CHIRAL TECHNOLOGIES EUROPE
SUBSIDIARY OF DAICEL CHEMICAL INDUSTRIES, LTD.

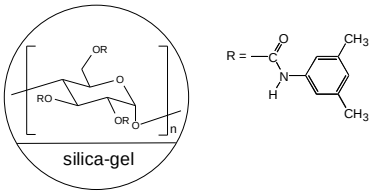
Parc d'Innovation
Bd. Gonthier d'Andernach, BP 80140
F-67404 Illkirch Cedex, FRANCE
Tel: +33-(0)3 88 79 52 00, Fax: +33-(0)3 88 66 71 66
e-mail: cte@chiral.fr; http://www.chiral.fr

INSTRUCTION MANUAL FOR CHIRALPAK® IA COLUMNS

Please read this instruction sheet completely before using this column

Column description

Chemical composition: **CHIRALPAK® IA** **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 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 4.6mm 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 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.

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

Method Development

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.

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/ Alcohol •	Ethanol/Methanol Ž	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	MtBE Œ	CH_2Cl_2 Œ	Ethyl acetate	THF
Modifying solvent	Ethanol •	Alkane Ž	Alkane Ž	Alkane Ž
Typical starting conditions (Primary / Modifying)	98:2	50:50	40:60	30:70
Advised optimisation range	80:20 to 100:0	25:75 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.
• 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.

ð Several solvent mixtures are described, together with the typical starting conditions and advised optimisation ranges. *Solvents are arranged according to their eluting strength*.
ð Toluene, MtBE and chlorinated solvents can be used in their pure form in the mobile phase.
ð For fast eluting solvents, such as THF, we recommend that it be used in combination with modifying solvents (especially alkanes) in order to modulate the retention.

Method Development – Compound Specific

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:

(*) 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.

Table 3.

Primary solvent	MtBEŒ	$CHCl_3$Œ	CH_2Cl_2Œ	Ethyl acetate	THF	1,4-Dioxane	Acetone
Modifying solvent	Ethanol •	Alkane Ž	Alkane Ž	Alkane Ž	Alkane Ž	Alkane Ž	Alkane Ž
Typical starting conditions (Primary / Modifying)	98:2	50:50	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.

Based on our extensive experience, the solvent versatility in terms of enantioselectivity can be sorted in the following order:

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

However, the separation ability of the chiral support may be different depending on the sample.

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*

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 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.

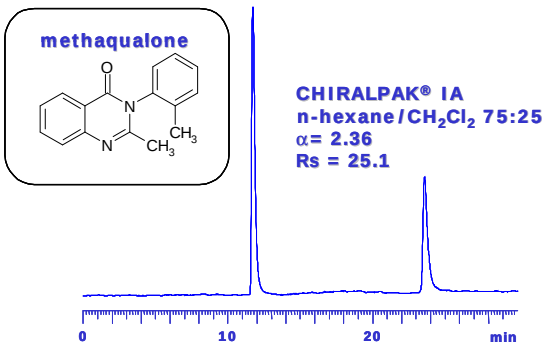
Column storage

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

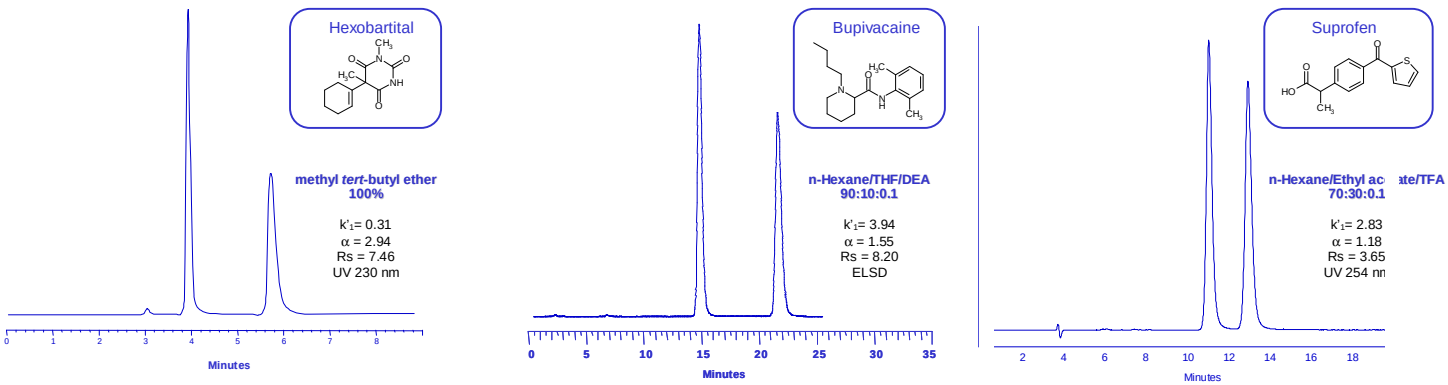
Solvent effects on CHIRALPAK® IA separations



ð Separation examples for racemic methaqualone on CHIRALPAK® IA (250 x 4.6 mm, 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

CHIRALPAK® IA
Analytical HPLC applications



General conditions: CHIRALPAK® IA 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., J. Chromatogr. A 1075 (2005) 65.*

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