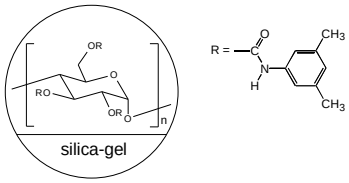
INSTRUCTION MANUAL FOR CHIRALPAK® IA COLUMNS

Please read this instruction sheet completely before using this column

Column description

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



Shipping solvent: **n-Hexane / ethanol solvent mixture (90/10 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 restrictions

	150 x 2.0 mm ID 250 x 2.0 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 (~700 psi) *f* for maximum column life Adapt flow rates to the size of the column. Do not exceed 100 Bar (~1400 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 pressure upper's limit (i.e. 100 Bar).**

Examples	Column 250 x 4.6mm ID	Column 250 x 20mm ID
Alkane/organic modifier~ 90:10	0.5 to 1.5 ml/min	18 to 25 ml/min
100% EtOH	~ 0.5 ml/min	~ 5 to 8 ml/min
100% 2-propanol	~ 0.2-0.3 ml/min	~ 3 to 5 ml/min

, **The back pressure value that should be taken into account 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.**

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

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

Operating procedure

A - Mobile phases

CHIRALPAK® IA allows *free choice* of any miscible solvents to compose the mobile phase. 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 alkanes/alcohol, pure alcohol or acetonitrile) to mobile phases containing ethyl acetate, tetrahydrofurane (THF), methyl *tert*-butyl ether (MtBE), dichloromethane (CH_2Cl_2) and chloroform($CHCl_3$), among others.

Two groups of solvents can be identified with regards to CHIRALPAK® IA:

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

In the following tables *some guidelines* will be given in order to assist the user in the method development. In Table 1 several solvent mixtures of both groups are described, together with the typical starting conditions and advised optimisation ranges. *Solvents are arranged according to their eluting strength*. Toluene, MtBE 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 solvents of group A (especially alkanes) in order to modulate the retention. In Table 2 the solvent mixtures of group A are shown following the same criteria as in Table 1. *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	MtBEŒ	$CHCl_3$Œ	CH_2Cl_2Œ	Ethyl acetate	THF	1,4-Dioxane	Acetone
Group A solvents	Ethanol •	AlkaneŽ	AlkaneŽ	AlkaneŽ	AlkaneŽ	AlkaneŽ	AlkaneŽ
Typical starting conditions (solvent B/solvent A)	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

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.
• Organic modifiers in MtBE can also be: 2-propanol, methanol, THF, ethyl acetate, methyl acetate, 1,4-dioxane or acetone.
Ž Alkane: n-hexane, iso-hexane or n-heptane. Some small selectivity differences may sometimes be found.

Table 2. Typical mobile phases containing solvents of group A

Group A solvent mixtures	Ethanol •	Methanol •	Acetonitrile	alkaneŽ / EtOH •	AlkaneŽ / 2-PrOH •	AlkaneŽ / MeOH •
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 ‘

• Certain alcohol mixtures have a higher viscosity. Pressure should be controlled and flow rate reduced if necessary.
• The retention is generally shorter with a higher alcohol content. The use of other alcohols such as 1-propanol, 1-BuOH, 2-BuOH etc...is possible.
‘ 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.

Based on our extensive experience the above mentioned solvents and their mixtures can be classified in two groups in terms of enantioselectivity. However, the separation ability of the chiral support may be different depending on the sample.

Alcohols, THF, MtBE, CH_2Cl_2 > Ethyl acetate, acetonitrile, $CHCl_3$, toluene, 1,4-dioxane, acetone

We would recommend THF, MtBE, dichloromethane or alcohols (pure or in alkane mixtures) to begin the development of an analytical method'. The solvent leading to a higher solubility of your sample will be the first choice when this is a limiting factor.

' 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:

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA Butyl amine" Ethanol amine"	TFA CH_3COOH HCOOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

" For primary amines mainly
" For primary amino alcohols mainly

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 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.**

If this is not successful, then try with 100% N,N-dimethylformamide or N,N-dimethylacetamide at 0.3 ml/min for 3 hours instead of the THF flush.

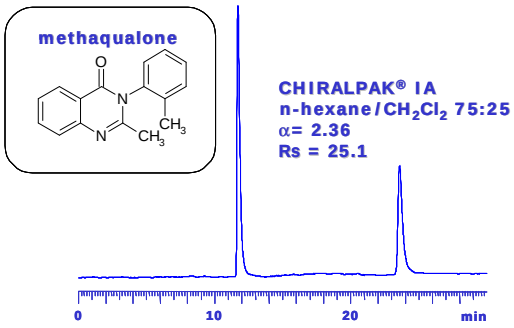
Column storage

q **Ethanol can be used as universal storage solvents. However, if you are working with alkane containing mobile phases, the column can be kept in n-hexane / ethanol 90/10 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.

