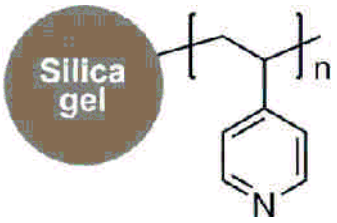
INSTRUCTION MANUAL FOR DAICEL DCpak® P4VP

Please read these instructions completely before using this column

Column Description

DAICEL DCpak® P4VP
Poly(4-vinylpyridine) **immobilized** on **5µm and 3µm silica** 

Shipping solvent: **100% Ethanol**

Every column has been examined and quality control tested before shipping. Please refer to the Column Performance Report and test parameters for results.

CAUTION

The column is designed for 34.35MPa maximum pressure and for 30MPa daily pressure.
Please use the column **neither** at a pressure over 30MPa **nor** at a temperature over 40 °C.

Please flush the residual solvent in the SFC instrument with a recommended mobile phase (see page 2) before connecting the column to the instrument.
Please be sure to flush the auto-sampler, syringe, needle, and injection loop as well.

Operating Conditions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical columns	**50 x 3.0 mm i.d.** **100 x 3.0 mm i.d.** **150 x 3.0 mm i.d.** Analytical columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical columns	**250 x 10 mm i.d.** **250 x 20 mm i.d.** **250 x 30 mm i.d.** **250 x 50 mm i.d.** Semi-prep/prep columns
Column Fittings	Waters			
Flow Rate Direction	As indicated on the column label			
Pressure Limitations*	30MPa (~ 305 kgf/cm^2 or ~ 4350 psi)			
Temperature	0 to 40 °C			

*Pressure means the pressure at the column head, which is nearly equal to the pump pressure. The recommended back pressure regulator (BPR) setting is 8 – 20MPa. If the BPR setting is too low, an unstable chromatogram may result.

Important Notice

- **This column is not for chiral separations.**
- **Do not attempt to disassemble the column.**
- **This instruction sheet for DCpak® P4VP is not applicable to any other Daicel column.**

Please contact your local Chiral Technologies office for further assistance before trying any solvents not mentioned below.

A – SFC Mobile Phases

	CO_2 / co-solvent
Composition	**100 / 0 to 40 / 60**

- Methanol is typically used as a co-solvent. Ethanol, 2-propanol, ethyl acetate, THF, and dichloromethane can also be used.
- The eluotropic strength of the alcoholic co-solvents are methanol>ethanol>2-propanol if the same volume percentage is applied. This tendency becomes remarkable for a polar analyte.
- A higher co-solvent content results in a shorter retention time.
- A mixed co-solvent of the above organic solvents can also be applied. When an **aprotic** co-solvent is employed, the addition of alcohol in a small amount may improve peak shape.
- An increase of the co-solvent content increases the column head pressure. **Pressure should not exceed 30 MPa**.

B – Additives

- Initial method development can be performed without the addition of any additives. If needed, the recommended additives as illustrated in the table may help sharpen the peak shape.
- Typical concentration is 0.1 vol% of the total mobile phase. (e.g. use co-solvent containing 0.5% of additive if CO_2/ co-solvent ratio is 80/20 v/v).

Additive for basic analyte	Additive for acidic analyte
Diethylamine Ammonium formate Ammonium acetate	Trifluoroacetic acid
~0.1 vol% of total mobile phase	~0.1 vol% of total mobile phase

- After a basic or acidic additive has been used, wash the column with more than 10 column volumes of mobile phase without additive, and then flush the column with ethanol.

Sample Preparation

- The sample should be dissolved in the mobile phase co-solvent, i.e. methanol, ethanol, etc., and should be filtered through a membrane filter of approximately 0.5µm porosity.

