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# Solvent versatility of immobilized 3,5-dimethylphenylcarbamate of amylose in enantiomeric separations by HPLC

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**Abstract**

CHIRALPAK® IA is a new chiral stationary phase containing amylose 3,5-dimethylphenylcarbamate immobilized onto silica gel. It is compatible with the whole range of organic solvents. Its solvent versatility has been thoroughly investigated. The option to use a wide range of solvents, especially the "non-standards" ones, in the mobile phase enables the enhancement of chiral separation methods in terms of enantioselectivity, resolution, analysis time, sample injection and sample solubility. Parameters such as the mobile phase type, the nature of modifier and eluting strength of various solvents are examined and discussed. A guideline for method development and optimization on CHIRALPAK® IA is also proposed.


*Keywords:* CHIRALPAK® IA; 3,5-Dimethylphenylcarbamate of amylose; Immobilized polysaccharide-based chiral stationary phase; Enantiomeric separation; Non-standard mobile phases; HPLC

## 1. Introduction

Polysaccharide-derived chiral stationary phases (CSPs) have been recognized as the most powerful packing materials for the chromatographic separation of enantiomers in analytical and preparative applications due to their broad application field and their remarkable loading capacity [1–7]. In practice, more than 95% of racemic compounds can be successfully resolved by chromatography using one of the currently commercially available polysaccharide-based CSPs. Specifically, about 90% of racemates can be separated analytically on just four CSPs, i.e. tris(3,5-dimethylphenylcarbamate) of amylose (CHIRALPAK® AD), tris(3,5-dimethylphenylcarbamate) of cellulose (CHIRALCEL® OD), tris((S)-methylbenzylcarbamate) of amylose (CHIRALPAK® AS), and tris(*p*-methylbenzoate) of cellulose (CHIRALCEL® OJ). All these CSPs are produced by physical coating of the chiral polymers on a matrix [8–10].

Due to their coated nature, these CSPs can only be used with a limited range of solvents such as the polar solvents (e.g. acetonitrile, alcohols) or non-polar solvents (e.g. alkanes) in combination with some polar components as modifiers (mainly alcohols). Solvents with intermediate polarities, such as methyl *t*-butyl ether, ethyl acetate, tetrahydrofuran, acetone, 1,4-dioxane and chlorinated solvents can partially or totally dissolve the chiral polymers. Therefore, they must be excluded in the optimization of chromatographic parameters on these CSPs.

Immobilization of a polysaccharide-derivative on the support is an evolutionary strategy to make a CSP compatible with the whole range of organic solvents, which will consequently extend its application scope. An efficient immobilization method should imply a sufficient immobilization degree, an easy and reproducible synthetic route leading to a scalable process whilst guaranteeing the high enantioselective performance and stability of the resulting CSPs. For one

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and half decades, significant effort has been applied in this area [11–29,31–39], as reviewed by Franco et al. [30]. Although the immobilization methods reported in the literature are very different, many pieces of work focus on immobilization of 3,5-dimethylphenylcarbamate of amylose or cellulose [11,13–16,18,21,25,27,33,36–38], the two chiral selectors which exhibit very high potential in enantiomeric separation.

CHIRALPAK® IA is a new immobilized CSP based on 3,5-dimethylphenylcarbamate of amylose. It has been developed and recently commercialized by Daicel Chemical Industries Ltd. using an improved proprietary technology that is applicable to both analytical and preparative materials.

CHIRALPAK® IA has been extensively investigated and proved to be versatile in enantiomeric separation and excellent in solvent compatibility. In the present work, the key role of mobile phase in the enantioselectivity shown by this CSP will be discussed. Solvent effects on the separation of a broad variety of chiral compounds will be reported.

## 2. Experimental

### 2.1. Chemicals

The analytical CHIRALPAK® IA columns, sized 250 mm × 4.6 mm (I.D.) were supplied by Daicel Chemical Industries Ltd. (Japan).

Mobile phases for chromatography were prepared from HPLC grade solvents. Hexane, methanol (MeOH), ethanol (EtOH), iso-propanol (IPA), acetonitrile (ACN), methyl *t*-butyl ether (MtBE), ethyl acetate, tetrahydrofuran (THF), acetone, 1,4-dioxane, chloroform ($CHCl_3$), dichloromethane ($CH_2Cl_2$) and toluene, were purchased from S.D.S. (Seltz, France). Diethylamine (DEA, the basic mobile phase additive), 1,3,5-tri-tert-butylbenzene (the void time marker) and the majority of the test racemic samples were obtained from Sigma-Aldrich (Saint Quentin Fallavier, France). EMD 53986 (**30**) was a sample from the R&D group of Merck KGaA Darmstadt, Germany. The structures of all test compounds are presented in Fig. 1.

### 2.2. Instrumentation

Two chromatographic instruments were used in this study. The first was a Merck Hitachi HPLC system and consisted of an interface (D-7000), an auto-sampler (L-7200), a pump (L-7100), an ultraviolet detector (L-7400), a column oven (L-7300) and the Hitachi D-7000 HPLC System Manager program. An Evaporative Light Scattering Detector (ELSD 2000 from Alltech, France) was attached to this HPLC unit for detection when highly UV-absorbing mobile phases were used or samples with poor UV absorption were in test. The second HPLC unit was an Agilent 1100 series apparatus equipped with a quaternary pump, a vacuum degasser, a column oven, a multiple wavelength

