

**CHIRALPAK® IA is the first in a series of polysaccharide-derived chiral chromatographic columns from DAICEL compatible with all ranges of organic miscible solvents. This new immobilised chiral stationary phase shows a unique solvent flexibility and excellent chiral recognition ability. CHIRALPAK® IA expands the application coverage of polysaccharide supports and broadens the choice of solvents to be used as mobile phases and/or sample solvents.**

### Introduction

Polysaccharide derivatives coated on a silica matrix have been extensively used as chiral stationary phases (CSPs) for their high selective and loading capacity in enantioseparation by HPLC. Immobilization of the polymeric chiral selectors on the support has been considered as a direct approach to confer a universal solvent compatibility to this kind of CSP, thereby broadening the choice of solvents able to be used as mobile phases<sup>1-3</sup>. In this context, Daicel Chemical Industries Ltd. has recently developed a new generation of CSPs for HPLC using a novel immobilization technology. CHIRALPAK® IA, a 3,5-dimethylphenylcarbamate derivative of amylose, immobilized onto silica, is the first of this series of CSPs to become commercially available.

The immobilisation of the amylose derivative on the silica gel support allows *free choice* of any miscible solvents to compose the mobile phase and enlarges the application domain of the polysaccharide-derived chiral selector. The column can be used with all ranges of organic miscible solvents, progressing from the traditional mobile phases used with other Daicel columns (mixtures of alkane/alcohol, pure alcohols or acetonitrile) to mobile phases containing ethyl acetate, tetrahydrofuran, methyl *tert*-butyl ether (MtBE), dichloromethane and chloroform, among others.

The outstanding solvent versatility of CHIRALPAK® IA and its robustness makes it a powerful tool for chiral method development. The extended choice of solvents opens up new possibilities for unique selectivities in the separation of enantiomers, together with enhanced solubility of samples.

The following features make CHIRALPAK® IA an ideal choice for chiral separations:

1. High solvent versatility in the selection of the mobile phase composition.
2. Solvent flexibility for the resolution of compounds with limited solubility.
3. High selectivity and broad application domain in the resolution of enantiomers.
4. Robustness and extended durability.
5. Excellent column efficiency.
6. Easy use of the column.

The present document will focus on the chromatographic method development and optimisation with CHIRALPAK® IA.

The enantioseparations of a wide range of compounds have been investigated and data on the selectivity available from different eluent systems will be presented. Some key factors and particular features of CHIRALPAK® IA will also be discussed.

### Method development on CHIRALPAK® IA

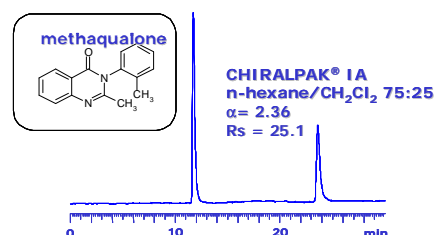
The major advantage of CHIRALPAK® IA is that it can be used with any organic miscible solvent combination in the mobile phase. This flexibility not only broadens the choice of mobile phase compositions, but also the type of solvents that can be used for sample injection in order to enhance the solubility. The only limitations will be *high pressure drop* (max. pressure 100 bar) or *extreme pH ranges* which must be avoided because they can damage the silica gel used in this column.

Based on our extensive experience the most commonly used chromatographic solvents and their mixtures can be classified in two groups in terms of **enantioselectivity**:

The mixtures containing solvents of the first group are usually leading to better enantioselectivities, although the separation

| Group 1                           | Group 2             |
|-----------------------------------|---------------------|
| ↑ Alcohols                        | ↑ Ethyl acetate     |
| ↑ THF                             | ↑ Acetonitrile      |
| ↑ MtBE                            | ↑ CHCl <sub>3</sub> |
| ↑ CH <sub>2</sub> Cl <sub>2</sub> | ↑ 1,4-Dioxane       |
|                                   | ↑ Acetone           |
|                                   | ↑ Toluene           |

ability of the chiral support may be different depending on the sample. In general terms, we would recommend THF, MtBE, alcohols or dichloromethane (pure solvents of group 1 or their alkane mixtures) to begin the development of an analytical method<sup>4</sup>. The solvent leading to a *higher solubility* of your sample will be the first choice when this is a limiting factor. *Temperature* can be adjusted between 0°C and 40°C.