Gradient and LC

- Analysis can be performed by gradient elution, however baseline instability may occasionally occur, in particular after using basic or acidic additives. Before performing a gradient analysis, complete a "test run" to verify baseline stability.
- While these columns were initially designed for use under SFC separation conditions, they can also be used under LC conditions in both Normal Phase (hexane/alcohol) and Reversed Phase modes. Please contact our technical assistance for suggested starting conditions.

Column Care / Maintenance

- After performing analyses which contain additives, it is good practice to flush the column with mobile phase which does not contain any additives. If removing the column from the system, flush with 100% ethanol or isopropanol first, and then remove the column following the notes below.
- Remove the column from the instrument **ONLY** after the inner pressure is completely released. Removing the column under a high inner pressure may cause hazards by rapid releasing CO_2, and can cause a deterioration of the column seal. Be sure to slowly loosen the connection to avoid possible release of CO_2.
- The column can be stored long term at ambient temperature.
- If reproducibility has been compromised, clean the column with more than 10 column volumes of ethanol at 1.0 mL/min. In addition, if the reproducibility has been compromised **AND** trifluoroacetic acid has been used repeatedly as an additive, it might be necessary to perform the column cleaning with 0.1vol% diethylamine in the mobile phase.

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 this column, 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
Tel: 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
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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

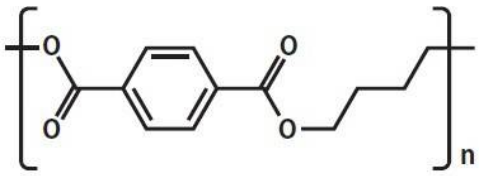
INSTRUCTION MANUAL FOR DAICEL DCpak® PBT

Please read these instructions completely before using this column

Column Description

DAICEL DCpak® PBT

Polybutylene terephthalate **coated** on **5µm and 3µm silica**



Shipping solvent: **100% Ethanol**

Every column has been examined and quality control tested before shipping. Please refer to the Column Performance Report and test parameters for results.

CAUTION

The column is designed for 34.35MPa maximum pressure and for 30MPa daily pressure.
Please use the column **neither** at a pressure over 30MPa **nor** at a temperature over 40 °C.

Please flush the residual solvent in the SFC instrument with a recommended mobile phase (see page 2) before connecting the column to the instrument.

Please be sure to flush the auto-sampler, syringe, needle, and injection loop as well.

Operating Conditions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical columns	**50 x 3.0 mm i.d.** **100 x 3.0 mm i.d.** **150 x 3.0 mm i.d.** Analytical columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical columns	**250 x 10 mm i.d.** **250 x 20 mm i.d.** **250 x 30 mm i.d.** **250 x 50 mm i.d.** Semi-prep/prep columns
Column Fittings	Waters			
Flow Rate Direction	As indicated on the column label			
Pressure Limitations*	30MPa (~ 305 kgf/cm^2 or ~ 4350 psi)			
Temperature	0 to 40°C			

* Pressure means the pressure at the column head, which is nearly equal to the pump pressure. The recommended back pressure regulator (BPR) setting is 8 – 20MPa. If the BPR setting is too low, an unstable chromatogram may result.

Important Notice

- **This column is not for chiral separations.**
- **Do not attempt to disassemble the column.**
- **This instruction sheet for DCpak® PBT is not applicable to any other Daicel column.**

Please contact your local Chiral Technologies office for further assistance before trying any solvents not mentioned below.

A – SFC Mobile Phases

	CO_2 / co-solvent
Composition	**100 / 0 to 70 / 30**

- Methanol is typically used as a co-solvent. Ethanol, 2-propanol, ethyl acetate, THF, and dichloromethane can also be used.
- The eluotropic strength of the alcoholic co-solvents are methanol>ethanol>2-propanol if the same volume percentage is applied. This tendency becomes remarkable for a polar analyte.
- A higher co-solvent content results in a shorter retention time.
- A mixed co-solvent of the above organic solvents can also be applied. When an **aprotic** co-solvent is employed, the addition of alcohol in a small amount may improve peak shape.
- An increase of the co-solvent content increases the column head pressure. **Pressure should not exceed 30 MPa**.

