the sample solubility or for the reason of incompatibility of the CSP with such solvents. The separation of EMD 53986 on CHIRALPAK® AD is a typical example of the second scenario.

CHIRALPAK® AD consists of the chiral selector of amylose 3,5-dimethylphenylcarbamate which is physically coated on the silica gel. The commonly used mobile phases for this kind of CSPs are pure methanol, ethanol, acetonitrile and the mixtures of alkanes with alcohols. All solvents that dissolve the chiral polymer are prohibited as mobile phase, mobile phase component or even as the sample diluent.

CHIRALPAK® IA is the immobilized version of CHIRALPAK® AD and has been introduced in the market in 2004. Owing to its immobilized nature, it offers the possibility to develop separation methods with no constraint on mobile phase selection [11,12]. Taking advantage of this "universal" solvent compatibility, the chiral separation of EMD 53986 has been optimized on this new immobilized CSP in terms of sample solubility, enantioselectivity and preparative productivity. In the current report, the rational design of the mobile phase and the method optimization strategies will be discussed. The estimation of productivity, as well as the high potential of CHIRALPAK® IA in preparative chiral separations will be demonstrated.

2. Experimental

2.1. Chemicals

An analytical column of CHIRALPAK® IA, sized 250 mm × 4.6 mm I.D. and packed with amylose 3,5-dimethylphenylcarbamate immobilized on 5 μm silica, was used for method development and optimization. The loading study was carried out on a column (250 mm × 4.6 mm I.D.) packed with 20 μm CHIRALPAK® IA. Both materials were supplied by Daicel Chemical Industries (Tokyo, Japan). The productivity for simulated moving bed (SMB) was calculated by a modelling software HELP from NovaSep (Pompey, France).

Mobile phases for chromatography were prepared from HPLC-grade solvents. Methanol (MeOH), ethanol (EtOH), tetrahydrofuran (THF), 1,4-dioxane and dichloromethane (CH_2Cl_2) were purchased from S.D.S. (Seltz, France). EMD 53986 was a sample from the R&D group of Merck (Darmstadt, Germany).

2.2. Instrumentation

The analytical injections were undertaken on an Agilent 1100 series apparatus composed of a quaternary pump, a vacuum degasser, a column oven, an injector, a multiple wavelength UV detector and a HP Chemstation software. The loading study was carried out with a Merck Hitachi HPLC system consisted of an interface (D-7000), an auto-sampler (L-7200) equipped with a sample loop of 1000 μl, a pump (L-7100), a multiple wavelength UV detector (L-7400) with a semi-preparative cellule, a column oven (L-7300) and a Hitachi D-7000 HPLC System Manager program.

3. Results and discussion

The determination of sample solubility is an essential step for selection of the most suitable eluent for preparative chromatography. Table 1 summarizes the solubility values of EMD 53986 in various solvents. The highest solubility values of this compound were found in tetrahydrofuran and 1,4-dioxane, followed by chloroform and dichloromethane. The presence of such solvents in the mobile phase will undoubtedly be beneficial for the solubility enhancement of EMD 53986. While the use of these solvents is destructive for the coated polysaccharide-derived CSPs, the corresponding immobilized ones constitute a good alternative for the

Table 1
Solubility of EMD 53986 in various solvents

Solvent	Viscosity (cP, 20 °C) [13]	Solubility (g/l)
THF	0.55	85
1,4-Dioxane	1.37	75
Chloroform	0.57	38
Dichloromethane	0.44	35
Methyl acetate		15
Ethyl acetate		10
Acetonitrile		10
Methanol	0.55	10
Ethanol	1.19	8
Methyl *tert*-butyl ether		<5
Toluene		<5