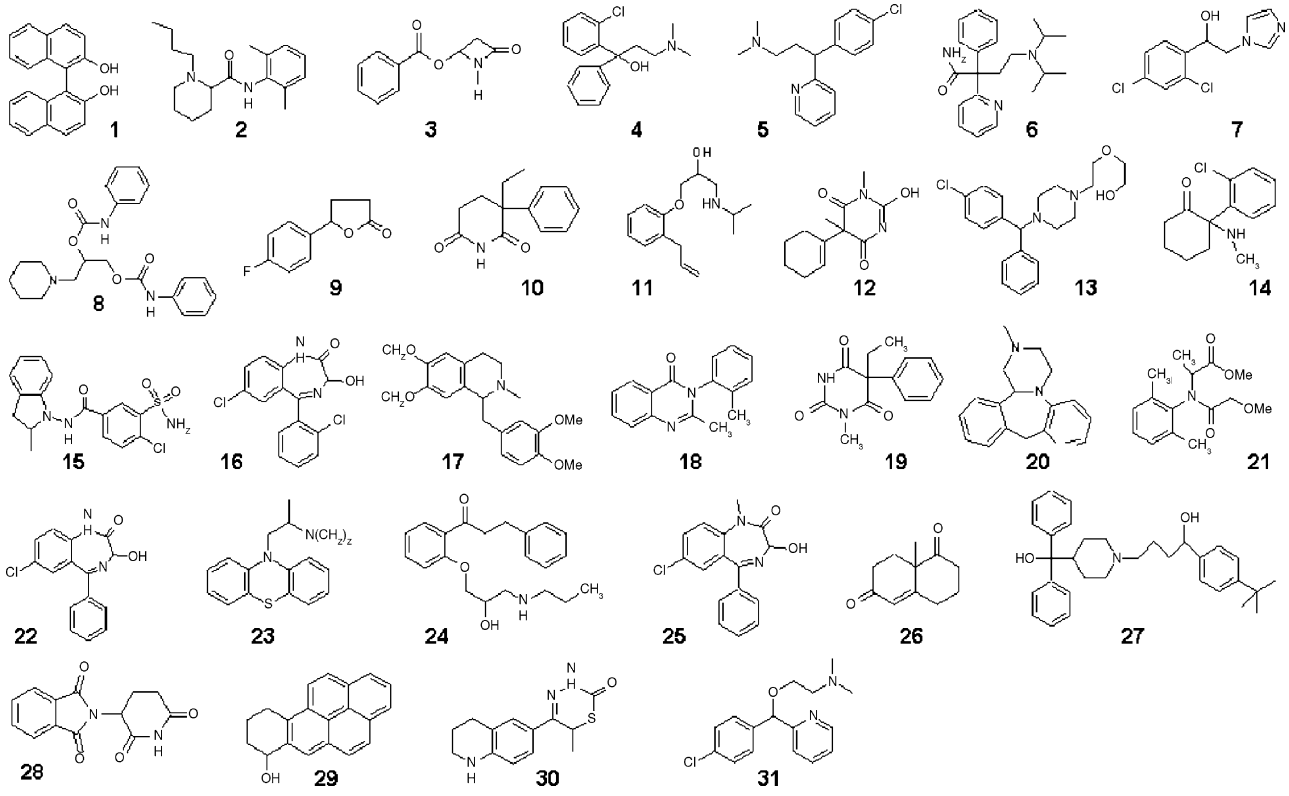


Fig. 1. Structures of the chiral compounds tested.

UV detector, an auto-sampler and a HP Chemstation software.

## 3. Results and discussion

### 3.1. Main features of the CSP

In CHIRALPAK® IA, the chiral selector 3,5-dimethylphenylcarbamate of amylose is immobilized onto silica gel. Owing to its immobilized nature, this CSP has excellent solvent versatility: it can be used with mobile phases of various natures, ranging from the so-called "standard solvents" which are recommended for the coated CSPs (ACN, alcohols, and their mixtures in alkanes) to the mobile phases containing "non-standard solvents" such as MtBE, chlorinated solvents, ethyl acetate and THF, among many others [40]. However, chromatographic conditions such as extreme pH ranges (pH < 2 or pH > 9) or high pressure (>100 bar) may be destructive for the silica support or for the packing of the column.

The option to use solvents in the expanded range (non-standard solvents) on immobilized CSPs offers several advantages. First, there are no longer constrains in choosing