B – Additives

- Add a small amount of additive to the co-solvent in the analysis of basic or acidic analytes as illustrated in the table.
- Typical concentration is 0.1 vol% of the total mobile phase. (e.g. use co-solvent containing 0.5% of additive if CO_2/co-solvent ratio is 80/20 v/v).

Additive for basic analyte	Additive for acidic analyte
Diethylamine	Trifluoroacetic acid
~0.1 vol% of total mobile phase	~0.1 vol% of total mobile phase

- After a basic or acidic additive has been used, wash the column with more than 10 column volumes of mobile phase without additive, and then flush the column with ethanol.

Sample Preparation

- The sample should be dissolved in the mobile phase co-solvent, i.e. methanol, ethanol, etc., and should be filtered through a membrane filter of approximately 0.5µm porosity.

Gradient and LC

- Analysis can be performed by gradient elution, however baseline instability may occasionally occur, in particular after using basic or acidic additives. Before performing a gradient analysis, complete a “test run” to verify baseline stability.
- While these columns were initially designed for use under SFC separation conditions, they can also be used under LC conditions in both Normal Phase (hexane/alcohol) and Reversed Phase modes. Please contact our technical assistance for suggested starting conditions.

Column Care / Maintenance

- After performing analyses which contain additives, it is good practice to flush the column with mobile phase which does not contain any additives. If removing the column from the system, flush with 100% ethanol or isopropanol first, and then remove the column following the notes below.
- Remove the column from the instrument **ONLY** after the inner pressure is completely released. Removing the column under a high inner pressure may cause hazards by rapid releasing CO_2, and can cause a deterioration of the column seal. Be sure to slowly loosen the connection to avoid possible release of CO_2.
- The column can be stored long term at ambient temperature.
- If reproducibility has been compromised, clean the column with more than 10 column volumesof ethanol at 1.0 mL/min.

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 this column, or encounter a problem, contact:

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

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Parc d'Innovation
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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

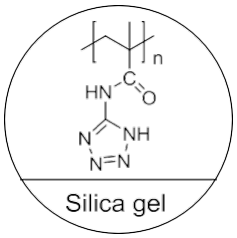
INSTRUCTION MANUAL FOR DAICEL DCpak® PTZ

Please read these instructions completely before using this column

Column Description

DAICEL DCpak® PTZ

Poly N-(1H-tetrazole-5-yl)-methacrylamide **immobilized** on **5µm and 3µm silica**



Shipping solvent: **10% Water / 90% Acetonitrile**

Every column has been examined and quality control tested before shipping. Please refer to the Column Performance Report and test parameters for results.

Operating Conditions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical columns	**50 x 3.0 mm i.d.** **100 x 3.0 mm i.d.** **150 x 3.0 mm i.d.** Analytical columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical columns	**250 x 10 mm i.d.** **250 x 20 mm i.d.** **250 x 30 mm i.d.** **250 x 50 mm i.d.** Semi-prep/prep columns
Column Fittings	Waters			
Flow Rate Direction	As indicated on the column label			
Pressure Limitations	30MPa (~ 305 kgf/cm^2 or ~ 4350 psi)			
Temperature	5 to 70°C			
pH Range①	Between pH 2.0 and 8.0			

①When this column is used at pH > 7, the temperature should be maintained between 5ºC and 40ºC, and the use of a guard cartridge is essential to maximize column life.

Important Notice

- **This column is not for chiral separations.**
- **Do not attempt to disassemble the column.**
- **This instruction sheet for DCpak® PTZ is not applicable to any other Daicel column.**

Please contact your local Chiral Technologies office for further assistance before trying any solvents not mentioned below.