| Mobile phase                            | k' <sub>1</sub> | a    | Rs   |      |
|---|-----------------|------|------|------|
| methyl <i>tert</i> -butyl ether/ethanol | 95:5            | 0.73 | 2.81 | 13.1 |
| <i>n</i> -hexane/dichloromethane        | 75:25           | 2.90 | 2.36 | 25.1 |
| toluene/ <i>n</i> -hexane/ethanol       | 70:25:5         | 0.54 | 1.96 | 9.28 |
| <i>n</i> -hexane/chloroform             | 50:50           | 0.64 | 1.79 | 7.84 |
| <i>n</i> -hexane/methyl acetate         | 80:20           | 1.87 | 1.70 | 9.45 |
| <i>n</i> -hexane/ <i>Z</i> -propanol    | 80:20           | 1.45 | 1.65 | 7.08 |
| <i>n</i> -hexane/tetrahydrofuran        | 85:15           | 3.14 | 1.63 | 11.3 |
| <i>n</i> -hexane/acetone                | 85:15           | 1.42 | 1.33 | 5.82 |

**Figure 1.** Separation of methaqualone on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C).

<sup>1</sup> E. Yashima, H. Fukaya, Y. Okamoto, *J. Chromatogr. A* **677** (1994) 11-19.

<sup>2</sup> P. Franco, A. Senso, L. Oliveros, C. Minguillón, *J. Chromatogr. A*, **906** (2001) 155-170.

<sup>3</sup> E. Francotte, T. Zhang, PCT International Patent Application WO 9704011.

<sup>4</sup> Detection with a regular UV detector may become difficult depending on a combination of sample and mobile phase (e.g. acetone, ethyl acetate, toluene, high percentages of chloroform). In those cases an alternative detector, such as RI detector or ELSD (Evaporative Light Scattering Detector), may be more effective than the UV.

In Figure 1 the separation of racemic methaqualone on CHIRALPAK® IA with an *n*-hexane/dichloromethane mixture is shown. This solvent combination led to an excellent resolution between the two enantiomers of this chiral drug, but a number of different mobile phases were also able to achieve interesting selectivity and resolution values. In the attached tables a range of separations of methaqualone are presented (arranged according to their  $\alpha$  values).

In order to assist you with some *typical starting conditions* and *ranges for optimisation* we would like to propose the following guidelines. As previously mentioned the column allows the use of all solvent ranges, but you should find the right composition leading to good selectivity and suitable retention times. In Table 1 you will find the classical alkane/alcohol mixtures and the pure polar solvent combinations (alcohols and acetonitrile) being already used for coated-type polysaccharide-derived columns. In Table 2 an extended series of some solvents only compatible with this new immobilised chiral support (see graphical representation in Figure 2).

In Tables 1 and 2 solvents are arranged *according to their eluting strength*. MtBE, toluene and chlorinated solvents can be used in their pure form in the mobile phase. For fast eluting solvents, such as THF, 1,4-dioxane or acetone, we

recommend to be used in combination with alkanes in order to modulate the retention (Figure 3-5). Alkanes, such as *n*-hexane, *iso*-hexane or *n*-heptane can be used (some small selectivity differences may sometimes be found).

If we take the case of methaqualone depicted in Figure 1 we can see that the best selectivity values were achieved with MtBE and dichloromethane solvent mixtures. Our approach to proceeding with MtBE would be to start the screening with MtBE/EtOH 98:2 (see Table 2) and then modulate the retention and/or selectivity with the addition of the alcohol. Please note that selectivity and peak shape can drastically change in the case of MtBE by the simple addition of a small percentage (usually 1-5%) of alcohol or any other of the organic modifiers indicated in Table 2 (Figure 6). This is also the case for the screening with the chlorinated solvents.

A screening with dichloromethane would be started with a 50:50 mixture in an alkane and then the retention and selectivity would be optimised within the advised range (between 25% and 100% of CH<sub>2</sub>Cl<sub>2</sub>, see Table 2).

Table 1.

|                             | Ethanol <sup>CE</sup>          | Methanol <sup>CE</sup>         | Acetonitrile                    | Alkane / EtOH <sup>CE</sup> | Alkane / 2-PrOH <sup>•</sup> | Alkane / MeOH <sup>•</sup>    |
|-----------------------------|--------------------------------|--------------------------------|---------------------------------|-----------------------------|------------------------------|-------------------------------|
| Typical starting conditions | 100                            | 100                            | 100                             | 90:10                       | 90:10                        | 95:5                          |
| Advised optimisation range  | 100-50% in MeOH, 2-PrOH or ACN | 100-50% in EtOH, 2-PrOH or ACN | 100-80% in MeOH, EtOH or 2-PrOH | 99:1 to 50:50               | 99:1 to 50:50                | 99:1 to 50:25:25 <sup>Z</sup> |

<sup>CE</sup> Certain alcohol mixtures have a higher viscosity. Pressure should be controlled and flow rate reduced if necessary.

