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

Please read this instruction sheet completely before using these columns

< Supercritical Fluid Chromatography (SFC) >

Column description

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

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

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).

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.

Operating Instructions

	100 x 4.6 mm ID **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	**250 x 30 mm ID** 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 - 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

⇨ *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.

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

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.

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

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 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 ID). 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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