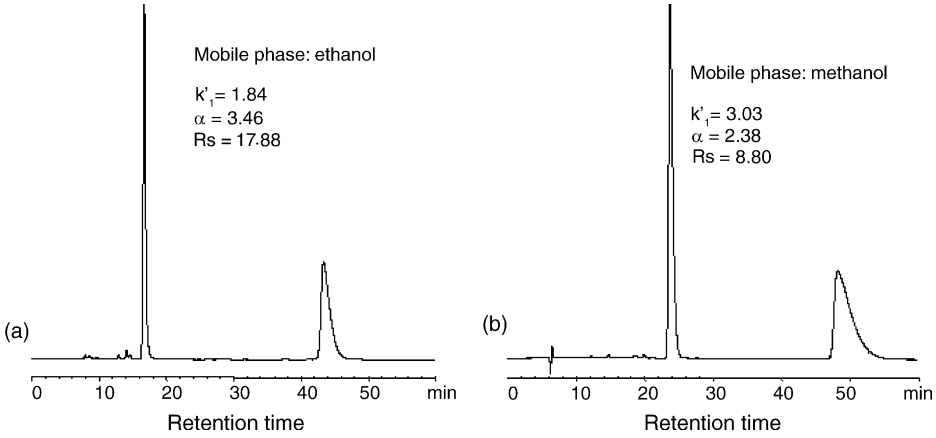

Fig. 2. Separation of EMD 53986 on CHIRALPAK® IA with alcohols. Flow rate: 0.5 ml/min, temperature: 25 °C, detection: UV 260 nm, (a) 100% EtOH; (b) 100% MeOH.

implementation of such "unusual" solvents. Driven by the outstanding separation of EMD 53986 on CHIRALPAK® AD, CHIRALPAK® IA (5 μm) was chosen to carry out the method optimization.

3.1. Mobile phase design and method optimization

As previously mentioned, the best chromatographic separation of EMD 53986 was identified on CHIRALPAK® AD with 100% ethanol as eluent [5,6,8]. In order to get a direct comparison between the two CSPs (CHIRALPAK® IA and CHIRALPAK® AD) with regard to the target sample, EMD 53986 was first injected onto CHIRALPAK® IA with 100% ethanol as eluent (Fig. 2a). The enantioselectivity obtained was 3.46, even higher than that with CHIRALPAK® AD under the same conditions ($\alpha = 3.10$ [8]). In comparison with ethanol, methanol led to a less important selectivity (Fig. 2b). Owing to its lower viscosity (see Table 1), however, methanol offers the possibility to be combined with 1,4-dioxane, the solvent offering an excellent solubility to EMD 53986 but being of high viscosity. Methanol was thus chosen as the starting mobile phase for the first optimization.

The challenge at this step is to keep the enantioselectivity large enough and the retention times in a reasonable range by adding to the mobile phase the solvents of high dissolving power. As shown in Table 1, the first choices for mobile phase composition are obviously THF and 1,4-dioxane. Due to their strong eluotropic strength, however, both THF and 1,4-dioxane were used as modifiers rather than as major components in the mobile phase. The chromatographic results obtained with various proportions of THF or 1,4-dioxane are summarized in Table 2. As found for 1,4-dioxane, THF in combination with methanol resulted in significant decreases in enantioselectivity and in retention factors, as depicted in Fig. 3. However, a totally different tendency occurred when THF or 1,4-dioxane was replaced by dichloromethane. It is worth mentioning that dichloromethane has much weaker eluotropic strength than THF and 1,4-dioxane on CHIRALPAK® IA, consequently it may be used at much higher percentages in the mobile phase or even in its pure form.

Fig. 4 displays the variation of the retention factors and the enantioselectivity values with the percentage

Table 2
Chromatographic results with THF or 1,4-dioxane in methanol

	k'_1	k'_2	α	Rs
MeOH/THF				
90/10	1.13	2.61	2.32	12.06
80/20	0.51	1.03	2.01	8.08
70/30	0.27	0.49	1.77	4.07
60/40	0.16	0.26	1.63	4.04
MeOH/1,4-dioxane				
90/10	0.78	1.50	1.92	9.25
80/20	0.38	0.62	1.64	4.45
70/30	0.23	0.34	1.53	2.38

Temperature: 25 °C, flow rate: 0.5 ml/min.

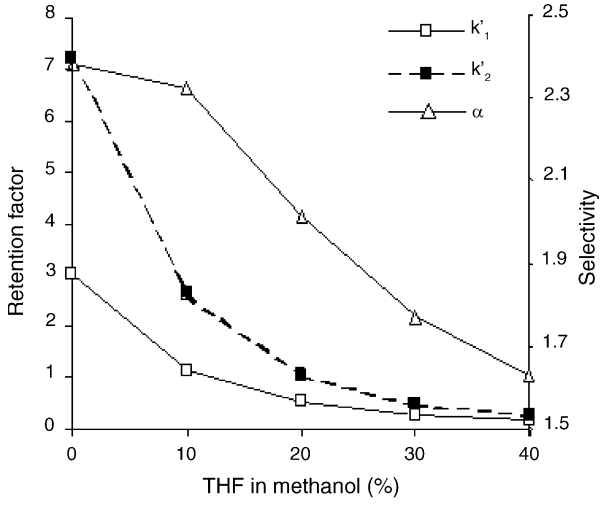

Fig. 3. Variation of chromatographic parameters with the percentage of THF in methanol. Flow rate: 0.5 ml/min, temperature: 25 °C, detection: UV 280 nm.

