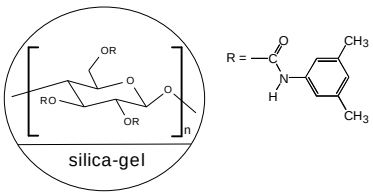
# INSTRUCTION MANUAL FOR CHIRALPAK® IB COLUMNS

CHIRAL TECHNOLOGIES EUROPE
Pure simplicity

**Please read this instruction sheet completely before using CHIRALPAK® IB columns**

## Column description

**Packing composition:** **Cellulose tris(3,5-dimethylphenylcarbamate) immobilized on 5µm silica-gel.**




**Shipping solvent:** **n-hexane/2-propanol 95/5 (v/v)**

**CHIRALPAK® IB columns are for analytical and semi-preparative applications.**

**All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.**

**THIS INSTRUCTION SHEET IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS**

## Operating restrictions

| | 150 x 2.1 mm ID Analytical columns | 150 x 4.6 mm ID 250 x 4.6 mm ID Analytical columns | 250 x 10 mm ID „ Semi-prep. columns | 250 x 20 mm ID „ Semi-prep. columns |
|---|---|---|---|---|
| Flow rate direction | As indicated on the column label | | | |
| Typical Flow rate • | ~ 0.1 - 0.2 ml/min Do not exceed 0.3 ml/min | ~ 1 ml/min Do not exceed 1.5 ml/min | ~ 5 ml/min Do not exceed 7 ml/min | ~ 18 ml/min Do not exceed 25 ml/min |
| Pressure limitation , | Should be maintained < 50 bar (5 MPa or 775 psi) *f* for maximum column life Adapt flow rates to the size of the column. Do not exceed 100 bar (10 MPa or 1450 psi ) | | | |
| Temperature | 0 to 40°C | | | |

• **The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the upper pressure limit (i.e. 100 Bar).**

| Examples | Column 250 x 4.6 mm ID | Column 250 x 20 mm ID |
|---|---|---|
| ethyl acetate | ~ 1.0 ml/min. | ~18 ml/min. |
| 100% EtOH | ~ 0.5 ml/min | ~ 5 to 8 ml/min |
| alkane/organic modifier~ 90:10 | 0.5 to 1.5 ml/min | 18 to 25 ml/min |

, **The backpressure value that should be assessed is the one generated by the column itself. This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.**

*f* **Ideal value for maximum column life, but stable up to 100 Bar.**

„ **When you use a semi-preparative column, it is highly recommended to discard at least the first 150ml (for 250 x 10 mm ID) or 500ml (for 250 x 20 mm ID) of eluent at the beginning of each preparative work.**

- The use of a guard cartridge is highly recommended for maximum column life.
- Samples should be filtered through a membrane filter of approximately 0.5µm porosity.

## Operating procedure

### *A - Mobile phases*

**CHIRALPAK® IB is compatible with all types of organic miscible solvents, progressing from the traditional mobile phases used with Daicel coated-type polysaccharide-derived columns (mixtures of alkanes/alcohol, pure alcohol or acetonitrile) to mobile phases containing chloroform ($CHCl_3$), ethyl acetate (EA), tetrahydrofuran (THF), methyl *tert*-butyl ether (MtBE) and toluene, among others.**

**Two groups of solvents can be considered with regard to CHIRALPAK® IB:**

- **Group A: standard solvents commonly used for coated-type polysaccharide-derived columns.**
- **Group B: extended solvent range.**

**In the following tables *some guidelines* are given in order to assist the user in method development. In Table 1 several solvent mixtures of both groups are described, together with the typical starting conditions and advised optimisation ranges. Toluene, MtBE and chlorinated solvents can be used in their pure form in the mobile phase. For fast eluting solvents, such as THF or acetone, we recommend that they are used in combination with solvents from group A (especially alkanes) in order to modulate the solute retention. In Table 2 the solvent mixtures of group A are summarized. *Extreme pH ranges must be avoided because they can damage the silica gel used in this column.***

