**INSTRUCTION MANUAL FOR**


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

< Supercritical Fluid Chromatography (SFC) >

### Column description

<table>
<thead>
<tr>
<th>“Coated” Amylose-Based chiral phases</th>
<th>“Coated” Cellulose-Based chiral phases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHIRALPAK® AD-H</strong></td>
<td><strong>CHIRALCEL® OD-H</strong></td>
</tr>
<tr>
<td>Amylose tris(3,5-dimethylphenylcarbamate)</td>
<td>Cellulose tris(3,5-dimethylphenylcarbamate)</td>
</tr>
<tr>
<td><strong>CHIRALPAK® AS-H</strong></td>
<td><strong>CHIRALCEL® OJ-H</strong></td>
</tr>
<tr>
<td>Amylose tris[(S)-α-methylbenzylcarbamate]</td>
<td>Cellulose tris(4-methylbenzoate)</td>
</tr>
<tr>
<td><strong>CHIRALPAK® AY-H</strong></td>
<td><strong>CHIRALCEL® OZ-H</strong></td>
</tr>
<tr>
<td>Amylose tris(5-chloro-2-methylphenylcarbamate)</td>
<td>Cellulose tris(3-chloro-4-methylphenylcarbamate)</td>
</tr>
<tr>
<td><strong>CHIRALPAK® AZ-H</strong></td>
<td><strong>CHIRALCEL® OX-H</strong></td>
</tr>
<tr>
<td>Amylose tris(3-chloro-4-methylphenylcarbamate)</td>
<td>Cellulose tris(4-chloro-3-methylphenylcarbamate)</td>
</tr>
</tbody>
</table>

**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

<table>
<thead>
<tr>
<th></th>
<th>100 x 4.6 mm ID</th>
<th>150 x 4.6 mm ID</th>
<th>250 x 10 mm ID</th>
<th>250 x 20 mm ID</th>
<th>250 x 30 mm ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analytical columns</td>
<td>Semi-prep. columns</td>
<td>Semi-prep. columns</td>
<td>Semi-prep. columns</td>
<td></td>
</tr>
<tr>
<td>Flow rate direction</td>
<td>As indicated on the column label</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical Flow rate in SFC</td>
<td>~ 1 - 5 ml/min</td>
<td>~ 15 ml/min</td>
<td>~ 60 ml/min</td>
<td>~ 120 ml/min</td>
<td></td>
</tr>
<tr>
<td>Pressure limitation</td>
<td>Should be maintained &lt; 300 Bar (4350 psi) for maximum column life</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Adapt flow rates to column size.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>0 to 40°C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**

<table>
<thead>
<tr>
<th>Primary solvent mixtures</th>
<th>CO₂ / MeOH</th>
<th>CO₂ / EtOH</th>
<th>CO₂ / 2-PrOH</th>
<th>CO₂ / CH₃CN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical starting conditions</td>
<td>80:20</td>
<td>80:20</td>
<td>80:20</td>
<td>70:30</td>
</tr>
<tr>
<td>Advised optimisation range</td>
<td>99:1 to 40:60</td>
<td>99:1 to 40:60</td>
<td>99:1 to 40:60</td>
<td>99:1 to 40:60</td>
</tr>
</tbody>
</table>

Enumerations:
- For strongly retained compounds, an alcohol can be added into CH₃CN to enhance the eluting strength.
- 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**

стрелка 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**

стрелка 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 additive. Actually, the acidic properties of the carbon dioxide (CO₂) are sometimes enough to elute properly the product.

 назначение 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 CO₂ / EtOH : 90:10, with EtOH containing 1% of additive, then the mobile phase composition will be CO₂ / EtOH / additive : 90:10:0.1).

 назначение 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.

 назначение 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% CO₂ or CO₂+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.
For a storage period exceeding 2-3 days remove the acidic or basic additives by flushing the column with 100% 2-ProOH or 100% methanol (no additives). After flushing with 2-ProOH 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:

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