INSTRUCTION MANUAL FOR CHIRALPAK® IA, CHIRALPAK IB, CHIRALPAK IC, CHIRALPKA ID, CHIRALPAK IE, CHIRALPAK IF and CHIRALPAK IG

Please read this instruction sheet completely before using these

< Supercritical Fluid Chromatography (SFC) >

Column Description

"Immobilized" Amylose-Based chiral phases **5µm silica-gel support**	"Immobilized" Cellulose-Based chiral phases **5µm silica-gel support**
CHIRALPAK® IA Amylose tris(3,5-dimethylphenylcarbamate)	**CHIRALPAK® IB** Cellulose tris(3,5-dimethylphenylcarbamate)
CHIRALPAK® ID Amylose tris(3-chlorophenylcarbamate)	**CHIRALPAK® IC** Cellulose tris(3,5-dichlorophenylcarbamate)
CHIRALPAK® IE Amylose tris(3,5-dichlorophenylcarbamate)	
CHIRALPAK® IF Amylose tris(3-chloro-4-methylphenylcarbamate)	
CHIRALPAK® IG Amylose tris(3-chloro-5-methylphenylcarbamate)	

Shipping solvent:
1. Hexane / alcohol 90:10 for analytical columns 4.6mm i.d; 150 & 250mm length
2. 100%Methanol for analytical columns 4.6mm i.d; 100mm length
3. 100%Methanol for semi-prep. columns 10-20 and 30mm i.d; 250mm length

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.

The columns are shipped in solvent. To avoid any damages, we recommend flush them with 100% 2-PrOH before their first use in SFC mode (see column transfer conditions between LC and SFC on page 4).

THIS INSTRUCTION SHEET IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	100 x 4.6 mm i.d. **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical columns	**250 x 10 mm i.d.** Semi-prep. columns	**250 x 20 mm i.d.** Semi-prep. columns	**250 x 30 mm i.d.** Semi-prep. columns
Flow rate direction	As indicated on the column label			
Typical Flow rate **in SFC**	~ 1 - 5 ml/min	~ 15 ml/min	~ 60 ml/min	~ 120 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			

The relevant pressure value is the one generated by the column itself (pressure drop).
The pressure drop is the difference between the inlet pressure (Pinlet) and the outlet pressure (Poutlet) in the system. The pressure drop generated by the system alone (without any column) has to be subtracted from the total value (system + column).

The column can be operated up to 300 Bar (pressure drop). However it is necessary to check if the SFC system has been designed to stand these conditions.

The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependant upon flow rate, amount and type of co-solvent in the mobile phase).

Method Development / SFC Mode

A - Mobile phases

CHIRALPAK® IA, IB, IC, ID, IE, IF and IG can be used *with all ranges of organic miscible solvents as co-solvent combined with the carbon dioxide (CO_2)*, progressing from the traditional solvents used with other DAICEL columns (mixtures of CO_2 with alcohols or acetonitrile (CH_3CN)) to mobile phases containing CO_2 with 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 Primary Screening.
2. If the compound or compound series are not soluble in any of these mobile phases, we recommend to try the Primary Screening with the product dissolved in a stronger solvent (DCM/alcohol...).

Table 1. Immobilized Primary Screening Solvents

Primary solvent mixtures	**CO_2** / MeOH	**CO_2** / EtOH	**CO_2** /2-PrOH	**CO_2** / CH_3CN
Typical starting conditions	80:20	80:20	80:20	70:30
Advised optimisation range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60

❶ Alcohols can be added into CH_3CN to enhance the eluting strength for strongly retained compounds.

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

Table 2. Immobilized Secondary Screening Solvents

Secondary solvent mixtures	CO_2 / THF	CO_2 / (DCM+MeOH 90:10)	CO_2 / (EtOAc+MeOH 90:10)	CO_2 / (MtBE+MeOH 80:20)
Typical starting conditions	75:25	80:20	80:20	75:25
Advised optimisation range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60

❶ The alcohol content and type (MeOH, EtOH and 2-PrOH) can be used to modulate retention and recognition.
THF can be added into DCM and EtOAc to enhance the eluting strength for strongly retained compounds.

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

C – General Comments

⇨ Only the immobilized polysaccharide-derived phases (IA / IB / IC/ ID/ IE/ IF/ IG) are suitable for the Secondary Screening.

Additional co-solvent such as $CHCl_3$, 1,4-Dioxane, Toluene or Acetone can also be investigated with CHIRALPAK® IA, IB, IC, ID, IE, IF and IG columns.

⇨ The typical starting conditions consist in 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.

⇨ It is also important to check your SFC system (seals...) is compatible with all types of solvents and to take into account UV cut-off of certain solvents, in order to avoid detection issues.
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).

D – Additives

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

Basic Samples require Basic additives ❶❷	Acidic Samples require Acidic additives❶
Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid

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

Acidic samples **do not always** require the presence of an additive. Actually, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute properly the product.

❶ In practice: 1% of the additive is incorporated to the co-solvent.
The total amount of additive into the mobile phase will be dependant upon the percentage of co-solvent; for example: if the mobile phase is CO_2 / EtOH 90:10, with EtOH containing 1% of additive, then the mobile phase composition will be CO_2 / EtOH / additive 90:10:0.1).

❷ For preparative purposes, it is recommended to use DEA or TEA as additives, due to their easy removal from the products by standard evaporation and drying systems.

Column care / Maintenance

- ❑ The use of in-line filter is highly recommended for maximum column life.
- ❑ Samples should preferably be dissolved in the co-solvent.
- ❑ Sample solutions should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before use.

☞ ***Column cleaning and regeneration procedures – to be done in liquid mode***

Following extensive column use, impurities can be accumulated and resolution may be compromised. In order to ensure consistent performance and clean the column, a regeneration method may be implemented in liquid mode:

1. Flush with 100% ethanol at 0.5 ml/min(*) for 30 min
2. Flush with 100% THF at 0.5 ml/min(*) for 2 hours.
3. Flush with 100% ethanol at 0.5 ml/min(*) for 30 min
4. Flush with Heptane/2-PrOH 90:10 at 0.5 ml/min for 60 min
5. Flush with 100% 2-PrOH 0.3 ml/min for 30 min
6. Flush with 100% CO_2 0.3 ml/min for 30 min
7. Equilibrate with the mobile phase prior to retesting the column (**).

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

(*) *Recommended flow rate for analytical columns (4.6mm i.d.). For semi-prep. columns, the flow rate should be adjusted according to the column diameter* ***x 5*** *for a 10mm i.d column;*
x20 *for a 20mm i.d. column;*
x40 *for a 30mm i.d. column*

(**) *To check/compare the efficiency of the column, it is necessary to apply the original test conditions supplied with the column.*

☞ ***Column transfer between modes:***

From LC to SFC

- Flush with 100% 2-PrOH at 0.25 ml/min(*) for 45 min
- Flush with 100% CO_2 or CO_2+co-solvent at 0.25 ml/min(*) for 45 min

From SFC to LC

- Flush with 100% 2-PrOH at 0.25 ml/min(*) for 45 min
- Flush with the mobile phase at 0.25 ml/min(*) for 45 min

(*) *Recommended flow rate for analytical columns (4.6mm i.d.). For semi-prep columns, the flow rate should be adjusted according to the column diameter.*

Column storage

❑ For a storage period exceeding 2-3 days remove the acidic or basic additives by flushing the column with 100% 2-PrOH or 100% methanol (no additives).
After flushing with 2-PrOH or methanol, the columns can be stored end capped in a drawer.

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

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

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