**Table 1. Typical mobile phases containing solvents of group A and B**

| Group B solvents | $CHCl_3$Œ | MtBEŒ | EA | THF | $CH_2Cl_2$Œ | TolueneŒ | Acetone |
|---|---|---|---|---|---|---|---|
| Group A solvents | Alkane • | EthanolŽ | Alkane • | Alkane • | Alkane • | Alkane • | Alkane • |
| Typical starting conditions (solvent B/solvent A) | 50:50 | 98:2 | 40:60 | 30:70 | 40:60 | 70:30 | 25:75 |
| Advised optimisation range | 25:75 to 100:0 | 80:20 to 100:0 | 20:80 to 70:30 | 10:90 to 50:50 | 20:80 to 100:0 | 30:70 to 100:0 | 10:90 to 50:50 |

Œ Some solvents such as MtBE, $CHCl_3$, $CH_2Cl_2$ or toluene may need to be combined with alcohols (usually 1-5%) to modulate retention times and improve peak shape.
• Alkane: n-hexane, iso-hexane or n-heptane. Small selectivity differences may sometimes be found due to the alkane nature.
Ž Organic modifiers in MtBE may also be: 2-propanol, methanol, THF, ethyl acetate, methyl acetate, 1,4-dioxane or acetone.

**Table 2. Typical mobile phases containing solvents of group A**

| Group A solvent mixtures | Alkane • / EtOH | Alkane • / 2-PrOH • | Alkane • / MeOH • | Ethanol • | Methanol • | Acetonitrile • |
|---|---|---|---|---|---|---|
| Typical starting conditions | 90:10 | 90:10 | 95:5 | 100 | 100 | 100 |
| Advised optimisation range | 99:1 to 50:50 | 99:1 to 50:50 | 99:1 to 50:25:25 ‘ | 100-50% in MeOH, 2-PrOH or ACN | 100-50% in EtOH, 2-PrOH or ACN | 100-80% in MeOH, EtOH or 2-PrOH |

• The retention is generally shorter with higher alcohol content. The use of other alcohols such as 1-propanol, 1-BuOH, 2-BuOH etc...is possible.
• The retention times are often very short when the polar organic solvents are used as mobile phase.
Certain alcohol mixtures have a higher viscosity. Pressure should be controlled and flow rate reduced if necessary.
‘ 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.

**Among the usual HPLC solvents, chloroform, ethyl acetate, THF, MtBE or alcohols in alkane are those with the highest potential in terms of enantioselectivity for CHIRALPAK® IB. We recommend that these solvents are used as**

mobile phase or mobile phase component to start the development of an analytical method '. If no satisfactory separation is found after screening of these solvents, it may be worth trying other solvents like dichloromethane, toluene, acetonitrile, acetone and dioxane.

' 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.

### *B – Additives*

For basic or acidic samples, it is necessary to incorporate an additive into the mobile phase in order to optimize the chiral separation.

Among the basic additives listed in the adjacent table, ethylenediamine (EDA) is the most efficient, followed by ethanolamine (EtNA), n-butylamine (BuA) and diethylamine (DEA). The addition of a low percentage of an alcohol (e.g. 2% EtOH or MeOH) in the mobile phase may be helpful to ensure the miscibility of certain amines with the mobile phases of low polarity.

| Basic Samples require Basic additives | Acidic Samples require Acidic additives |
|---|---|
| ethylenediamine (EDA)<br>ethanolamine (EtNA)<br>n-butylamine (BuA)<br>diethylamine (DEA) | TFA<br>$CH_3COOH$<br>HCOOH |
| < 0.5%<br>Typically 0.1% | < 0.5%<br>Typically 0.1% |

ð STRONGLY BASIC solvent additives or sample solutions MUST BE AVOIDED, because they are likely to damage the silica gel used in this column.

## Column care / Maintenance

### F *Column cleaning and regeneration procedures*

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 performance 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 ethyl acetate at 1.0 ml/min for 30 min, (>2 hours if some additives are used in the mobile phase)
- Store the column at RT for 2 days or longer
- Flush with hexane/IPA 90/10 v/v at 1.0 ml/min for 1 hour prior to retesting the column.