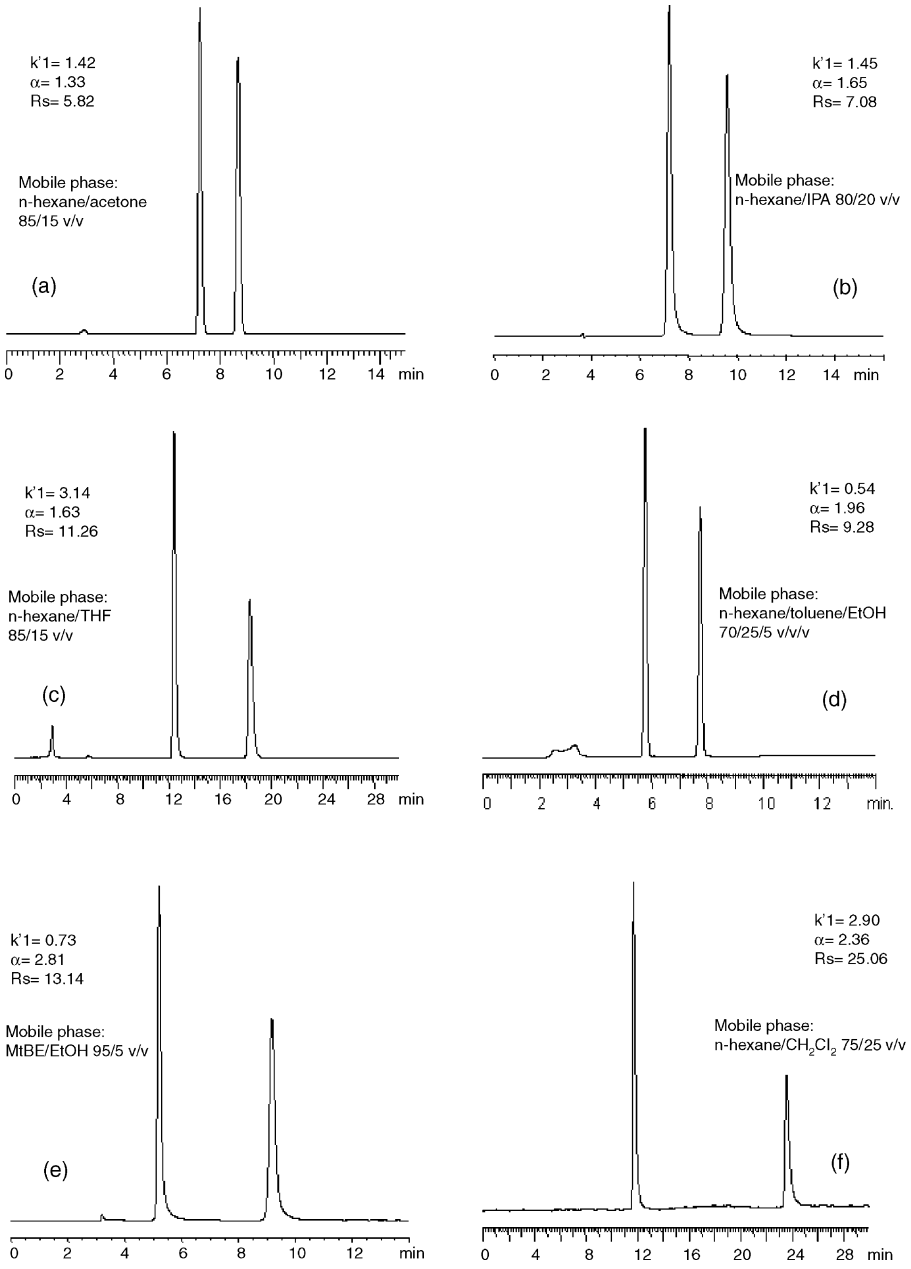


Fig. 2. Resolution of methaqualone (**18**) under various mobile phase conditions. Flow rate: 1.0 ml/min, temperature: 25 °C, detection: UV 280 nm for (b) and (e); ELSD for (a), (c), (d) and (f).

solvents for mobile phase composition. Therefore, it opens up possibilities for new selectivity profiles, as shown in Fig. 2 for the separation of racemic methaqualone (**18**) on CHIRALPAK® IA. Compounds very narrowly or not at all resolved with the standard mobile phases on the coated CSPs may now be fully or better separated using a similar or totally different solvent system on an immobilized CSP. For example, racemic bupivacaine (**2**) can only be partially resolved using ACN/DEA 100/0.1 v/v on CHIRALPAK® AD (the CSP having the same chiral selector nature as CHIRALPAK® IA but simply being coated on the support). Under exactly the same mobile phase condition, CHIRALPAK® IA gives better selectivity and increased resolution degree for the same compound, as demonstrated in Fig. 3(a–b). Furthermore, the separation of bupivacaine (**2**) can be improved to a great extent on CHIRALPAK® IA with a mobile phase switch to *n*-hexane/THF/DEA 90/10/0.1 (v/v/v) (Fig. 3(c)) or to *n*-hexane/$CH_2Cl_2$/DEA 50/50/0.1 (v/v/v) (Fig. 3(d)).

It has been shown that there is no limitation on the sample injection solvent with immobilized CSPs. Solvents such as THF, $CHCl_3$ or even dimethylsulfoxide (DMSO) [41] can be safely used as the sample diluents, making the automation of injections possible for samples directly issuing from various synthetic media. In addition, it is possible to design or modulate the mobile phase on immobilized CSPs in terms of sample solubility.

### 3.2. Mobile phase effects

Basically, all miscible solvents can be used with an immobilized CSP either in their pure form or as mobile phase components. In practice, the selection of mobile phase can be oriented by considering the solute nature, the purpose of the chromatographic operation and the general enantioselective properties of each solvent towards the CSP when they are used in the mobile phase.

The mobile phase chosen should have a minimum solubility towards the analyte so that the liquid chromatography process becomes feasible, at least at an analytical level. The sample solubility may become a determinant factor in solvent selection for the mobile phase when an ulterior preparative separation has to be considered. In this case, the solvents enabling good dissolution of the sample should first be considered as the mobile phase or the major component of the mobile phase. The exhaustive investigation of various solvents for their general behavior in enantioselective separations is undoubtedly essential in the mobile phase selection.

A large series of organic solvents have been investigated in our laboratories. It is found that, among the usual solvents for chromatography, MtBE, $CH_2Cl_2$, THF, ethyl acetate, together with the standard solvents are those with the highest potential in terms of enantioselectivity on CHIRALPAK® IA. Although this CSP can afford more or less comparable separations with standard mobile phases as CHIRALPAK® AD,

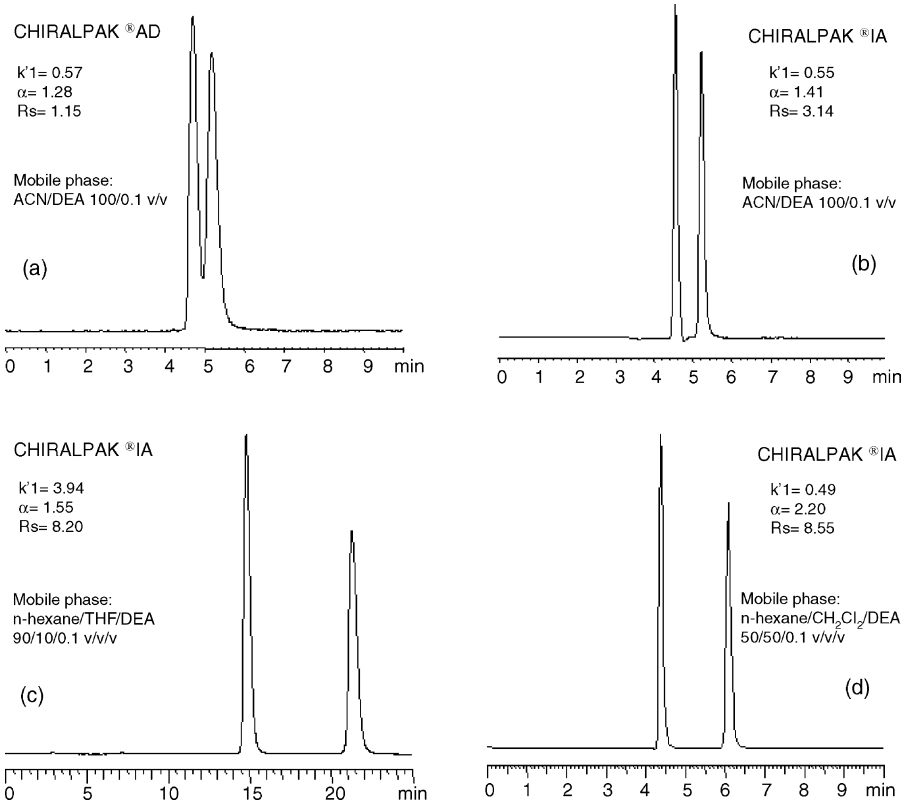


Fig. 3. Separation of bupivacaine (**2**) on CHIRALPAK® AD (a) and CHIRALPAK® IA (b–d). Flow rate: 1.0 ml/min, temperature: 25 °C, detection: 260 nm.