<sup>•</sup> The retention is generally shorter with a higher alcohol content. The use of other alcohols such as 1-propanol, 1- and 2-butanol etc...is possible.

<sup>Z</sup> No range limitation, but due to miscibility restrictions, mix methanol with an equal volume of ethanol when using with alkane mixtures, otherwise, separation of liquid phases might happen. A maximum of 5% methanol in *n*-hexane may be used without adding ethanol.

Table 2.

|                             | MtBE <sup>•</sup> / Ethanol <sup>•</sup> | CHCl <sub>3</sub> <sup>•</sup> / Alkane <sup>•</sup> | CH <sub>2</sub> Cl <sub>2</sub> <sup>•</sup> / Alkane <sup>•</sup> | Ethyl acetate / Alkane | THF / Alkane   | 1,4-Dioxane / Alkane | Acetone / Alkane |
|-----------------------------|--|--|--|------------------------|----------------|----------------------|------------------|
| Typical starting conditions | 98:2                                     | 60:40  | 50:50  | 40:60                  | 30:70          | 25:75                | 25:75            |
| Advised optimisation range  | 80:20 to 100:0                           | 25:75 to 100:0                                       | 25:75 to 100:0   | 20:80 to 70:30         | 10:90 to 50:50 | 10:90 to 40:60       | 10:90 to 40:60   |

<sup>•</sup> Some solvents such as MtBE, CHCl<sub>3</sub> or CH<sub>2</sub>Cl<sub>2</sub> may need the combination with alcohols (1-5%) to modulate retention times and improve peak shape.

<sup>•</sup> Organic modifiers in MtBE can also be: 2-propanol, methanol, THF, ethyl acetate, methyl acetate, 1,4-dioxane or acetone.

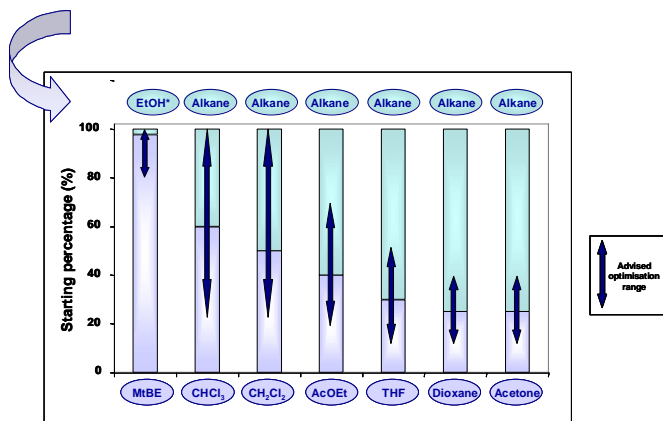
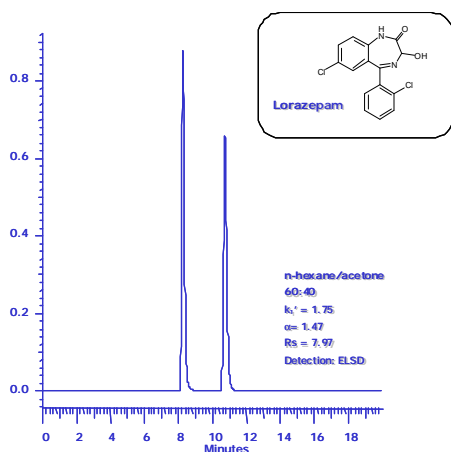


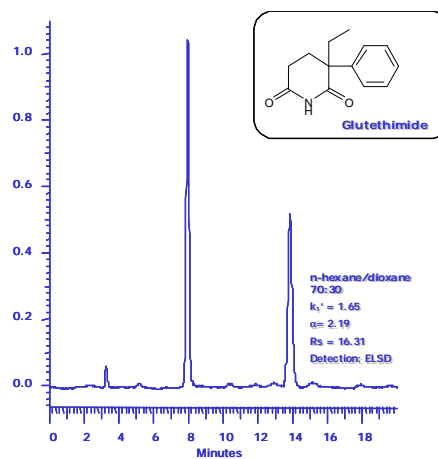
Figure 2. Graphical representation of the typical starting conditions and the advised optimisation ranges for the solvents presented in Table 2.