A – HILIC Mobile Phases

	Polar Organic Solvent / Aqueous Buffer
Typical Starting Conditions	**80/20 (v/v)**
Advised Optimization Range	**95/5 to 5/95**

- Acetonitrile is the recommended starting polar organic solvent, however DCpak® PTZ can be used with any water miscible polar organic solvent.
- The eluotropic strength for this column in descending order is acetonitrile > methanol.
- It is recommended to start with an acidic aqueous buffer, such as 20 mM ammonium acetate (pH 4.7), however water, or other aqueous buffers can be used.
- A higher water content results in a shorter retention time.

B – Additives

- Ammonium formate or ammonium acetate (typically 20 mM) are generally used as additives to the aqueous solution.
- Formic acid, acetic acid, or ammonia water can be used for pH control.

Additive for basic analyte	Additive for acidic analyte
20 mM ammonium formate or ammonium acetate	
Ammonia water (for basic pH control)	Formic acid or acetic acid (for acidic pH control)

Sample Preparation

- The sample should be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- Before storing the column, remove the acidic or basic additives by flushing the column with the same mobile phase without the additives. Columns can be stored with the additive free mobile phase at ambient temperature.

☞ ***Column cleaning and regeneration procedures***

Following extensive use of the column in a wide variety of mobile phase conditions, there may be a change in column reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any changes in recognition due to the history of the column (mobile phases, additives…).

- Flush with 100 mM aqueous ammonium acetate / acetonitrile = 20 / 80 (v/v) at 1.0 mL/min.(*) for 2 hours.

- Equilibrate with 20 mM aqueous ammonium acetate / acetonitrile = 10 / 90 (v/v) at 1.0 mL/min.(*) for 1 hour prior to retesting the column.

(*) Recommended flow rate for 4.6 mm i.d. analytical columns. Please see conversion table below for other i.d. column flow rates.

- If the peak shape is collapsed, flush the column with 10 or more column volumes of 100 mM aqueous ammonium acetate / acetonitrile = 10 / 90 (v/v). Then plug both ends of the column and let it stand, while heating to 70ºC, for 20 hours or more.

Conversion Table

❑ Column ID vs Flow rate

Column i.d. (mm)	2.1	3.0	4.6	10	20	30
Flow rate (mL/min)	0.21	0.43	1.0	4.7	19	43

❑ Pressure

MPa	bar	kg/cm^2	psi
1	10	10.197	145.038
0.1	1	1.020	14.504
9.807×10^{-2}	0.981	1	14.223
6.895×10^{-3}	6.895×10^{-2}	7.031×10^{-2}	1

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 this column, 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

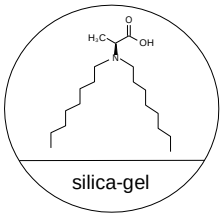
India
Daicel Chiral Technologies (India) Pvt Ltd
Survey No. 542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR CHIRALPAK® MA(+)

Please read this instruction sheet completely before using this column

Column Description

Packing composition: N,N-dioctyl-L-alanine coated on **3µm silica-gel**.



Column size: 50mm L x 4.6mm i.d.

Shipping solvent: The column is filled with 2mM $CuSO_4$ aq. solution.

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.

CAUTION

This column contains an amino acid derivative coated on silica-gel as the packing material. The entire HPLC system including the injector and the injection loop must be flushed with a mobile phase compatible with the column and its storage solvent prior to connecting.

Do not use mobile phases other than those listed in this document.

Otherwise, the column may be damaged by dissolving the amino acid derivative.

Strong-basic compounds are likely to deteriorate the silica-gel used in this column.

Standard Analytical Conditions

Mobile phase	: 2mM $CuSO_4$ aq. solution
Operating temperature	: 0 - 40°C
Column pressure	: Max. 150kg/cm^2
Flow rate	: 1.0mL/min
Flow direction	: As indicated on column label

Mobile Phases

Suitable mobile phases :	$CuSO_4$ aq. solution	= 0.1 - 2.0mM
	$CuSO_4$ aq. solution / methanol	= 100/0 - 85/15 (v/v)
	$CuSO_4$ aq. solution / acetonitrile	= 100/0 - 85/15 (v/v)

NOTES:

⇒ Retention time is generally shorter with acetonitrile than with methanol and with a higher content of organic solvent.