Table 1
Examples of enantiomeric separations using MtBE as major component in mobile phase on CHIRALPAK® IA

| Sample reference | Solute | Chromatographic parameter | | | Mobile phase composition (% by volume) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Name | $k'_1$ | $\alpha$ | $R_s$ | MtBE | EtOH | Acetone | 1,4-Dioxane | DEA |
| **12** | Hexobarbital | 0.31 | 2.94 | 7.46 | 100 | – | – | – | – |
| **18** | Methaqualone | 1.35 | 3.35 | 15.23 | 100 | – | – | – | – |
| **21** | Metalaxyl | 2.93 | 1.25 | 3.50 | 100 | – | – | – | – |
| **29** | 7,8,9,10-Tetrahydrobenzo(α)pyren-7-ol | 0.74 | 1.28 | 2.55 | 100 | – | – | – | – |
| **2** | Bupivacaine | 2.04 | 1.98 | 10.56 | 100 | – | – | – | 0.1 |
| **14** | Ketamine | 0.72 | 1.97 | 7.50 | 100 | – | – | – | 0.1 |
| **27** | Terfenadine | 1.78 | 1.53 | 2.85 | 100 | – | – | – | 0.1 |
| **16** | Lorazepam | 3.10 | 1.33 | 4.68 | 95 | 5 | – | – | – |
| **6** | Disopyramide | 2.04 | 1.20 | 2.69 | 90 | 10 | – | – | 0.1 |
| **15** | Indapamide | 0.67 | 1.75 | 4.32 | 80 | 20 | – | – | 0.1 |
| **22** | Oxazepam | 1.85 | 1.82 | 8.76 | 90 | – | 10 | – | – |
| **29** | Thalidomide | 3.12 | 1.65 | 8.83 | 90 | – | – | 10 | – |

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

its real interest lies in the use of non-standard ones. That is the reason why we focus in this article mainly on the effects of non-standard mobile phases on the enantiomeric separations.

### 3.2.1. MtBE in the mobile phase

Apart from alkanes, MtBE is the solvent with the weakest eluting strength among the solvents investigated in this study. Therefore, it is possible to use it as mobile phase in its pure form, as indicated in Table 1 for some of the compounds. Nevertheless, it has been proven that 100% MtBE may sometimes not be strong enough to have compounds eluted within a reasonable time length. Addition of some modifiers in MtBE is often required.

A separation of lorazepam (**16**) was found on CHIRALPAK® IA with 100% MtBE as mobile phase. However, the analysis time is very long (more than 40 min) and the peak shape is poor: broad peaks with large tailing were observed. The addition of 5% EtOH can reduce the analysis time by half and the peak shape is significantly improved without deteriorating the selectivity. Such a drastic modifier effect in MtBE is demonstrated in Fig. 4.

Several solvents of higher eluting strength, such as MeOH, EtOH, ACN, THF, acetone and dioxane, can be efficiently used as the modifier in MtBE to improve separations. It should be noted that the modifier providing the best separation results depends on the compound to be resolved. Although the percentage of a modifier is generally low (mostly 2–10% in MtBE), its nature can greatly affect the enantioselectivity of a given compound. Similar to lorazepam (**16**), long retention times and large peak tailing were found for the separation of thalidomide (**28**) with pure MtBE. The resolution of the enantiomers completely collapsed when adding 5% EtOH in MtBE (Fig. 5(a)). The substitution of THF (10%) or ACN (5%) for EtOH retrieved the separation (Fig. 5(b–c)). The best separation was eventually achieved with 10% 1,4-dioxane as modifier (Fig. 5(d)).

### 3.2.2. Chlorinated solvents in the mobile phase

Dichloromethane is also a versatile solvent for chiral separation on CHIRALPAK® IA. Table 2 summarizes some separation examples with $CH_2Cl_2$ in the mobile phase, some in comparison with the results from $CHCl_3$-contained mobile phases. Though $CH_2Cl_2$ has a stronger eluting strength than MtBE, it is still possible to use it in its undiluted form as for the enantiomeric separations of compounds 1, 3 and 28. The retentive behavior of CHIRALPAK® IA with $CH_2Cl_2$ varies significantly from one compound to another. If a solute is eluted too fast, addition of an alkane or MtBE to $CH_2Cl_2$

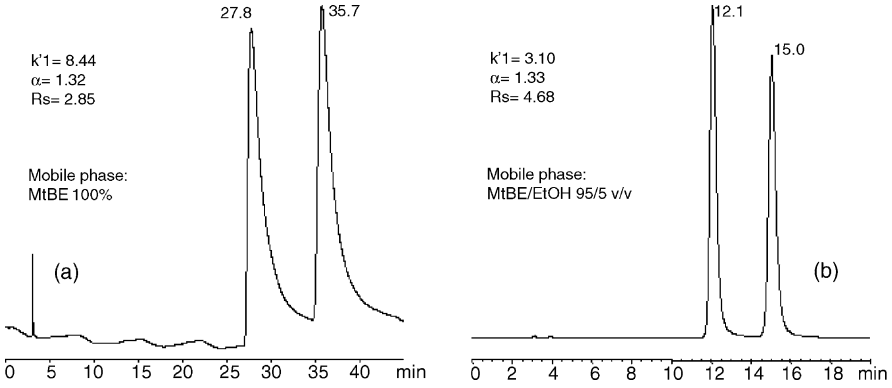


Fig. 4. Effect of modifier in MtBE on the separation of lorazepam (**16**). Flow rate: 1.0 ml/min, temperature: 25 °C, detection: UV 230 nm.

