# *INSTRUCTION MANUAL FOR CHIRALPAK® AD-3, CHIRALPAK AS-3, CHIRALPAK AY-3, CHIRALPAK AZ-3, CHIRALCEL® OD-3, CHIRALCEL OJ-3, CHIRALCEL OX-3 and CHIRALCEL OZ-3*

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

## < Supercritical Fluid Chromatography (SFC) >

## Column description

| "Coated" Amylose-Based chiral phases<br>***3µm silica-gel support*** | "Coated" Cellulose-Based chiral phases<br>***3µm silica-gel support*** |
|---|---|
| **CHIRALPAK® AD-3**<br>Amylose tris(3,5-dimethylphenylcarbamate) | **CHIRALCEL® OD-3**<br>Cellulose tris(3,5-dimethylphenylcarbamate) |
| **CHIRALPAK® AS-3**<br>Amylose tris[(S)- α-methylbenzylcarbamate] | **CHIRALCEL® OJ-3**<br>Cellulose tris(4-methylbenzoate) |
| **CHIRALPAK® AY-3**<br>Amylose tris(5-chloro-2-methylphenylcarbamate) | **CHIRALCEL® OZ-3**<br>Cellulose tris(3-chloro-4-methylphenylcarbamate) |
| **CHIRALPAK® AZ-3**<br>Amylose tris(3-chloro-4-methylphenylcarbamate) | **CHIRALCEL® OX-3**<br>Cellulose tris(4-chloro-3-methylphenylcarbamate) |

***THIS INSTRUCTION SHEET IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS***

**CAUTION**

**The entire SFC system including the injector and the injection loop must be flushed with a solvent compatible with the column and its storage solvent prior to connecting. Solvents such as acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride and THF may DESTROY the chiral stationary phase if they are present, even in residual quantities, in the system.**
**If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed and the relevant solvent lines flushed.**

**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.

## Operating Instructions

| | **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 **in SFC** | ~ 0.5 - 4 ml/min |
| Pressure limitation | Should be maintained < 300 Bar (4350 psi) for maximum column life<br>***Typical $CO_2$ backpressure (BPr) 110 – 150 Bar*** |
| 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: $\Delta P = P_{total} - P_{system}$ - 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 - Method Development - Screening*

| **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 For strongly retained compounds, an alcohol can be added into $CH_3CN$ to enhance the eluting strength.
❷ The retention is generally shorter with Ethanol or Methanol than with 2-propanol.
The use of other alcohols such as 1-propanol, 1-BuOH, 2-BuOH etc…is also possible.

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

## B – General Comments

⇨ 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.

## C – Additives

| Basic Samples require Basic additives ❶ | Acidic Samples require Acidic additives ❶ |
|---|---|
| Diethylamine (DEA)<br>Triethylamine (TEA) | Trifluoroacetic acid (TFA)<br>Acetic acid<br>Formic acid |

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

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 acidic 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 in $CO_2$; 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).

☞ ***Basic additives should be avoided on CHIRALPAK® AZ-3***

## 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 transfer between modes:***

***From LC to SFC***

- Flush with 100% EtOH at 0.2 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% EtOH at 0.2 ml/min for 45 min
- Flush with the mobile phase at 0.25 ml/min 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 this column 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 or call 800-6-CHIRAL
In the EU: cte@chiral.fr or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91-40-2338-3700

**Locations:**


**North/Latin America**
Chiral Technologies. Inc.
800 North Five Points Road
West Chester, PA 19380
800 6 CHIRAL
Tel: 610-594-2100
Fax: 610-594-2325
chiral@chiraltech.com
www.chiraltech.com

**Europe**
Chiral Technologies Europe
Parc d'Innovation
Bd Gonthier d'Andernach
67400 Illkirch Cedex, France
Tel: +33-388-795-200
Fax: +33-388-667-166
cte@chiral.fr
www.chiral.fr

**India**
Daicel Chiral Technologies (India) Pvt. Ltd.
Lab No. 4A, Phase III
IKP Knowledge Park
Genome Valley, Turkapally,
Shameerpet, Ranga Reddy Dist.
Hyderabad-500 078, Telangana
Tel: +91-40-2338-3700
Fax: +91-40-2348-0104
chiral@chiral.daicel.com