⇒ Eluent solution should be filtered through a 0.45 mm membrane filter.

⇒ High content of organic solvent may cause precipitation of $CuSO_4$ which makes the tubing clogged.

Sample

The sample solution should be filtered through a 0.45 mm membrane filter as a rule.

Column Cleaning

If the column has been contaminated, wash the column with 15% acetonitrile in H_2O (v/v) at a flow rate of 0.5mL/min for 3 hours.

Storage

The column can be stored at room temperature.
When the column is not in use, it is recommended to fill it with a 2mM $CuSO_4$ aq. solution.

Important Notice

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

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@cti.daicel.com or call 800-6-CHIRAL
In the EU: cte@cte.daicel.com or call +33 (0)3 88 79 52 00
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Locations:

North/Latin America
Chiral Technologies, Inc.
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West Chester, PA 19380
800 6 CHIRAL
Tel: 610-594-2100
Fax: 610-594-2325
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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
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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

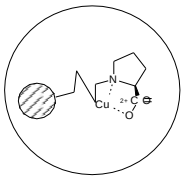
INSTRUCTION MANUAL FOR CHIRALPAK® WH

Analytical column for: Free Amino Acid
Amino Acid Derivatives
Carboxylic Acid

Please read this instruction sheet completely before using this column

Column Description

Chemical composition: Amino acid chemically bonded to a 10µm silica-gel support.



Column size: 250mm L x 4.6mm i.d.

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.

CAUTION

Some of the most popular buffer solutions and / or organic solvent remaining in your HPLC system may destroy this column.

Be careful not to connect the column to your HPLC system before the system is completely flushed with the appropriate eluent.

Do not use racemate of strong acid or base.

Operating Procedure

Typical mobile phase: 0.25mM $CuSO_4$ aq (up to 0.5mM)

Suitable mobile phase modifiers include CH_3CN and CH_3OH from 0 to 80% (v/v).

Notes:
- ☞ Retention time is generally shorter with higher $CuSO_4$ concentration and/or higher column temperature.
- ☞ High content of organic solvent may cause precipitation of $CuSO_4$ and clogging of the tubing.

Flow Direction:	As indicated by arrow on column label.
Flow Rate:	Typical flow rate is 1.0 ml/min. Maintain flow rate that will result in column pressures of less than 1400 psi. (100 kg/cm^2). DO NOT EXCEED 3.0 ml/min.
Temperature:	0°C – 50°C. ☞ If your sample is a free amino acid, column temperature should be maintained more than 40°C.
Pressure:	1400 psi, MAXIMUM; less than 700 psi is recommended for maximum column life.

☞ Sample/eluent solution should be filtered through a 0.45 m membrane filter.

☞ The use of a guard column is recommended for maximum column life.

Maintenance

- The mobile phase should be displaced with Storage Solvent (0.25mM $CuSO_4$ aq.) when stored more than a week.
- When washing is required, use 10mM $CuSO_4$ aq. + 10% MeOH at 0.3ml/min for 3 hours.

Important Notice

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

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@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 Tel: +91 84 1866 0700 & 703

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www.chiraltech.com

Europe
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Parc d'Innovation
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Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL - USE OF GUARD CARTRIDGES FOR CHIRALPAK®, CHIRALCEL®, CROWNPAK®, and DAICEL DCPak® ANALYTICAL COLUMNS

The purpose of a guard cartridge is to protect the analytical column from materials that would adsorb on the stationary phase. Guard Cartridges for CHIRALPAK®, CHIRALCEL®, CROWNPAK®, and DAICEL DCPak® analytical columns have been designed to fully protect your analytical column.