For basic or acidic samples, it may be necessary to incorporate an additive into the mobile phase in order to optimize the chiral separation. Basic samples may require a basic additive (diethylamine, butylamine, ethanolamine, ...) and acidic compounds the addition of an acid (TFA, acetic or formic acid, ...). The percentage needed is typically 0.1% and should not exceed 0.5% (see examples in Figure 5-7).

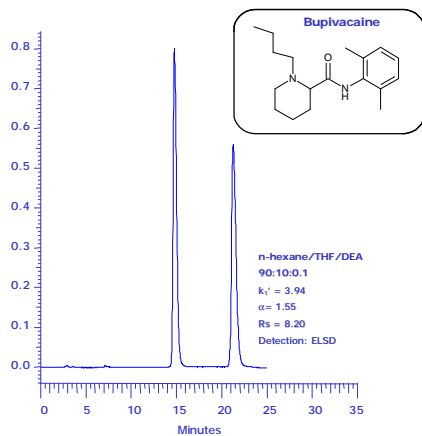
The use of CHIRALPAK® IA is possible under *reversed-phase conditions*. To make this type of operation we highly recommend a suitable transfer of the column through a series of miscible solvents and allow enough equilibration time, which may be longer than with organic solvents. However, it is important to point out that water can be adsorbed by the polymer and the silica gel and it may be difficult to remove it afterwards. The presence of water, even in traces, may modify the chiral recognition ability of the polysaccharide derivative. Therefore, dedicated columns for normal phase applications are strongly recommended.



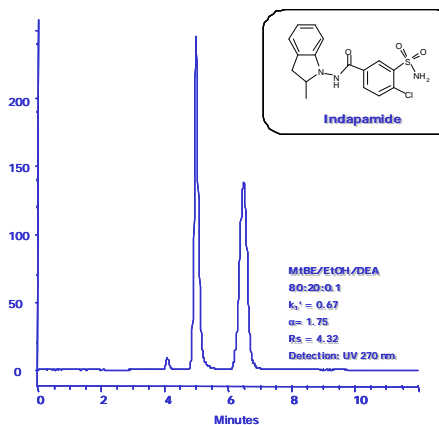
**Figure 3.** Separation of lorazepam on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).



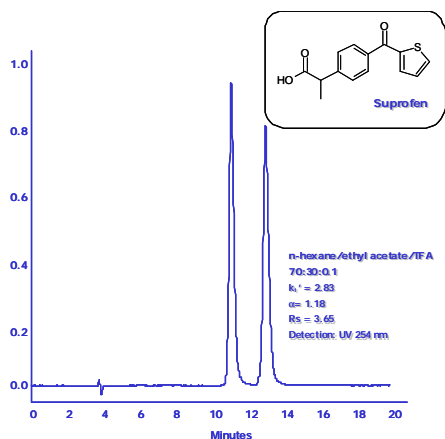
**Figure 4.** Separation of glutethimide on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).



**Figure 5.** Separation of bupivacaine on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).



**Figure 6.** Separation of indapamide on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).



**Figure 7.** Separation of suprofen on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).

Examples with other solvents are shown in Figures 8-11. The behaviour of chloroform is quite similar to dichloromethane, although its eluting strength is slightly lower. Toluene can sometimes add particular enantioselectivity features to the separation. Several excellent separations have been described with 100% toluene (i.e. bi-2-naphtol or flavanone enantiomers) and UV detection at 280 nm<sup>5</sup>. Nevertheless, certain separations are achieved with mixtures containing an alkane and a low percentage of alcohol (see examples Figures 1 and 9).

The main innovation of CHIRALPAK® IA is the possibility of using the extended series of solvents presented in Table 2. However, the column is also effective with the classical mixtures of Table 1. Two additional examples are presented in Figures 10 and 11 with polar solvents, such as pure alcohols or acetonitrile.

Reversal of the elution order can sometimes be observed with different solvents for the same compound. This feature may be perceived as an advantage when the determination of the enantiomeric excess of an enriched sample is envisaged.

<sup>5</sup> Several of the examples presented in this document with an ELSD detector can be also analysed with UV.

