

**INSTRUCTION MANUAL FOR  
CHIRALPAK® IA-3, CHIRALPAK® IB-3, CHIRALPAK® IC-3,  
CHIRALPAK® ID-3, CHIRALPAK® IE-3, CHIRALPAK® IF-3**

*For analytical purposes*

Supercritical Fluid Chromatography (SFC)

***Please read this instruction sheet completely before using these columns***

**Column Description**

Immobilized <u>Amylose</u> -Based chiral phases <b>3µm silica-gel support</b>	Immobilized <u>Cellulose</u> -Based chiral phases <b>3µm silica-gel support</b>
<p><b>CHIRALPAK® IA-3</b> Amylose tris(3,5-dimethylphenylcarbamate)</p> <hr/> <p><b>CHIRALPAK® ID-3</b> Amylose tris(3-chlorophenylcarbamate)</p> <hr/> <p><b>CHIRALPAK® IE-3</b> Amylose tris(3,5-dichlorophenylcarbamate )</p> <hr/> <p><b>CHIRALPAK® IF-3</b> Amylose tris(3-chloro-4-methylphenylcarbamate)</p>	<p><b>CHIRALPAK® IB-3</b> Cellulose tris(3,5-dimethylphenylcarbamate)</p> <hr/> <p><b>CHIRALPAK® IC-3</b> Cellulose tris(3,5-dichlorophenylcarbamate)</p>

**Shipping solvent:                      100% Methanol**

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 Conditions

	50 x 3 mm i.d. / 100 x 3 mm i.d. / 150 x 3 mm i.d. Analytical columns
Flow rate direction	As indicated on the column label
Typical flow rate <b>in SFC</b>	~ 0.5 - 4 ml/min
Pressure limitation	Should be maintained < 300 Bar (4350 psi) for maximum column life <b>Typical CO<sub>2</sub> backpressure (BPr) 110 – 150 Bar</b>
Temperature	0 to 40°C

The relevant pressure value is the one generated by the column itself (pressure drop over the column). The pressure drop is the difference between the column inlet pressure (P-inlet) and the column outlet pressure (P-outlet).

The pressure drop generated by the system alone (without any column) has to be subtracted from the total value (system + column). **It can be calculated:  $\Delta P = P_{\text{total}} - P_{\text{system}} - BPr$**

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, temperature, amount and type of co-solvent in the mobile phase).

## Method Development / SFC Mode

### A - Mobile phases

CHIRALPAK® IA-3, IB-3, IC-3, ID-3, IE-3 and IF-3 can be used **with all ranges of organic miscible solvents as co-solvent combined with the carbon dioxide (CO<sub>2</sub>)**, progressing from the traditional solvents (mixtures of CO<sub>2</sub> with alcohols or acetonitrile (CH<sub>3</sub>CN)) to mobile phases containing CO<sub>2</sub> with tetrahydrofuran (THF), dichloromethane (DCM), chloroform (CHCl<sub>3</sub>), ethyl acetate (EtOAc), methyl *tert*-butyl ether (MtBE), 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 <sub>2</sub> / MeOH	CO <sub>2</sub> / EtOH	CO <sub>2</sub> / 2-PrOH	CO <sub>2</sub> / CH <sub>3</sub> CN
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

① Alcohols can be added into CH<sub>3</sub>CN 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 <sub>2</sub> / THF	CO <sub>2</sub> / (DCM+MeOH 90:10)	CO <sub>2</sub> / (EtOAc+MeOH 90:10)	CO <sub>2</sub> / (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 separation. 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 (CHIRALPAK® IA-3, IB-3, IC-3, ID-3, IE-3 and IF-3) are suitable for the Secondary Screening.

Additional co-solvent such as CHCl<sub>3</sub>, 1,4-Dioxane, Toluene or Acetone can also be investigated with CHIRALPAK® IA-3, IB-3, IC-3, ID-3, IE-3 and IF-3 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**

For basic

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

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

Acidic samples **do not always** require the presence of an acidic additive. Actually, the acidic properties of the carbon dioxide (CO<sub>2</sub>) 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 in CO<sub>2</sub>; for example: if the mobile phase is CO<sub>2</sub> / EtOH 90:10, with EtOH containing 1% of additive, then the mobile phase composition will be CO<sub>2</sub> / EtOH / additive 90:10:0.1).

### 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 injection.

## ☞ 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**:

- Flush with 100% ethanol at 0.2 ml/min<sup>(\*)</sup> for 30 min, followed by 100% THF at 0.2 ml/min<sup>(\*)</sup> for 2 hours.
- Flush with 100% ethanol at 0.2 ml/min<sup>(\*)</sup> for 30 min and then equilibrate with the mobile phase prior to retesting the column<sup>(\*\*)</sup>.
- *If this is not successful, then try with 100% N,N-dimethylformamide (DMF) or N,N-dimethylacetamide (DMAC) at 0.15 ml/min<sup>(\*)</sup> for 3 hours instead of the THF flush<sup>(\*\*\*)</sup>.*

(\*) *Recommended flow rate for analytical columns (3.0mm i.d.).*

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

(\*\*\*) *For CHIRALPAK® IA-3 particularly, a column flushing with 100% EtOH at a very low flow rate (0.05 mL/min for 60min) is essential after THF (or DMF / DMAC) flushing and before EtOH flushing at 0.2 ml/min.*

## ☞ Column transfer between modes:

### From LC to SFC

- Flush with 100% EtOH at 0.2 ml/min<sup>(\*)</sup> for 45 min
- Flush with 100% CO<sub>2</sub> or CO<sub>2</sub>+co-solvent at 0.25 ml/min<sup>(\*)</sup> for 45 min

### From SFC to LC

- Flush with 100% EtOH at 0.2 ml/min<sup>(\*)</sup> for 45 min
- Flush with the mobile phase at 0.25 ml/min<sup>(\*)</sup> for 45 min

## **Column Storage**

- ☐ For a storage period exceeding 2-3 days remove the acidic or basic additives by flushing the column with 100% methanol (no additives).

***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:

In the USA: [questions@chiraltech.com](mailto:questions@chiraltech.com) or call 800-6-CHIRAL

In the EU: [cte@chiral.fr](mailto:cte@chiral.fr) or call +33 (0)3 88 79 52 00

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