The guard cartridge should contain the same packing material as the analytical column. Specific guard cartridges are available for all DAICEL columns.

The guard cartridge should be replaced regularly otherwise the column performance can be affected.
Loss in separations, an increase of peak broadening or tailing, a high pressure drop in your system, are all signals that indicate a guard cartridge may need to be replaced.

The device is comprised of two pieces: a guard cartridge and a holder.


		
Remove protective cover from cartridge, place the cartridge in the holder end fitting marked "IN", ensuring that the arrow marking the flow direction on the cartridge points away from the fitting.	Assemble the holder, tightening the end fittings snugly with appropriate wrenches to ensure a leak-free unit.	Connect the guard column unit to the analytical column with a short length of capillary tubing, taking care that the flow direction is correct.

Important Notice

- Ensure that guard cartridge contains the same chiral phase as the analytical column.
- Check that the flow direction as marked on the cartridge and holder is correct.
- Use a length of connection tubing as short as possible to connect the guard to the analytical column. Use a tubing of 0.25mm diameter or less.
- The cartridge lifetime is very dependent upon the operating conditions

⇨ If you have any questions about the use of these guard cartridges, 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

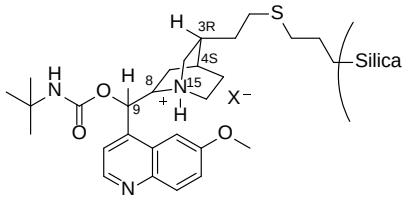
India
Daicel Chiral Technologies (India) Pvt Ltd
Survey No. 542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR CHIRALPAK® QN-AX and CHIRALPAK QD-AX

Please read this instruction sheet completely before using these columns

Column Description

Packing composition: O-9-(*tert*-butylcarbamoyl) quinine for CHIRALPAK® QN-AX
or
O-9-(*tert*-butylcarbamoyl) quinidine for CHIRALPAK® QD-AX
immobilized on **5µm silica-gel**.



CHIRALPAK® QN-AX: (8S,9R)
CHIRALPAK® QD-AX: (8R,9S)

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 with the column when purchased.

Operating Conditions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** Analytical Column	**150 x 21 mm i.d.** Semi-Prep Column
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**/ /**
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate ①	0.1-0.2 ml/min **Do not exceed 0.3 ml/min**	1.0 ml/min **Do not exceed 2 ml/min**	18.0 ml/min **Do not exceed 25 ml/min**
Pressure Limitation②	Should be maintained < 150 Bar (2100 psi)③ for maximum column life Adapt flow rates to column size. Do not exceed 180 bar (2500 bar)		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 180 Bar).

Examples	150 x 4.6 mm i.d.	150 x 21 mm i.d.
100% Methanol	0.5 to 2 ml/min	9 to 25 ml/min
Methanol/aqueous buffer~ 80:20	0.5 to 1.5 ml/min	9 to 18 ml/min
Alkane/organic modifier~ 90:10	0.5 to 2 ml/min	9 to 25 ml/min

② The back pressure value that should be taken into account is the one generated by the column itself. This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

③ Ideal value for maximum column life, but stable up to 180 Bar.

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ Samples should be filtered through a membrane filter of approximately 0.5 µm porosity.
- ❑ Mobile phases (in particular RP-mode) should be filtered through an appropriate filtration membrane.

Operating Procedure

CHIRALPAK® QN-AX and CHIRALPAK® QD-AX have been developed as enantioselective weak anion-exchange (AX) HPLC columns most useful for the separation of acidic chiral compounds, based on two complementary stereoisomeric quinine (QN) and quinidine (QD) derivatives. Owing to their pseudo-enantiomeric character, they usually reveal reversed elution order for opposite enantiomers.