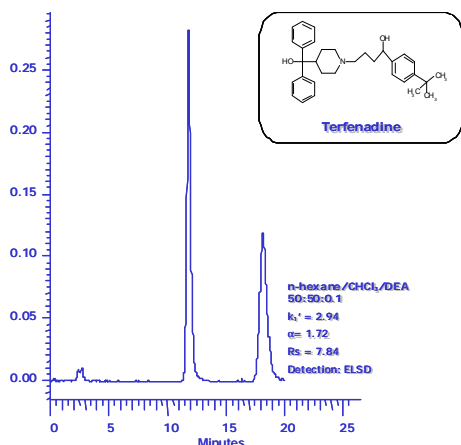


Figure 8. Separation of terfenadine on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).

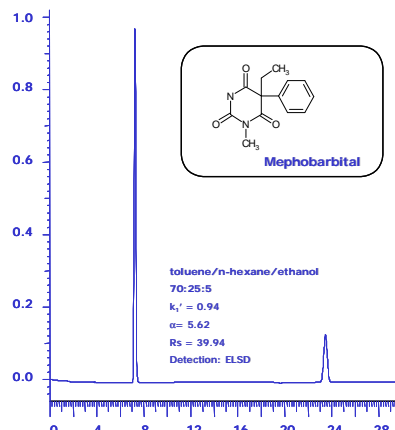


Figure 9. Separation of mephobarbital on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 0.8 mL/min).

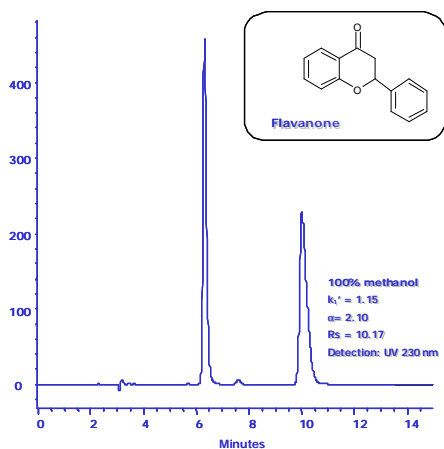


Figure 10. Separation of flavanone on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).

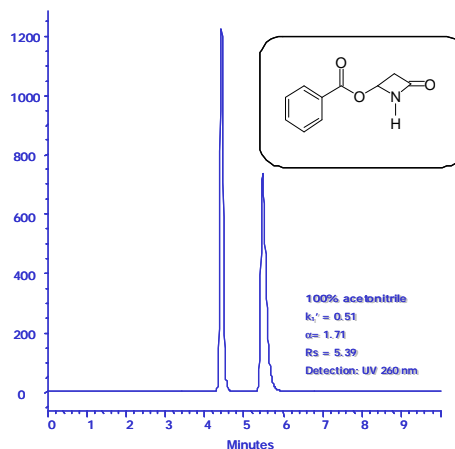


Figure 11. Separation of an azetidinone derivative on an analytical CHIRALPAK® IA column (25 x 0.46 cm, 25°C, 1 mL/min).

### Column cleaning and regeneration procedures

As described in the previous section, the enantioselectivity displayed by the chiral column strongly depends on the correct choice of the operating conditions (mobile phase composition, temperature, ...). Furthermore, the chiral recognition of polysaccharide type phases also depends on the *supramolecular structure* of the polymeric chiral selector. The molecular conformation can change in different solvating environments. In order to ensure consistent performances after extensive use with different mobile phases, a regeneration method may be necessary to eliminate any unexpected change of chiral recognition due to the history of the column (mobile phases, additives, ...).

- Flush with **ethanol** (0.5 ml/min for 30 min) followed by **100% THF** at 0.5 ml/min for 2 hours.
- Flush with **ethanol** (0.5 ml/min for 30 min) and then equilibrate with alkane / ethanol = 80 / 20 (v/v) prior to retesting the column.

This treatment with ethanol and THF recovers the initial conformation adopted by the polymer and can even "delete" memory effects of basic or acidic additives. The insertion of

this regeneration sequence after intensive changes between very different solvent mixtures will allow you to achieve very reproducible chromatographic results with your column.

**CHIRALPAK® IA, the new immobilised Daicel column, is able to achieve unique enantioselective separations with a broader range of solvents.**

### CHIRAL TECHNOLOGIES EUROPE

Parc d'Innovation  
Bd. Gonthier d'Andernach, BP 80140  
F-67404 Illkirch Cedex, FRANCE  
Tel: +33-(0)3 88 79 52 00, Fax: +33-(0)3 88 66 71 66  
e-mail: [cte@chiral.fr](mailto:cte@chiral.fr)

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