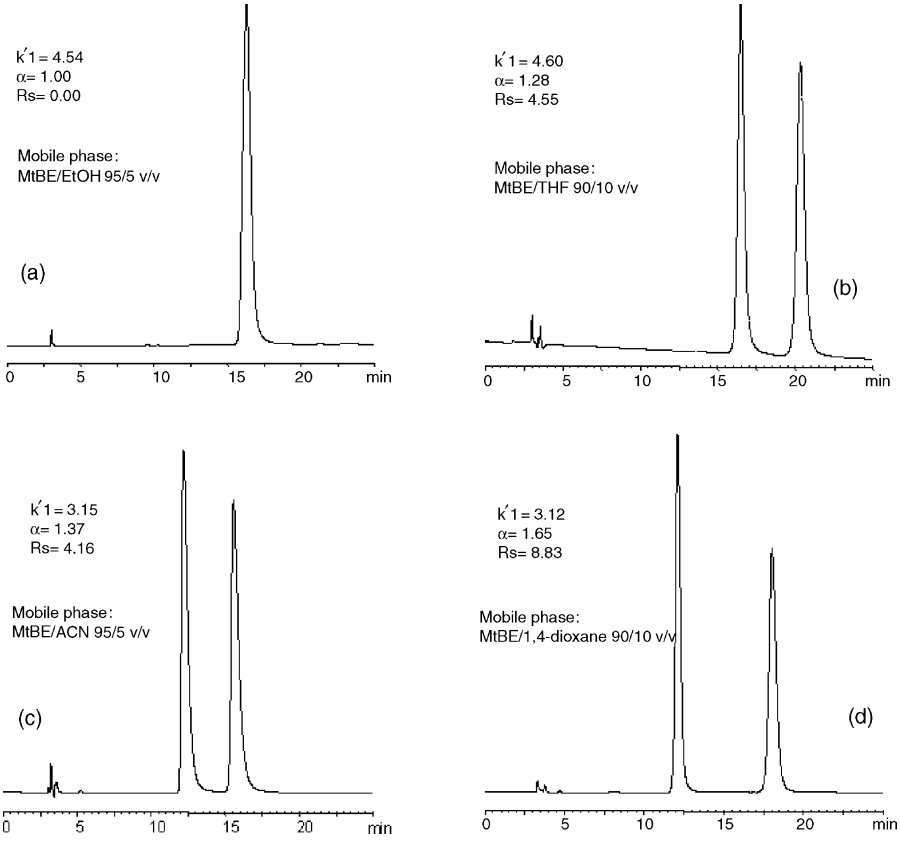


Fig. 5. Effect of modifiers in MtBE on the separation of thalidomide (**28**). Flow rate: 1.0 ml/min, temperature: 25 °C, detection: (a) UV 230 nm; (b) UV 250 nm; (c) UV 230 nm and (d) UV 300 nm.

may be helpful to return the retention to an appropriate time range. On the contrary, if the retention is too long, the eluting strength of the mobile phase should be enhanced by adding strong modifiers. That is the case for the separation of compound EMD 53986 (5-(1,2,3,4-tetrahydroquinoline-6-yl)-6-methyl-3,6-dihydro-1,3,4-thiadiazin-2-one, **30**), as shown in Fig. 6. An excellent separation of this compound is possible with 100% $CH_2Cl_2$. However, it requires more than 70 min for the second peak to be eluted. Such a separation is considered excessive and should undoubtedly be optimized for practical reasons. By adding 10% THF in $CH_2Cl_2$, the enantiomers of EMD 53986 are separated within 30 min with a slightly enhanced resolution degree. Although the selectivity is somewhat reduced, it still remains excellent. It was observed that the addition of 5% MeOH in $CH_2Cl_2$ could also shorten the retention time to a similar degree, but the selectivity was reduced by half ($\alpha = 5.40$).

Table 2
Examples of enantiomeric separations with $CH_2Cl_2$ or $CHCl_3$ in mobile phase

| Sample reference | Solute | $CH_2Cl_2$ in mobile phase | | | $CHCl_3$ in mobile phase | | | Mobile phase (% by volume) |
|---|---|---|---|---|---|---|---|---|
| | Name | $k'_1$ | α | $R_s$ | $k'_1$ | α | $R_s$ | X = $CHCl_3$ or $CH_2Cl_2$ |
| **21** | Metalaxyl | 3.19 | 1.37 | 5.58 | 2.17 | 1.28 | 3.75 | X/*n*-hexane 25/75 |
| **20** | Mianserin | 0.47 | 1.35 | 2.92 | 0.26 | 1.52 | 1.72 | X/*n*-hexane/DEA 25/75/0.1 |
| **14** | Ketamine | 1.38 | 1.88 | 9.96 | 0.58 | 1.59 | 4.51 | X/*n*-hexane/DEA 25/75/0.1 |
| **2** | Bupivacaine | 0.49 | 2.20 | 8.55 | 0.33 | 2.12 | 5.66 | X/*n*-hexane/DEA 50/50/0.1 |
| **8** | Diperodon | 2.28 | 1.58 | 7.68 | – | – | – | X/*n*-hexane/DEA 50/50/0.1 |
| **18** | Methaqualone | 0.80 | 2.03 | 9.29 | 0.64 | 1.79 | 7.84 | X/*n*-hexane 50/50 |
| **19** | Mephobarbital | 1.24 | 4.51 | 25.66 | 1.17 | 2.98 | 23.13 | X/*n*-hexane 75/25 |
| **1** | 1,1′-Bi-2-naphthol | 0.83 | 1.69 | 5.46 | – | – | – | X 100% |
| **3** | 4-Benzoyloxy-2-azetidinone | 1.95 | 1.54 | 7.20 | – | – | – | X 100% |
| **28** | Thalidomide | 1.98 | 1.61 | 6.22 | 1.63 | 1.48 | 6.82 | X 100% |
| **30** | EMD 53986 | 0.57 | 5.40 | 24.50 | – | – | – | X/MeOH 95/5 |
| **10** | Glutethimide | 0.47 | 2.84 | 11.78 | – | – | – | X/THF 90/10 |

Temperature: 25 °C, flow rate: 0.5 ml/min for EMD 53986; 1.0 ml/min for all other compounds.

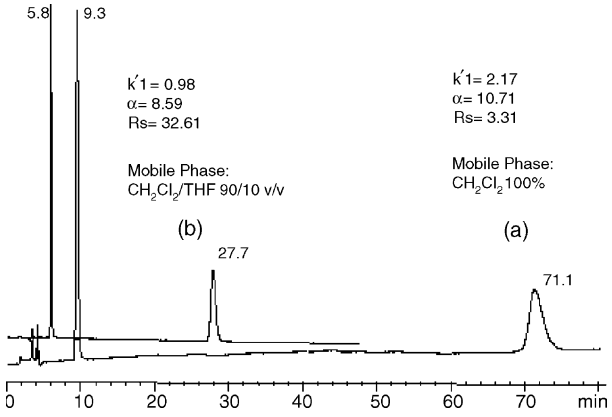


Fig. 6. Separation of compound (**30**) on CHIRALPAK® IA using $CH_2Cl_2$–based mobile phases. Flow rate: 1.0 ml/min, temperature: 25 °C, detection: UV 254 nm.

