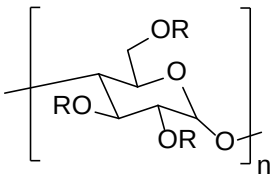
INSTRUCTION MANUAL FOR CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U

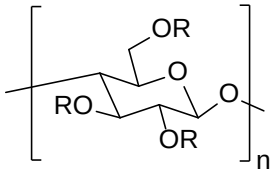
<Normal Phase>

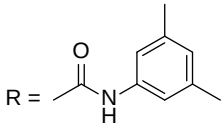
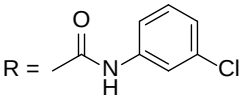
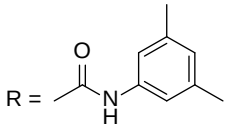
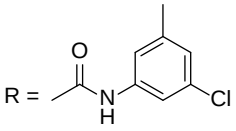
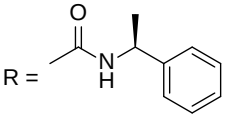
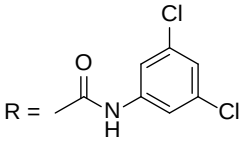
Please read this instruction manual completely before using these columns

These columns can also be used in reversed-phase and SFC. Please refer to the corresponding instruction manual for details.

Column Description

<table>
<tr><th colspan="2">AMYLOSE-BASED
 

Immobilized on 1.6 µm silica gel</th><th>CELLULOSE-BASED
 

Immobilized on 1.6 µm silica gel</th></tr>
<tr><th>CHIRALPAK® IA-U</th><th>CHIRALPAK® ID-U</th><th>CHIRALPAK® IB-U</th></tr>
<tr><td>Amylose tris(3,5-dimethyl-phenylcarbamate)
 
</td><td>Amylose tris(3-chloro-phenylcarbamate)
 
</td><td>Cellulose tris(3,5-dimethyl phenylcarbamate)
 
</td></tr>
<tr><th>CHIRALPAK® IG-U</th><th>CHIRALPAK® IH-U</th><th>CHIRALPAK® IC-U</th></tr>
<tr><td>Amylose tris(3-chloro-5-methyl-phenylcarbamate)
 
</td><td>Amylose tris[(S)- α-methylbenzylcarbamate]
 
</td><td>Cellulose tris(3,5-dichloro phenylcarbamate)
 
</td></tr>
<tr><td colspan="3">Shipping solvent: Hexane/IPA = 90:10 (v/v)

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.</td></tr>
</table>

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Although these columns can be used with an HPLC system, it is highly recommended that a UHPLC system be utilized to preserve the best separation performance of the column.

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 Limit①	700 bar (10150 psi)	
Column Fitting②	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 / Normal Phase

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*, progressing from the traditional mobile phases used with other DAICEL columns (mixtures of alkanes/alcohol, pure alcohol or acetonitrile (ACN)) to mobile phases containing methyl *tert*-butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform ($CHCl_3$), 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 progressing directly to the Secondary Screening (Table 2).

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	Alkane❶/2-PrOH	Alkane❶/EtOH	Alkane❶/MtBE/EtOH❷	Alkane❶/THF❸	Alkane/DCM❹/EtOH
Typical Starting conditions	80:20	80:20	0:98:2	70:30	50:50:2
Advised Optimization Range	99:1 to 50:50	99:1 to 50:50	80:20:0 to 0:40:60	95:5 to 0:100	85:15:0 to 0:80:20

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ In absence of alkane, methanol is more efficient than ethanol when combined with MtBE.
❸ In the case of no environmental restrictions, **use of DCM is preferred to THF** in terms of better enantioselectivity that the former may induce.
❹ For excessively retained samples, addition of ethanol up to 20% in pure DCM would be helpful.

If a suitable chiral separation is not found using the Immobilized Primary Screening strategy, we recommend an Immobilized Secondary Screening to be applied using the following conditions:

Table 2. Immobilized Secondary Screening Solvents

Secondary Solvent Mixtures	EtOAc❶/Alkane❷	ACN❸/Alcohol❹
Typical Starting Conditions	50:50	100:0
Advised Optimization Range	20:80 to 100:0	100:0 to 0:100

❶ Alcohols (❹) or THF can be added into EtOAc to enhance the eluting strength for strongly retained compounds.

❷ Alkane: n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❸ Transfers between alkane mixtures and ACN are preferably made with a transition in alcohol in order to avoid miscibility issues.
❹ Alcohol: MeOH, EtOH and 2-PrOH.

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

C – General Comments

⇨ Additional solvent combinations such as $CHCl_3$/Alkane, 1,4-Dioxane/Alkane, Toluene/Alkane or Acetone/Alkane can also be investigated with CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U columns.

⇨ The typical starting conditions represent the 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.

⇨ Toluene, MtBE and chlorinated solvents can be used in their pure form as the mobile phase.

