

## INSTRUCTION MANUAL FOR CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U

### <Supercritical Fluid Chromatography (SFC)>

**Please read this instruction manual completely before using these columns.** These columns can also be used in normal phase or reversed-phase. Please refer to the corresponding instruction manual for details.

#### Column Description

AMYLOSE-BASED		CELLULOSE-BASED
Immobilized on 1.6 µm silica gel		Immobilized on 1.6 µm silica gel
<b>CHIRALPAK® IA-U</b>	<b>CHIRALPAK® ID-U</b>	<b>CHIRALPAK® IB-U</b>
Amylose tris(3,5-dimethyl-phenylcarbamate)	Amylose tris(3-chloro-phenylcarbamate)	Cellulose tris(3,5-dimethyl-phenylcarbamate)
<b>CHIRALPAK® IG-U</b>	<b>CHIRALPAK® IH-U</b>	<b>CHIRALPAK® IC-U</b>
Amylose tris(3-chloro-5-methyl-phenylcarbamate)	Amylose tris[(S)-α-methylbenzylcarbamate]	Cellulose tris(3,5-dichloro-phenylcarbamate)
Shipping solvent: <b>Hexane/IPA = 90:10 (v/v)</b>		
All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, were included with the column when purchased.		

\*Because these columns are shipped in hex/IPA, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage (see column transfer conditions between LC and SFC on page 4).\*

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

## Operating Instructions

	50 x 3.0 mm i.d. Analytical Columns	100 x 3.0 mm i.d. Analytical Columns
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.6 to 5.0 ml/min	0.6 to 2.6 ml/min
Temperature	0 to 40°C	
Column Pressure Limit <sup>①</sup>	700 bar (10150 psi)	
Column Fitting <sup>②</sup>	Upchurch or Parker-type	

① The column pressure is the total pressure minus the system pressure. At a given temperature, the column back pressure is linearly proportional to the flow rate.

② **It is highly recommended the matching fitting be used.** Improperly matched fittings can create a void at the inlet, leading to significant extra-column band broadening. This effect is much more pronounced on these small, sub-2 µm particles, compared to larger particle sizes. Additionally, fitting mismatch can lead to significant leaking.

## Method Development / SFC

### A - Mobile Phases

CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U can be used *with all ranges of organic miscible solvents as modifiers combined with supercritical carbon dioxide (CO<sub>2</sub>)*, progressing from the traditional solvents used with other DAICEL columns (mixtures of CO<sub>2</sub> with alcohols or acetonitrile (CH<sub>3</sub>CN)) to mobile phases containing CO<sub>2</sub> with methyl *tert*-butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform (CHCl<sub>3</sub>), and 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 a **Primary Screening**.
2. If the compound or compound series are not soluble in any of these mobile phases, we recommend trying 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 <sup>①</sup>
Typical Starting Conditions	80:20	80:20	80:20	70:30 <sup>①</sup>
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60 <sup>①</sup>

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 Immobilized Primary Screening strategy, we recommend progressing to an Immobilized Secondary Screening 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 Optimization 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 immobilized CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U are suitable for the Secondary Screening.

Additional modifiers such as CHCl<sub>3</sub>, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U.

⇒ The typical starting conditions consist of 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 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.**

For basic samples, it is necessary to incorporate an additive into the mobile phase in order to optimize 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 additive. In fact, the acidic properties of the carbon dioxide (CO<sub>2</sub>) are sometimes enough to elute the product properly.

❶ In practice, 1% of the additive is incorporated with the modifier. The total amount of additive into the mobile phase will be dependent upon the percentage of modifier. 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

- ❑ Samples should preferably be dissolved in the modifier.
- ❑ Sample solutions should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before use.

Following extensive use of the column in multiple solvents, there may be a change in separation reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in chiral recognition due to the history of the column (mobile phases, additives...). This procedure should also be used when switching from reversed-phase to normal phase or SFC.

**For detailed Regeneration Procedures, please [click here](#)**

## ☞ Column transfer between modes:

### From LC to SFC

- Flush with 100% 2-PrOH at 0.10 ml/min for 45 min
- Flush with 100% CO<sub>2</sub> or CO<sub>2</sub>+modifier at 0.10 ml/min for 45 min

### From SFC to LC

- Flush with 100% 2-PrOH at 0.10 ml/min for 45 min
- Flush with the mobile phase at 0.10 ml/min for 45 mi

## Column Storage

- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% 2-PrOH or 100% methanol, without additives.
- ❑ Columns can be stored with ends capped in the additive-free mobile phase, or the shipping solvent, at room temperature.

***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@cti.daicel.com](mailto:questions@cti.daicel.com) or call 800-6-CHIRAL

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

In India: [chiral@chiral.daicel.com](mailto:chiral@chiral.daicel.com) or call +91 84 1866 0700 & 703

## Locations:

### **North/Latin America**

Chiral Technologies, Inc.  
1475 Dunwoody Dr. Ste 310  
West Chester, PA 19380  
800 6 CHIRAL  
Tel: 610-594-2100  
Fax: 610-594-2325  
[chiral@cti.daicel.com](mailto:chiral@cti.daicel.com)  
[www.chiraltech.com](http://www.chiraltech.com)

### **Europe**

Chiral Technologies Europe SAS  
Parc d'Innovation  
160, Bd Gonthier d'Andernach CS 80140  
67404 Illkirch Cedex France  
Tel: +33 (0) 3 88 79 52 00  
Fax: +33 (0) 3 88 66 71 66  
[cte@cte.daicel.com](mailto:cte@cte.daicel.com)  
[www.chiraltech.com](http://www.chiraltech.com)

### **India**

Daicel Chiral Technologies (India) Pvt Ltd  
Survey No. 542/2 IKP Knowledge Park, Turkapally,  
Shamirpet Mandal, Medchal-Malkajgiri District,  
Hyderabad-500101. Telangana, India  
Tel: +91 84 1866 0700 & 703  
Fax: +91 84 1866 0730  
[chiral@chiral.daicel.com](mailto:chiral@chiral.daicel.com)  
[www.chiraltech.com](http://www.chiraltech.com)

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