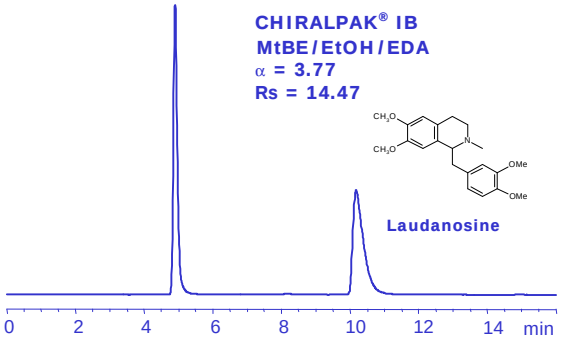
## Column storage

q Ethanol can be used as universal storage solvent. However, if you are working with alkane containing mobile phases, the column can be kept in a n-hexane/alcohol mixture (e.g. n-hexane/2-propanol 90/10 v/v) when stored for more than one week.

q For columns used with acidic or basic additives, flush the column with the same mobile phase without the modifier before storage.

Operating this column in accordance with the guidelines outlined here will result in a long column life.

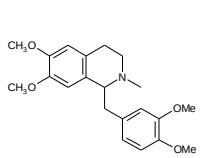
## Solvent effects on CHIRALPAK® IB separations




ð Separation examples for racemic laudanosine on CHIRALPAK® IB (250 x 4.6 mm ID, 25°C, 1.0ml/min.)

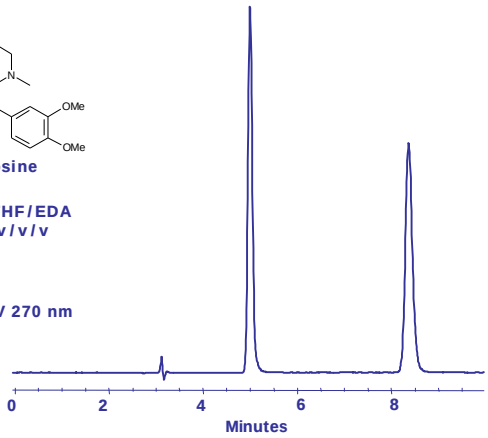
| Mobile phase | | $k'_1$ | a | Rs |
|---|---|---|---|---|
| MtBE/EtOH/EDA | 95/5/0.1 | 0.64 | 3.77 | 14.47 |
| n-hexane/THF/EDA | 70/30/0.1 | 0.67 | 2.68 | 15.41 |
| n-hexane/toluene/EDA | 20/80/0.1 | 0.39 | 2.73 | 8.44 |
| n-hexane/$CHCl_3$/EtNA | 65/35/0.1 | 1.32 | 1.28 | 5.75 |
| n-hexane/2-propanol/EDA | 80/20/0.1 | 1.33 | 2.76 | 14.62 |

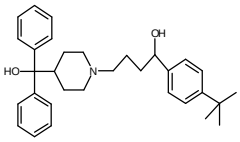
# SEPARATION EXAMPLES ON CHIRALPAK® IB



**Laudanosine**

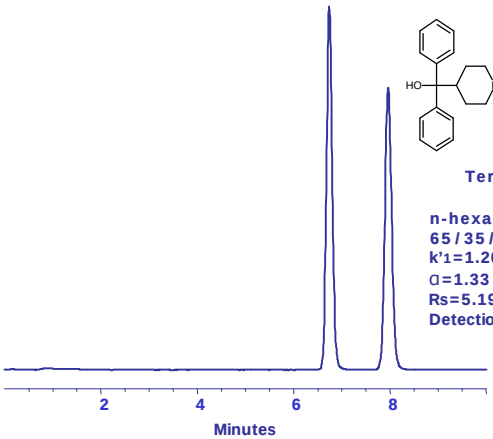
n-hexane / THF / EDA
70 / 30 / 0.1 v / v / v
k'1=0.67
α=2.68
Rs=15.41
Detection: UV 270 nm