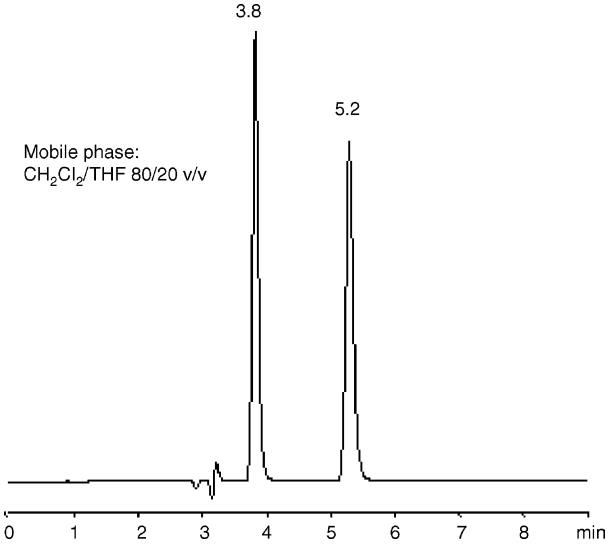


Fig. 7. Separation of glutethimide (**10**) on CHIRALPAK® IA. Flow rate: 0.5 ml/min, temperature: 25 °C, detection: UV 260 nm.

The need for a large selectivity may be less important when an analytical method only is required. In this case, a good resolution degree and a short analysis time become more essential. An example can be given with the enantiomeric analysis of glutethimide (**10**). The separation of this compound can be achieved on CHIRALPAK® IA by various mobile phase conditions, but is characterized by very long retention times. However, a mixture of $CH_2Cl_2$/THF 80/20 (v/v) provides an efficient analytical method of gluthetimide on this column in terms of high resolution degree ($R_s$ = 8.2) and low analysis time (run time = 6 min), as presented in Fig. 7.

Chloroform shows similar properties to dichloromethane on CHIRALPAK® IA, but generally leads to lower enantioselectivity on this CSP as compared in Table 2.

In the case of chlorinated solvents, it is recommended that they are used in their stabilized state for safety reasons. Depending on the solvent supplier, different substances (in the range of 0.0005%–0.2%) may be added as the stabilizer. They can be MeOH, EtOH, amylene, cyclohexane, amines, etc. It has been noticed that the nature of the stabilizer in a chlorinated solvent may influence chiral separations, especially in terms of retention times. For example, a significant retention shift was observed when $CH_2Cl_2$ solvents stabilized respectively with EtOH and with amylene were used as the mobile phase. The presence in trace amount of EtOH could markedly shorten retention times. This factor should not be ignored if the reproducibility of separations has to be considered.

### *3.2.3. Ethyl acetate and THF in the mobile phase*

Ethyl acetate and THF are faster eluting solvents compared to MtBE and $CH_2Cl_2$. They can be used as good alternative mobile phase components for enantiomeric separation on CHIRALPAK® IA. Both of them often require a co-eluent. In Table 3 are summarized some chiral separations with ethyl acetate, generally combined with n-hexane. The percentage of ethyl acetate in the mobile phase can be very variable (from 20 to 100%) to separate various compounds within 30 min.

Successful separations can sometimes be achieved with a high concentration of ethyl acetate in an alkane or even with

Table 3
Examples of enantiomeric separations with ethyl acetate in mobile phase

| Sample reference | Solute | Chromatographic parameter | | | Mobile phase composition (% by volume) | | |
|---|---|---|---|---|---|---|---|
| | Name | $k'_1$ | $\alpha$ | $R_s$ | Ethyl acetate | *n*-Hexane | DEA |
| **18** | Methaqualone | 2.72 | 1.95 | 8.20 | 20 | 80 | – |
| **21** | Metalaxyl | 2.06 | 1.21 | 6.19 | 30 | 70 | – |
| **12** | Hexobarbital | 1.25 | 4.09 | 32.02 | 30 | 70 | – |
| **15** | Indapamide | 4.94 | 1.23 | 3.99 | 40 | 60 | 0.1 |
| **6** | Disopyramide | 4.52 | 1.39 | 7.98 | 40 | 60 | 0.1 |
| **16** | Lorazepam | 3.34 | 1.85 | 13.81 | 50 | 50 | – |
| **22** | Oxazepam | 3.03 | 1.69 | 10.82 | 50 | 50 | – |
| **25** | Temazepam | 3.47 | 1.59 | 13.44 | 50 | 50 | – |
| **5** | α-(2,4-DCP)-1H-imidazole-1-ethanol | 3.29 | 1.54 | 6.47 | 60 | 40 | 0.1 |
| **13** | Hydroxyzine | 2.90 | 2.22 | 19.64 | 60 | 40 | 0.1 |
| **19** | Mephobarbital | 0.29 | 4.69 | 16.60 | 70 | 30 | – |
| **10** | Glutethimide | 0.47 | 4.00 | 16.37 | 100 | 0 | – |

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

Table 4
Examples of enantiomeric separations with THF in mobile phase

| Sample reference | Solute | Chromatographic parameter | | | Mobile phase composition (% by volume) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Name | $k'_1$ | $\alpha$ | $R_s$ | THF | *n*-Hexane | EtOH | MeOH | DEA |
| **11** | Alprenolol | 2.06 | 1.42 | 3.95 | 10 | 90 | – | – | 0.1 |
| **4** | Chlophedanol | 1.00 | 1.24 | 3.74 | 10 | 90 | – | – | 0.1 |
| **5** | Chlopheniramine | 1.83 | 1.37 | 3.24 | 10 | 90 | – | – | 0.1 |
| **23** | Promethazine | 0.91 | 1.59 | 7.57 | 10 | 90 | – | – | 0.1 |
| **26** | WMK | 0.43 | 1.26 | 2.20 | 10 | | | 90 | – |
| **8** | Diperodon | 1.75 | 1.24 | 3.76 | 20 | 80 | – | – | 0.1 |
| **9** | 4-Fluorophenyl-γ-butyrolactone | 2.04 | 1.43 | 6.96 | 20 | 80 | – | – | – |
| **10** | Glutethimide | 0.60 | 2.52 | 12.54 | 20 | – | 80 | – | – |
| **17** | Laudanosine | 1.99 | 1.25 | 2.46 | 20 | 80 | – | – | 0.1 |
| **18** | Methaqualone | 0.20 | 1.82 | 3.86 | 30 | – | 70 | – | – |
| **25** | Temazepam | 3.49 | 1.68 | 11.45 | 30 | 70 | – | – | – |
| **24** | Propafenone | 0.51 | 1.43 | 3.30 | 40 | 60 | – | – | 0.1 |

Temperature: 25 °C, flow rate: 0.5 ml/min for compounds 10 and 25; 1.0 ml/min for all other compounds.