They can be used in polar organic mode (non-aqueous, polar organic solvents containing organic acids and bases as buffer constituents), reversed-phase (RP) mode, or SFC (super-critical fluid) mode. CHIRALPAK® QN-AX and CHIRALPAK® QD-AX are designed specifically for enantioselective HPLC of chiral acids and possess exceptional enantiomer separation capabilities for acidic chiral compounds containing carboxylic, phosphonic, phosphinic, phosphoric, or sulfonic acid groups. In some cases, weakly acidic compounds such as phenols (e.g. coumarols) can also be separated.

They are compatible with all common HPLC solvents (e.g. methanol, acetonitrile, tetrahydrofuran, dioxane, and chloroform) as well as in a wide pH range spanning from pH 2 to 8. Typical buffers used in hydro-organic mode are acetate, formate, citrate and phosphate. They are capable for use in LC-MS detection of chiral acids with compatible mobile phases and buffers (e.g. ammonium acetate, ammonium formate).

In addition, the separation of chiral basic and neutral compounds may also be possible, but usually under normal phase (NP) conditions, or if preferred, in RP-mode with higher aqueous content. In NP-mode, CHIRALPAK® QN-AX and CHIRALPAK® QD-AX behave like a standard Pirkle-type chiral stationary phase.

Column Care / Maintenance

Before initial use, the column should be flushed with at least 20 column volumes (ca. 30 ml for a 150 x 4.6 mm i.d. column) of 1% (v/v) acetic acid in methanol. The column should then be equilibrated with 20 column volumes of the initial mobile phase.

☞ ***Column cleaning and regeneration procedures***

- ❑ After use of counter-ions with high affinity towards the selector such as *citrate and phosphate,* or when *multiply charged solute species* from the sample are trapped on the column and do not come off due to the use of a weak counter-ion in the eluent, it is recommended to rinse the column for 30 min with a solution of **methanol/triethylamine (TEA) (100:2, v/v)** before switching back to an eluent with a counter-ion having low affinity to the selector such as acetate. After the methanol/TEA rinsing, the column should be washed with methanol/water (50:50, v/v) or acetonitrile/water (50:50, v/v) before it is equilibrated with the new mobile phase or stored.

- ❑ If *highly hydrophobic compounds* are trapped on the column by virtue of strong hydrophobic interactions, they can be eluted with **acetonitrile/acetic acid (100:1, v/v)** or with **THF**, **dioxane** or **dimethylsulfoxide**. The column should then be washed with methanol/water (50:50, v/v) or acetonitrile/water (50:50, v/v) before it is equilibrated with the new mobile phase or stored.

Column Storage

- After use, the column should be washed for 5-10 min with a mixture of methanol/water (50:50, v/v) or pure methanol (or acetonitrile) for overnight storage (this is in particular recommended if phosphate or citrate is used as buffer).

- If a high content of salts have been used, rinse the column first with an organic/aqueous mixture, before switching to pure organic solvents in order to avoid salt precipitation.

- For long-term storage, the column should be flushed with 20 column volumes of pure methanol or acetonitrile, but it can also be stored in a methanol or acetonitrile mixture in water (50:50, v/v). It can be stored at room temperature.

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

Practical Method Development Scheme

The solute to be separated into individual enantiomers is:

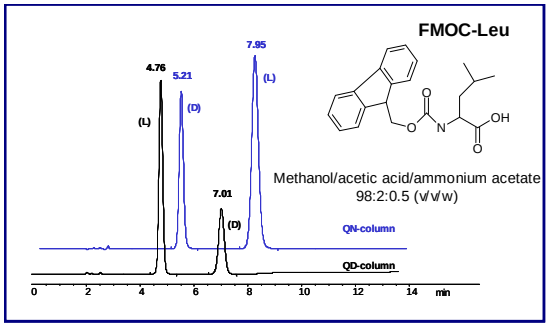
- a chiral **acidic** compound: Use polar organic mode or RP-mode (Charts 1 and 2).
- a chiral **sulfonic**, **phosphonic**, **phosphoric acid** and **di- or multicarboxylic acid**: Use RP-mode (retention will be too strong in the polar organic mode) (Chart 2).
- an **amphoteric** chiral compound: Use RP-mode, but study extended pH-range up to pH 8 and possibly lower flow rates e.g. 0.5 ml/min for analytical columns (Chart 2).
- a **neutral** or **basic** chiral compound: Use NP-mode or, if preferred, try RP-mode with higher water content.