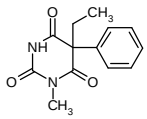


**Terfenadine**

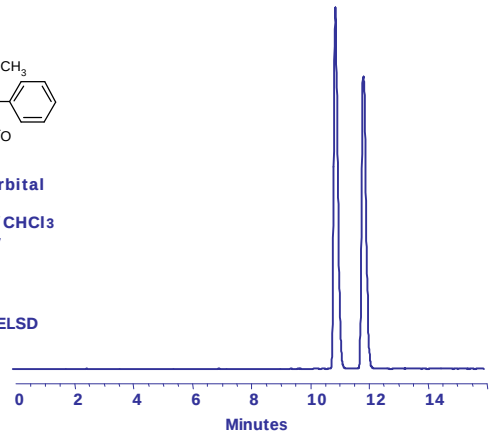
n-hexane / CHCl3 / EtNA
65 / 35 / 0.1 v / v / v
k'1=1.20
α=1.33
Rs=5.19
Detection: ELSD

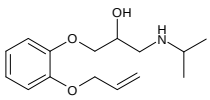


**Mephobarbital**

n-hexane / CHCl3
50 / 50 v / v
k'1=2.44
α=1.12
Rs=3.68
Detection: ELSD

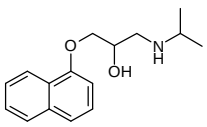


**Oxprenolol**

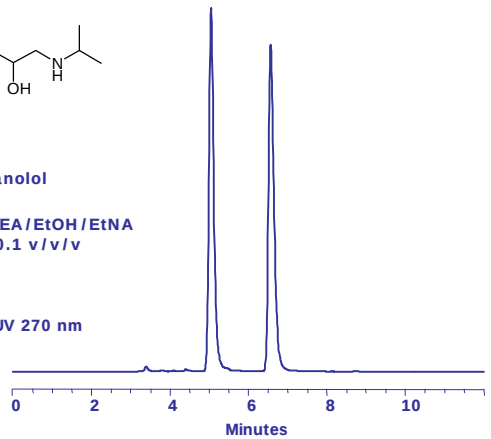
MtBE / EtOH / EDA
95 / 5 / 0.1 v / v / v
k'1=0.45
α=2.98
Rs=12.39
Detection: UV 270 nm

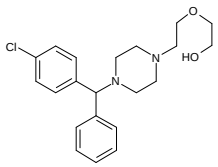


**Propranolol**

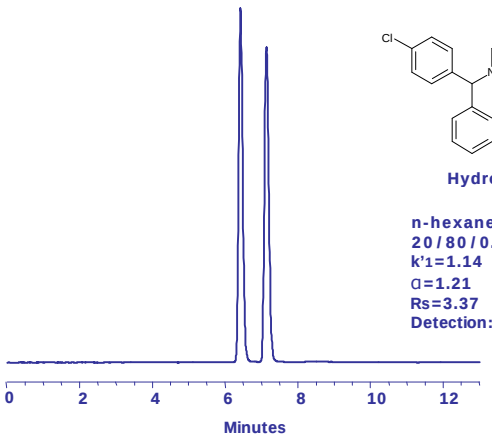
n-hexane / EA / EtOH / EtNA
50 / 50 / 2 / 0.1 v / v / v
k'1=0.69
α=1.74
Rs=6.49
Detection: UV 270 nm



**Hydroxyzine**

n-hexane / toluene / EDA
20 / 80 / 0.1 v / v / v
k'1=1.14
α=1.21
Rs=3.37
Detection: ELSD




**General conditions: CHIRALPAK® IB 250 x 4.6 mm ID, Flow rate: 1.0 ml/min, 25°C**

**ð If you have any questions about the use of this column, or encounter a problem, please contact CHIRAL TECHNOLOGIES EUROPE for assistance (cte@chiral.fr).**

**For more detailed information about this column and other Daicel supports, refer to our catalogue available on our website: http://www.chiral.fr or contact CHIRAL TECHNOLOGIES EUROPE.**