100% ethyl acetate. However, THF should always be diluted either by a non-polar solvent such as hexane or by a very polar component, e.g. MeOH or EtOH. Among the solvents investigated in this study, THF appears to be the solvent of the highest eluting strength on CHIRALPAK® IA. The use in its pure form leads quasi systematically to no retention of solutes regardless of their natures. In a large series of compounds tested, 70% THF in n-hexane is the highest proportion for the most retained solute. Therefore, THF is often used as a modifier rather than as a major component in mobile phases. Some enantiomeric separations using THF are presented in Table 4. Fig. 8 shows the separations of 4-fluorophenyl-γ-butyrolactone (**9**) and glutethimide (**10**) by 20% THF in the mobile phase, but with two different co-eluents.

To understand the particular eluting behavior of THF on CHIRALPAK® IA, we need to review the usual chromatographic modes. In reversed phase (RP) chromatography, a non-polar adsorbent operates in combination with polar eluents. The contrary is true for normal phase (NP) chromatography: the adsorbent is polar but the mobile phases are apolar. Like all phenylcarbamates or benzoates of amylose or cellulose, the chiral selector on CHIRALPAK® IA is a material of mid-polarity. It is certainly more polar than C18 or C8 adsorbents, but less polar than bare silica. This makes it possible to operate in a kind of "polytypic mode ": by switching the mobile phase from one extreme to the other in the polarity range, the mode of separation can be changed and the separation can be achieved by different mechanisms. That also explains why the polysaccharide-derived CSPs generally work well for chiral separations either with alkanes modified by alcohols or with polar solvents like pure MeOH or ACN. The key point for mobile phase selection is that, in order to maximize the interactions between the stationary phase and solutes, the polarity of the mobile phase should distinguish itself to a sufficient extent from that of the stationary phase. By extrapolation, the interactions stationary phase-solute would collapse if the stationary phase and the mobile phase have very similar properties in terms of polarity. This may be the case for THF and CHIRALPAK® IA. "Like cancels like" can precisely describe this special mobile phase-stationary phase relationship. Such a statement is actually a variation on the adage "like dissolves like" which describes the dissolution of a substance by a solvent. Even though the retention mechanisms may be more complex and may not simply follow the polarity rule, this theory may explain the lack of retention of analytes on CHIRALPAK® IA with pure THF.

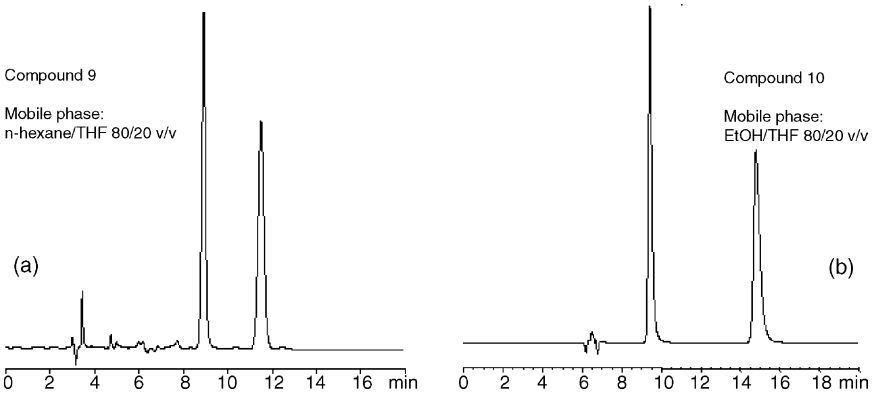


Fig. 8. Separation examples on CHIRALPAK® IA using THF in the mobile phase. Temperature: 25 °C, detection: (a) UV 220 nm; (b) UV 260 nm, flow rate: (a) 1.0 ml/min; (b) 0.5 ml/min.

Table 5
Typical starting conditions and optimization ranges for method development

| Non standard solvent (NSS) | MtBE[a] | $CH_2Cl_2$[a] | Ethyl acetate | THF[b] |
|---|---|---|---|---|
| Co-eluent (CoE) | EtOH | Alkane | Alkane | Alkane |
| NSS/CoE | 98:2 | 50:50 | 40:60 | 30:70 |
| Optimization range | 80:20–100:0 | 25:75–100:0 | 20:80–70:30 | 10:90–50:50 |

[a] (Other) useful modifiers: MeOH, acetone, 1,4-dioxane, THF.
[b] Other co-eluents are possible: MtBE, $CH_2Cl_2$, alcohols, ACN.

### 3.2.4. Method development

As demonstrated, MtBE, $CH_2Cl_2$, THF and ethyl acetate are the major non-standard solvents which afford excellent enantioselectivity on CHIRALPAK® IA. This can undoubtedly simplify to a significant degree the approach to mobile phase selection and consequently shorten the time needed for method development. Table 5 summarizes the typical starting conditions and solvent ranges for a quick method development and optimization of enantiomeric separations on CHIRALPAK® IA.

If an exhaustive investigation is to be carried out with a given compound or the results from the first screening of the four types of mobile phases are not satisfactory, it may be worth considering expanding the solvent range. For example, acetone, 1,4-dioxane or toluene may also be useful mobile phase components for successful chiral separations. The chromatograms demonstrated in Fig. 9 are the best separations for disopyramide (**6**), laudanosine (**17**), chlophedianol (**4**) and carbinoxamine (**31**) that could be identified with the whole series of solvents examined in our study, including those which are in the standard category.