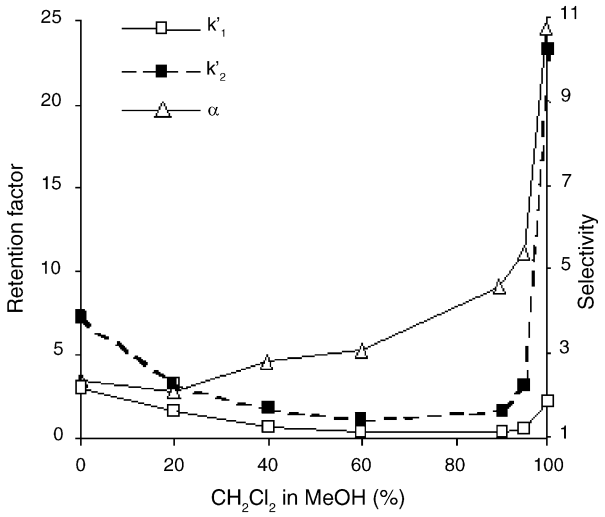

Fig. 4. Variation of chromatographic parameters with the percentage of dichloromethane in methanol. Flow rate: 0.5 ml/min (1.0 ml/min for 100% CH_2Cl_2), temperature: 25 °C, detection: UV 280 nm.

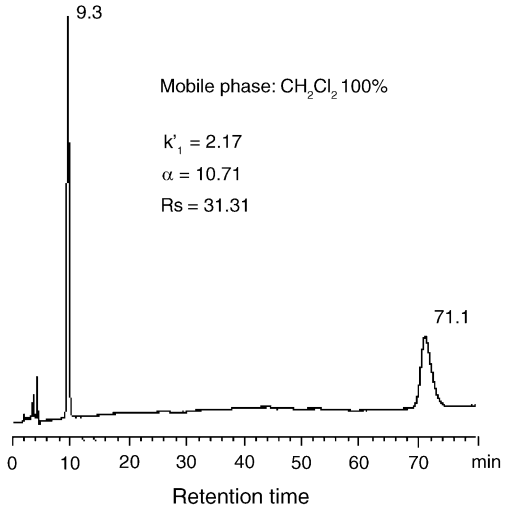

Fig. 5. Separation of EMD 53986 with 100% dichloromethane. Flow rate: 1.0 ml/min, temperature: 25 °C, detection: UV 254 nm.

of dichloromethane in methanol. While the selectivity value was gradually increasing, the retention factors were significantly reduced with the addition of dichloromethane up to 40%. Retention for both enantiomers was somewhat stabilized in the range from 40 to 90%. Beyond mix ratio of 90% dichloromethane, a steep rise in retention factor for the second enantiomer (k'_2) was observed while k'_1 remained below 3.0. As a consequence, a very large separation between the two enantiomers was generated with 100% dichloromethane as demonstrated in Fig. 5. Because of the extended run-time, such a separation is hardly applicable for the preparative purpose. Nevertheless, it gave an indication of the possible combination of dichloromethane with THF or 1,4-dioxane, whilst keeping the separation large enough and enhancing the sample solubility in the mobile phase.

As expected, EMD 53986 was well resolved into enantiomers by adding THF or 1,4-dioxane into dichloromethane. Two chromatograms obtained with 20% THF and 1,4-dioxane, respectively, in dichloromethane are illustrated in Fig. 6. Compared with 1,4-dioxane, THF was definitively the mobile phase modifier of choice for the larger selectivity it could induce, for the higher sample solubility it could offer and for its much lower viscosity (0.55 cP against 1.37 cP for 1,4-dioxane).

