INSTRUCTION MANUAL FOR CHIRALPAK® IF COLUMNS

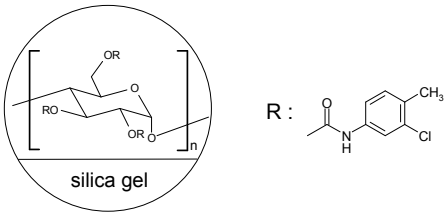
Please read this instruction sheet completely before using this column

IF columns can also be used in reversed phase mode.
Please refer to the corresponding instruction sheet for details.

Column description

CHIRALPAK® IF

Amylose tris(3-chloro-4-methylphenylcarbamate) immobilised on 5µm silica gel.



Shipping solvent: **n-Hexane / 2-Propanol 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

	2.1 x 150 mm **2.1 x 250 mm** Analytical columns	**4.6 x 150 mm** **4.6 x 250 mm** Analytical columns	**10 x 250 mm** Semi-prep. columns	**20 x 250 mm** Semi-prep. columns
Flow rate direction	As indicated on the column label			
Typical flow rate ①	~ 0.1 - 0.2 ml/min	~ 1 ml/min	~ 5 ml/min	~ 18 ml/min
Pressure limitation	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.			
Temperature	0 to 40°C			

① 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. 300 Bar).

Method Development / Normal Phase

A - Mobile phases

CHIRALPAK® IF 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 (CH_3CN)) to mobile phases containing methyl *tert*-butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc) among others.

B - Method Development - Screening

When developing methods we would recommend a screening approach.

1. The conditions described in Table 1 should be used as a Primary Screening.
2. If the compound or compound series are not soluble in any of these mobile phases, we recommend progressing directly to the Secondary Screening (Table 2).

Table 1. Immobilised Primary Screening Solvents

Primary solvent mixtures	Alkane❶/2-PrOH	Alkane❶/EtOH	Alkane❶/MtBE/EtOH❷	Alkane❶/THF❸	Alkane/DCM❹/EtOH
Typical starting conditions	80:20	80:20	0:98:2	70:30	50:50:2
Advised optimisation range	99:1 to 50:50	99:1 to 50:50	80:20:0 to 0:40:60	95:5 to 0:100	85:15:0 to 0:80:20

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ In absence of alkane, methanol is more efficient than ethanol when combined with MtBE.
❸ In the case of no environmental restrictions, **use of DCM is preferred to THF** in terms of better enantioselectivity that the former may induce.
❹ For excessively retained samples, addition of ethanol up to 20% in pure DCM would be helpful.

If a suitable chiral separation is not found using the Immobilised Primary Screening strategy, we recommend an Immobilised Secondary Screening to be applied using the following conditions:

Table 2. Immobilised Secondary Screening Solvents

Secondary solvent mixtures	EtOAc❶/Alkane❷	CH_3CN❸/Alcohol❹
Typical starting conditions	50:50	100:0
Advised optimisation range	20:80 to 100:0	100:0 to 0:100

❶ Alcohols (❹) or THF can be added into EtOAc to enhance the eluting strength for strongly retained compounds.
❷ Alkane: n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❸ Transfers between alkane mixtures and CH_3CN are preferably made with a transition in alcohol in order to avoid miscibility issues.
❹ Alcohol: MeOH, EtOH and 2-PrOH.

Note: All solvent proportions indicated in this manual are by volume.

C – General Comments

⇨ Additional solvent combinations such as $CHCl_3$/Alkane, 1,4-Dioxane/Alkane, Toluene/Alkane or Acetone/Alkane can also be investigated with CHIRALPAK® IF column.

⇨ The typical starting conditions represent the mobile phases of upper middle eluting strength. Under such conditions, most of the analytes can be eluted within a reasonable time range with a good probability of full resolution of the enantiomers.

⇨ Toluene, MtBE and chlorinated solvents can be used in their pure form as the mobile phase.

⇨ For fast eluting solvents, such as THF, we recommend to add alkane in order to modulate the retention.

⇨ Detection with a regular UV detector may become difficult depending on a combination of sample and mobile phase (e.g. EtOAc, high percentages of DCM). In these cases an alternative detector, such as RI detector or ELSD (Evaporative Light Scattering Detector), may be more effective than the UV detector.

D – Additives

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

❶ It has been found that certain amines, such as EDA and AE induce much better behaviour for certain basic compounds than the most commonly used 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 EDA and AE with the low polarity mobile phases.*

Basic Samples require Basic additives	Acidic Samples require Acidic additives
Diethylamine (DEA) Ethylenediamine (EDA)❶ 2-Aminoethanol (AE)❶ Butyl amine (BA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

⇨ *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

- The use of a guard cartridge is highly recommended for maximum column life.
- Samples should be dissolved in the mobile phase. The mobile phase and the sample solution should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.

☞ ***Column cleaning and regeneration procedures***

Following extensive use of the column in multiple solvents there may be a change in separation 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 at 0.5 ml/min(*) for 30 min, followed by 100% N,N-dimethylformamide (DMF) at 0.3 ml/min(*) for 3 hours.
- Flush with ethanol at 0.3 ml/min(*) for 50 min and then equilibrate with n-Hexane/IPA = 90/10 (v/v) prior to retesting the column.

(*) *Recommended flow rate for analytical columns (4.6mm ID).*

Column storage

- For column storage, remove the acidic or basic additives by flushing the column with the same mobile phase without the additive. Columns can be stored with additive-free mobile phases.

⇨ If you have any questions about the use of these columns, or encounter a problem, please contact DAICEL CORPORATION for assistance (chiral@jp.daicel.com).

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

Columns packed with 20µm material dedicated to preparative scale applications are also available from DAICEL CORPORATION.

Column Name	Ref.	Product Type	Internal Diameter (mm)	Column Length (mm)
CHIRALPAK® IF 5µm	86311	Guard cartridge (x3)	4.0	10
	86324	Analytical	4.6	150
	86325	Analytical	4.6	250
	86394	Analytical	2.1	150
	86395	Analytical	2.1	250
	86335	Semi prep	10	250
	86345	Semi prep	20	250
	86337	Guard column for Semi prep	10	20

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