⇨ For fast eluting solvents, such as THF, we recommend to add alkane in order to modulate the retention.

⇨ 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). In these cases an alternative detector, such as an RI detector or an ELSD (Evaporative Light Scattering Detector), may be more effective than the UV detector.

D – Additives

For basic or acidic samples, it is necessary to incorporate an additive into the mobile phase in order to optimize the chiral separation.

❶ It has been found that certain amines, such as EDA and AE induce much better behaviour for certain basic compounds than the most commonly used DEA.

☞ *The addition of a low percentage of an alcohol (e.g. 2% EtOH or MeOH) in the mobile phase may be helpful to ensure the miscibility of EDA and AE with the low polarity mobile phases.*

Basic Samples require Basic additives	Acidic Samples require Acidic additives
Diethylamine (DEA) 2-Aminoethanol (AE)❶ Ethylenediamine (EDA)❶ Butyl amine (BA)	Trifluoroacetic acid (TFA) Acetic acid (AA) Formic acid (FA)
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

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

Column Care / Maintenance

- ❑ Samples should preferably be dissolved in the mobile phase.
- ❑ The mobile phase and the sample solution should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.

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.

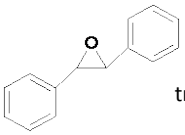
For detailed Regeneration Procedures, please click here

Column Storage

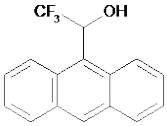
- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% 2-PrOH or 100% methanol, without additives.
- ❑ Columns can be stored with ends capped in the additive-free mobile phase, or the shipping solvent, at room temperature.

Operating these columns in accordance with the guidelines outlined here will result in a long column life.

Column Performance – Examples



trans-Stilbene oxide



2,2,2-trifluoro-1-(9-anthryl)ethanol

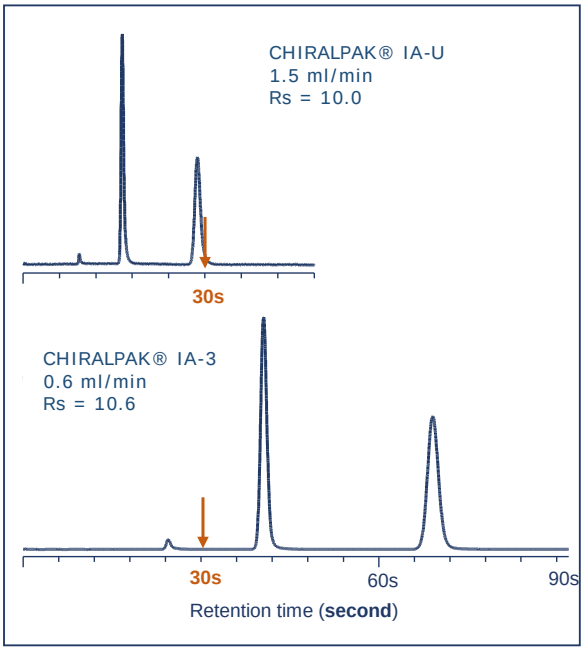


Figure 1

Common conditions

Column size: 50x3 mm i.d.
Mobile phase: Hexane/2-PrOH 90:10 v/v
Temperature: 25°C
UV 230nm

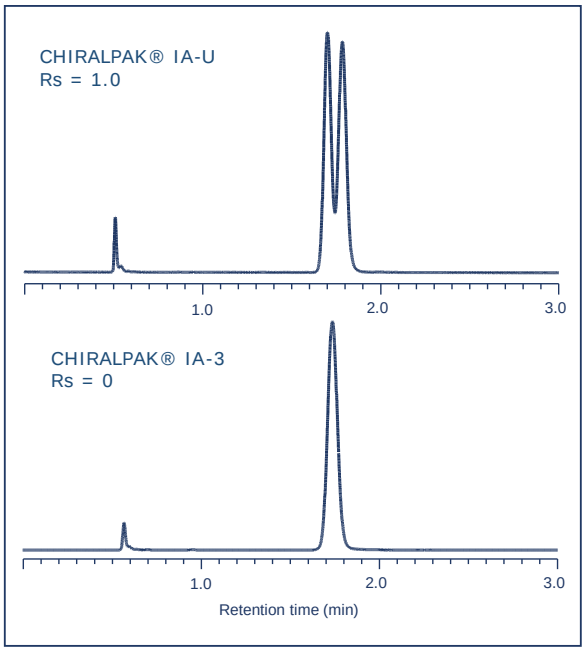


Figure 2

Common conditions

Column size: 50x3 mm i.d.
Flow rate: 0.425 mL/min
Mobile phase: Hexane/2-PrOH 90:10 v/v
Temperature: 25°C
UV 230nm

⇨ If you have any questions about the use of these columns, or encounter a problem, contact:

In the USA: questions@cti.daicel.com or call 800-6-CHIRAL

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

In India: 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
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
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
www.chiraltech.com