Table 3
Chromatographic results with THF in dichloromethane

CH_2Cl_2/THF	k'_1	k'_2	α	Rs
100/0	2.17	23.20	10.70	31.32
90/10	0.98	8.42	8.59	32.61
85/15	0.70	5.36	7.69	28.72
80/20	0.54	3.62	6.70	24.76
70/30	0.34	1.83	5.37	17.85

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

Table 3 details the separation results by varying the percentage of THF in dichloromethane. Though the selectivity was decreasing continuously by increasing the percentage of THF in dichloromethane, the separation was

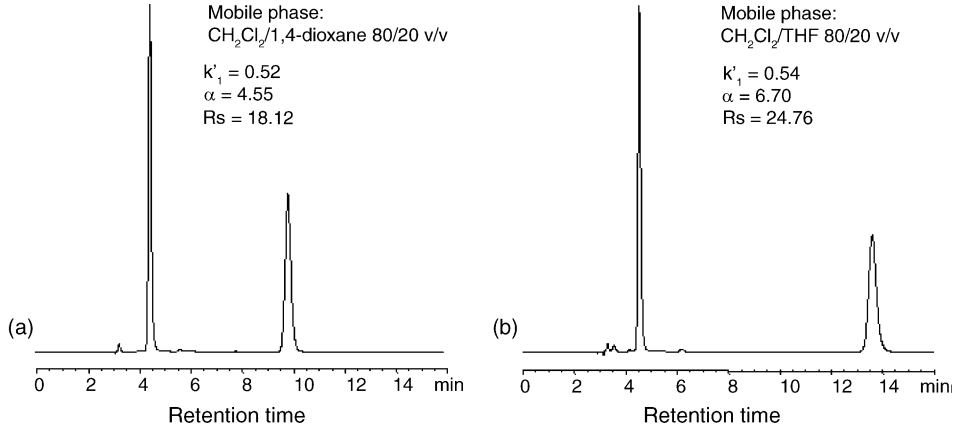

Fig. 6. Separation of EMD 53986 with 20% 1,4-dioxane (a) or 20% THF (b) in dichloromethane. Flow rate: 1.0 ml/min, temperature: 25 °C, detection: UV 280 nm.

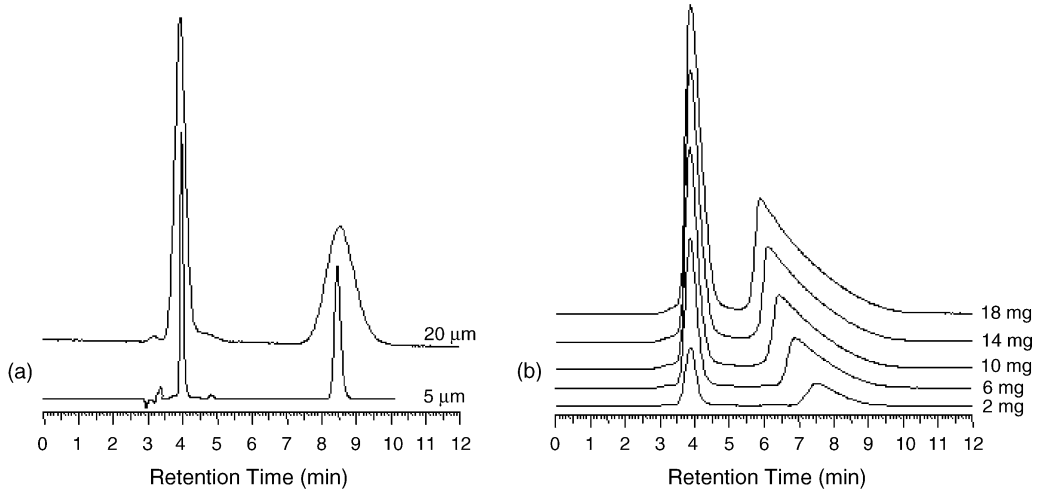

Fig. 7. Chromatograms of EMD 53986 obtained with CH_2Cl_2/THF 70/30 (v/v). Flow rate: 1.0 ml/min, temperature: 25 °C, (a) analytical injections on 20 and 5 μm CHIRALPAK® IA, UV 254 nm; (b) loading on 20 μm CHIRALPAK® IA, UV 395 nm.

still excellent at 30% THF. Furthermore, the short retention times (tr_1 = 3.9 min; tr_2 = 8.3 min) made this method ideal for its application in a preparative separation.