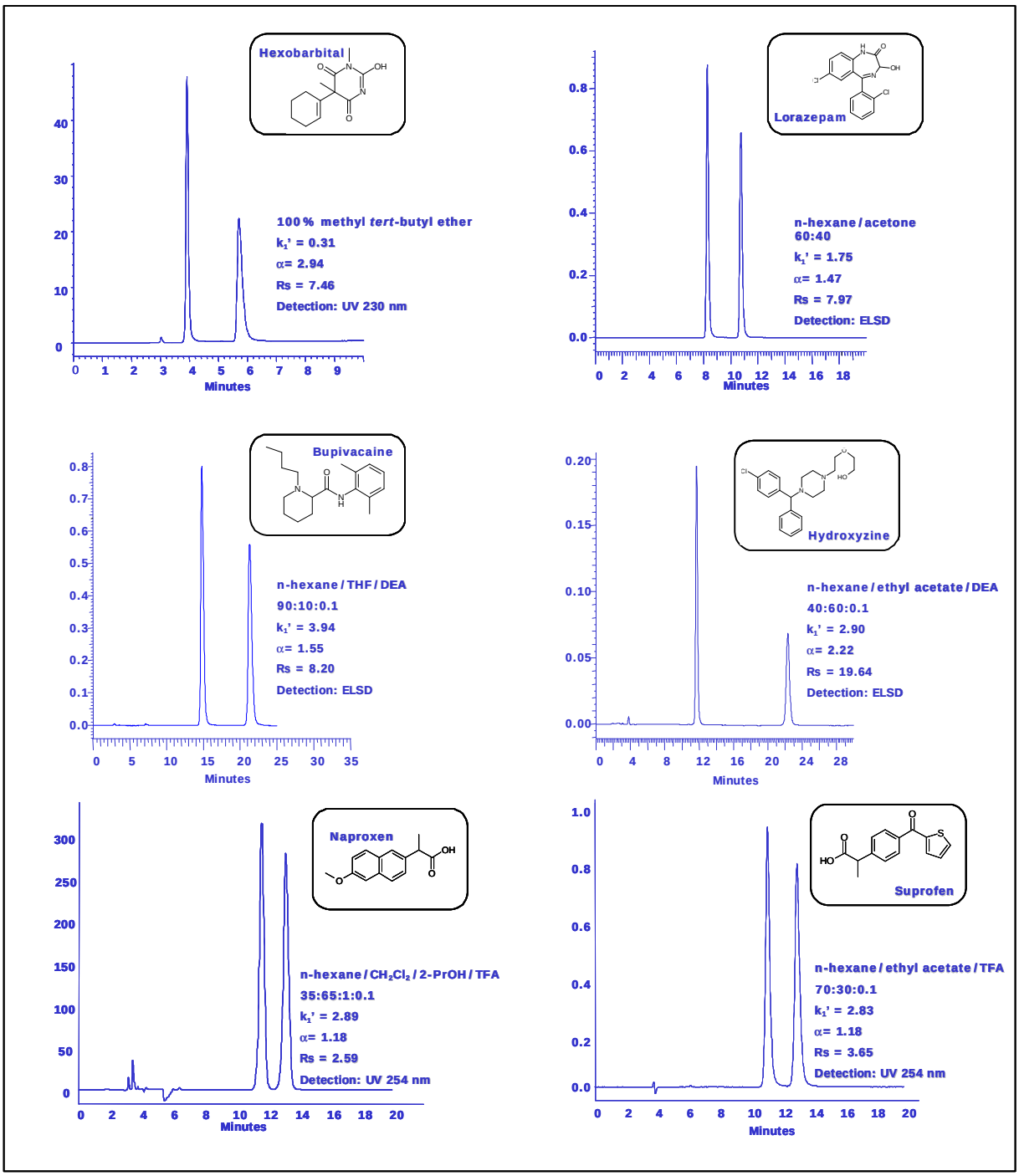
Solvent effects on CHIRALPAK® IA separations



ð **Separation examples for racemic methaqualone on CHIRALPAK® IA (25 x 0.46 cm, 25°C)**

Mobile phase		k'_1	a	Rs
n-hexane/2-propanol	80:20	1.45	1.65	7.08
n-hexane/methyl acetate	80:20	1.87	1.70	9.45
n-hexane/chloroform	50:50	0.64	1.79	7.84
n-hexane/dichloromethane	75:25	2.90	2.36	25.1
n-hexane/acetone	85:15	1.42	1.33	5.82
n-hexane/tetrahydrofuran	85:15	3.14	1.63	11.3
methyl *tert*-butyl ether/ethanol	95:5	0.73	2.81	13.1
toluene/n-hexane/ethanol	70:25:5	0.54	1.96	9.28

SEPARATION EXAMPLES ON CHIRALPAK® IA



General conditions: CHIRALPAK® IA 25 x 0.46 cm, 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).

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