### 3.3. CSP stability towards the non-standard solvents

As previously stated, in the traditionally coated CSPs, there are no chemical linkages either between the polymer and the matrix or among the polymeric chains. The polymer may be stripped off from the CSP if non-standard solvents are used as mobile phase components or as sample diluents. As a consequence, the CSP may be damaged or completely destroyed. In contrast to the coated CSPs, CHIRALPAK® IA is resistant to all kinds of solvents owing to its immobilized nature.

Ethyl acetate is an excellent solvent which will readily and totally dissolve the polymer of tris(3,5-dimethylphenylcarbamate) of amylose in its pure form. In order

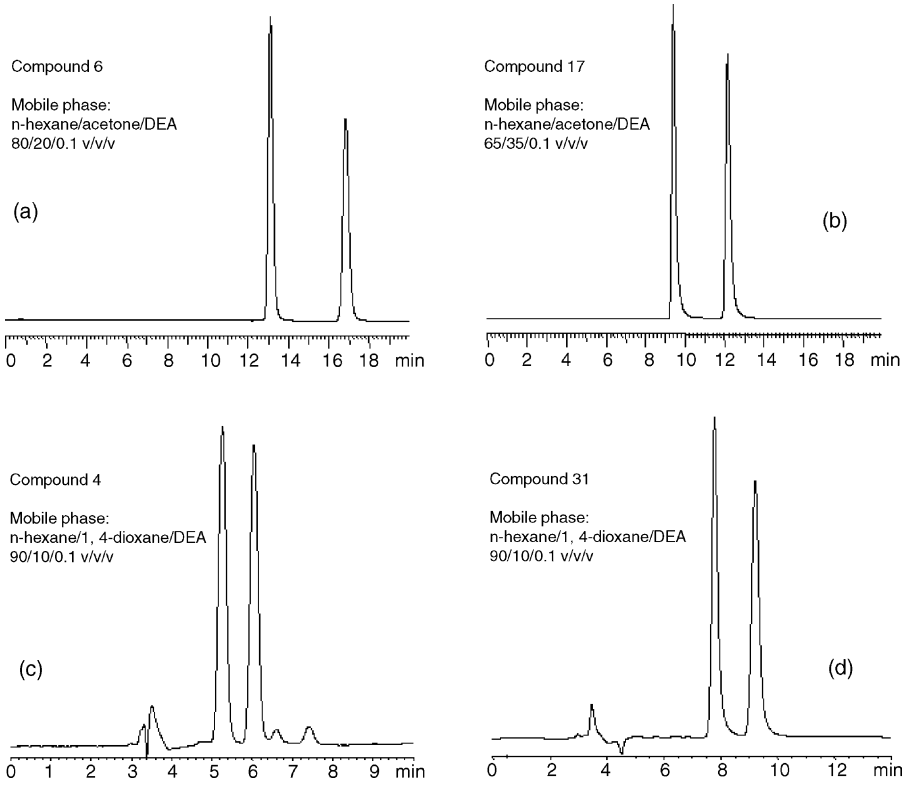


Fig. 9. Separation examples on CHIRALPAK® IA using acetone and 1,4-dioxane in the mobile phase. Flow rate: 1.0 ml/min, temperature: 25 °C, detection: ELSD.

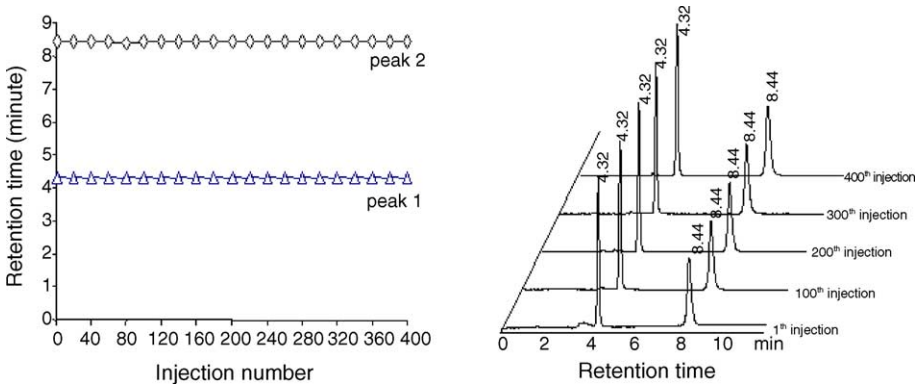


Fig. 10. Separation repeatability of glutethimide (**10**) on CHIRALPAK® IA with 100% ethyl acetate. Flow rate: 1.0 ml/min, temperature: 25 °C, detection: UV 274 nm.

to examine the CSP stability, pure ethyl acetate was used as mobile phase to repeat the separation of glutethimide (**10**) on a CHIRALPAK® IA column. Four hundred injections of glutethimide were carried out within 80 h. In Fig. 10, the repeatability of the separation is depicted and the chromatograms from the first, 100th, 200th, 300th and 400th injections are overlaid. It can be observed that, even with a non-standard solvent like ethyl acetate, the separation is completely repeatable. The CSP is proved to be totally compatible with non-standard solvents.

## 4. Conclusions

The advanced immobilization technologies give an impetus to the modern chiral stationary phases for their applicability and reliability in enantiomeric separations.

CHIRALPAK® IA is the first in a new generation of immobilized polysaccharide-based supports. Owing to the specific structure of its chiral selector as well as its immobilized nature, it exhibits both high enantioselective performance and excellent solvent compatibility.

The implementation of non-standard mobile phases makes it extremely versatile in method development and in separation of various chiral compounds. It provides the possibility to enhance chiral separations or to create totally new enantioselectivity profiles without compromising the CSP stability.

Among many solvents that may be used in mobile phase, four non-standard ones are particularly versatile. They are MtBE, $CH_2Cl_2$, THF and ethyl acetate. It is highly recommended to screen these solvents first for enantioselectivity. The choice of mobile phase should be steered to the solvents that enable good dissolution of the solute if the product solubility is a limiting factor for preparative applications.

The chromatographic properties of CHIRALPAK® IA in reverse phase mode, in gradient mode and in SFC mode are currently under investigation in our laboratories. Its loading capacity, a crucial parameter for preparative applications, has also been in examination for different chiral separation projects and will be the topic of further publications.

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