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SUBSIDIARY OF DAICEL CHEMICAL INDUSTRIES, LTD.

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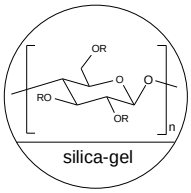
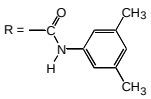
INSTRUCTION MANUAL FOR CHIRALPAK® IB COLUMNS

Please read this instruction sheet completely before using this column

Column description

Chemical composition: **CHIRALPAK® IB** **Cellulose tris (3,5-dimethylphenylcarbamate) immobilized on 5µm silica-gel.**





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

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 Instructions

	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) ƒ 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 is dependant upon the mobile phase viscosity (mobile phase composition), and should be adjusted in line 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 relevant back pressure value 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.
ƒ Ideal value for maximum column life, but stable up to 100 Bar.
„ When using 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.

1 **The use of a guard cartridge is highly recommended for maximum column life.**

1 **Samples should be filtered through a membrane filter of approximately 0.5µm porosity.**

Method Development

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.

Extreme pH ranges must be avoided because they can damage the silica gel used in this column.

Method Development - Screening

When developing methods for several compounds at once we would recommend a screening approach:

F Screening – Initial

It is our recommendation that the following conditions are used as an Initial Screen. If the compound or compound series are not soluble in any of these mobile phases we recommend progressing directly to the Extended Screen.

Table 1. Typical Initial Screen mobile phases

Primary solvent mixtures	Alkane Œ/ Alcohols •	Ethanol/Methano Žl	Acetonitrile
Typical starting conditions	90:10	50:50	100
Advised optimisation range	99:1 to 50:50	100% of MeOH, to 100% of EtOH	100-80% in MeOH, EtOH or 2-PrOH

Œ Alkane: n-hexane, iso-hexane or n-heptane. Some small selectivity differences may sometimes be found.
• The retention is generally shorter with a higher alcohol content. Ethanol and 2-Propanol are usually chosen first to start the screening. The use of other alcohols such as 1-propanol, 1-BuOH, 2-BuOH etc. is possible.
Ž Certain alcohol mixtures have a higher viscosity. Pressure should be controlled and flow rate reduced if necessary.

F Screening – Extended

If a suitable chiral separation is not found using the Initial Screening strategy we recommend an Extended Screen be applied using the following conditions:

Table 2. Typical Extended Screen mobile phases

Primary solvent	$CHCl_3$ Œ	MtBE Œ	Ethyl acetate	THF
Modifying solvent	Alkane •	Ethanol Ž	Alkane •	Alkane •
Typical starting conditions (Primary / Modifying)	50:50	98:2	40:60	30:70
Advised optimisation range	25:75 to 100:0	80:20 to 100:0	20:80 to 70:30	10:90 to 50:50

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

ð 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.

ð 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.

If a chiral separation method needs to be developed for use with a specific compound, for use in a preparative separation or because of limited solubility, then we recommend selection of the mobile phases based upon an HPLC solvent in which the compound is soluble.
When a suitable dissolution solvent has been found, the mobile phase can be determined as described in Table 3 to start the investigation:

Table 3.

Primary solvent	$CHCl_3$ Œ	MtBE Œ	Ethyl acetate	THF	CH_2Cl_2 Œ	Toluene Œ	Acetone
Modifying solvent	Alkane •	Ethanol Ž	Alkane •	Alkane •	Alkane •	Alkane •	Alkane •
Typical starting conditions (Primary / Modifying)	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.

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).

F 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) ethanolamine (EtNA) n-butylamine (BuA) diethylamine (DEA)	TFA CH_3COOH HCOOH
< 0.5% Typically 0.1%	< 0.5% 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 procedure*

Following extensive use of the column in multiple solvents there may be a reduction in column reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in 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

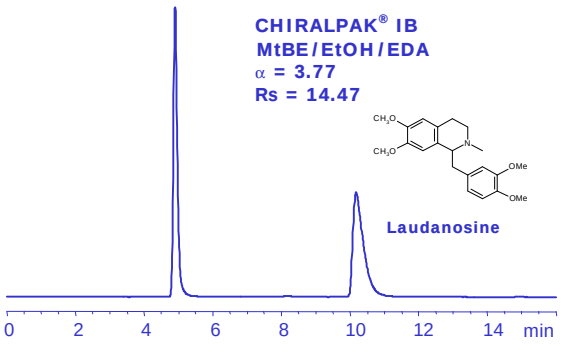
- **Ethanol can be used as universal storage solvent. However, if you are working with alkane containing mobile phases, the column can be kept in a n-hexane/alcohol mixture (e.g. n-hexane/2-propanol 90/10 v/v) when stored for more than one week.**

- **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.

For more detailed information, refer to our catalogue also available on our website http://www.chiral.fr or contact Chiral Technologies Europe.

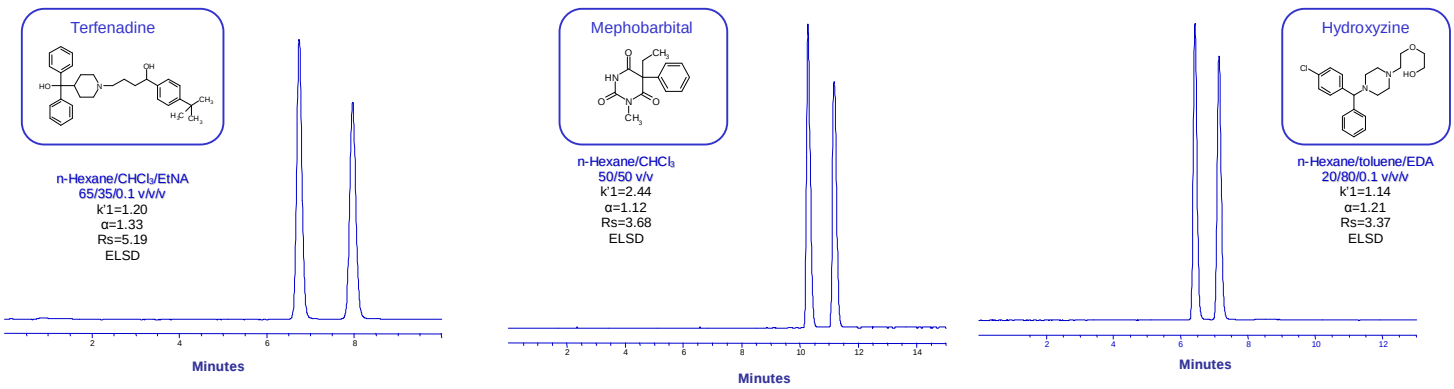
Solvent effects on CHIRALPAK® IB separations



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

CHIRALPAK® IB
Analytical HPLC applications



General conditions: CHIRALPAK® IB 250 x 4.6 mm, Flow rate: 1 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).

ð Ref: T.Zhang et al., Anal. Chim. Acta 557 (2006) 221.

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