3.2. Preparative potential of the optimized chromatographic method on CHIRALPAK® IA

As a result of method optimization for the separation of EMD 53986, the mixture of CH_2Cl_2/THF 70/30 (v/v) was chosen for a loading study on the column packed with 20 μm CHIRALPAK® IA. The successful transfer of the separation from 5 to 20 μm packing material is demonstrated in Fig. 7a.

The purpose of the loading study was to determine the adsorption isotherm parameters of EMD 53986 on CHIRALPAK® IA under the chosen chromatographic conditions. Experimentally, a series of injections is carried out by gradually increasing the injection volume of the sample solution at a given concentration. As the amount injected increases, the peak maxima shift towards shorter time. The profile of the retention shift characterizes the adsorption isotherm [14], with which a modelling of a SMB process can be performed via the NovaSep SMB simulation software HELP. In the current study, EMD 53986 was dissolved in the mixture of CH_2Cl_2/THF 70/30 (v/v) at the concentration of 40.0 g/l and then injected sequentially from 50 to 450 μl with the interval of 50 μl. Some chromatograms from this loading injection series are displayed in Fig. 7b. It is worth noting that a complete separation of the two enantiomers could be observed even at the load as high as 18 mg racemate with the 20 μm material packed into the analytical column.

Table 4 summarizes, under Method 1, the results from the current optimization study and, under Method 2, the literature data [8]. From a direct comparison, Method 1 prevails over Method 2:

(1) The enantioselectivity is remarkably enlarged (α = 5.78 against α = 3.10 in Method 2). To a certain extent, larger

Table 4
Comparison of two chromatographic methods for enantiomeric separation of EMD 53986

Parameter	Method 1 (from current study)	Method 2 (literature data [8])
CSP	CHIRALPAK® IA (20 μm)	CHIRALPAK® AD (20 μm)
Mobile phase	CH_2Cl_2/THF 70/30 (v/v)	Ethanol
Selectivity	5.78	3.10
k'_1/k'_2	0.32/1.85	6.14/19.03
Sample solubility (g/l)	45.5	8
Column number and dimension	8 × (100 mm × 48 mm I.D.)	8 × (50 mm × 26 mm I.D.)
Amount of CSP (g)	800	100
Feed concentration (g/l)	41.0	6.0
Flow rates (ml/min)		
Feed	57.3	10.0
Extract	223.6	68.0
Raffinate	65.7	10.0
Eluent	232.0	68.0
Period time (min)	7.8	2.1
Expected purity of enantiomers (%)	>99	>99
Productivity (g enantiomer/kg CSP/day)	2834	430

selectivity allows higher load of the compound without compromising the purity of each separated enantiomer.

(2) The retention factors are considerably reduced ($k'_1 = 0.32$ against $k'_1 = 6.14$ in Method 2). It may be expected that Method 1 will lead to a separation process much more cost-effective by reducing the consumption of solvents.
(3) The sample solubility in mobile phase is enhanced to a great degree (almost six-fold).
(4) The viscosity of the eluent CH_2Cl_2/THF 70/30 (v/v) is much lower than that of ethanol. As a consequence, Method 1 should allow to conduct the SMB separation with higher flow rates to achieve a higher production output.
(5) The specific productivity in Method 1 is estimated as high as 2.8 kg enantiomer/kg CSP/day. This value is 6.5-fold the one from Method 2.

Needless to say, the significant improvement in productivity is the result of the simultaneous optimizations in selectivity, product solubility and mobile phase nature. However, the contribution of product solubility in the gain of productivity is undoubtedly essential because the loading capacity of the CSP can be fully exploited only if the solute concentration is in a reasonable range.

4. Conclusion

Although this piece of work deals with the optimization of a specific chiral separation for a given compound, some general conclusions can be derived.

Sample solubility in mobile phase has a direct impact on productivity in preparative separation of enantiomers by chromatography. In practice, the solvents having high dissolution power for the solute should be considered as mobile phases or mobile phase components. From the method development viewpoint, a compromise of sample solubility, enantioselectivity, resolution and retention time is often needed.

Owing to its solvent versatility and high chromatographic performance, CHIRALPAK® IA allows method development directly guided by sample solubility with the possibility to improve the enantioselectivity. This CSP has proven itself to be versatile for both analytical and preparative applications.

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