Acidic Compounds in Polar Organic (PO) Mode *(Chart 1)*

1) Test a mobile phase of methanol/acetic acid/ammonium acetate (98:2:0.5, v/v/w). Start the screening at 1 ml/min (at 0.1 ml/min for microbore columns) and 25 °C.

2) A mixture of acetonitrile/methanol 50:50 (v/v) containing the same amount of organic acid and ammonium acetate can be tested. If with this mixture higher separation factors are obtained continue with it and optimize the percentage of methanol in acetonitrile. For *N-derivatized amino acids,* methanol proved in general to be the better choice, while for *arylcarboxylic acids* acetonitrile turned out to generate higher enantioselectivity values. For *hydroxyl carboxylic acids,* a mixture of acetonitrile/methanol may be the preferred solvent.

⇨ As soon as a separation is detected, the **elution order** can be checked. If necessary, switch from CHIRALPAK® QN-AX to QD-AX and vice versa to pursuit the method development, as they show inversion of elution order in most cases.



3) If a **baseline separation** is achieved but **retention times are too long**, select the mobile phase combination that gave suitable selectivity for further optimization and adjust retention factors by:

   a. *Increasing the concentration of the buffer in the eluent*: a change of the concentration of the competing acid (counter-ion) in the mobile phase at identical acid-basic ratio usually has a negligible or only minor effect on the enantioselectivity. It will, however, affect retention considerably by modulation of the actual ion-exchange capacity.
   Increasing counter-ion concentration will decrease retention. For example, a mixture of methanol/acetic acid/ammonium acetate (98:4:1, v/v/w) or simply methanol/acetic acid/triethylamine (TEA) 100:3:1 (v/v/v) (or methanol/acetic acid/concentrated aqueous ammonia (NH_3) 100:3:0.7 (v/v/v)) can be tested.
   b. *Changing the type of counter-ion (competing acid)*: the buffer type may have a strong influence on retention and can potential have a significant effect on enantioselectivity. If retention is too long, the acetate buffer may often be replaced conveniently by formate buffer, which has a higher elution strength (Try for example, methanol/formic acid/TEA 100:1:3 (v/v/v) or methanol/formic acid/NH_3 100:1:1.5 (v/v/v)).

c. *Modifying acid-basic ratio (apparent pH or pHa):* by variation of the acid-base ratio (e.g. the acetic acid to TEA or NH_3 ratio), the apparent pH can be altered. This may have a significant effect on retention and enantioselectivity. With increasing excess of acid, the retention will increase. The optimal concentration in terms of selectivity will depend on the solute structure. For example, compare first the best mobile phase at pH 5, 6 and 7 by adjusting with TEA or NH_3 (see Table for advised acid-basic ratios).

Acid-base ratios (examples)

	pHa < 6	pHa ≈ 6	pHa ≈ 6.5	pHa > 7
Acetic acid/TEA	2 : 0.2	3 : 1 2 : 0.6 1 : 0.3	2 : 1.6 1 : 0.8	2 : 3 1 : 1.5
Acetic acid/NH_3	2 : 0.2	3 : 0.7 2 : 0.5 1 : 0.2	2 : 1.2 1 : 0.5	2 : 2 1 : 1
Formic acid/TEA	0.5 : 0.5	1 : 3 0.5 : 1.5	1 : 3.5	1 : 4 0.5 : 1.8
Formic acid/NH_3	0.5 : 0.3	1 : 1.5 0.5 : 0.8	0.5 : 1	0.5 : 1.5

⇩ **Starting conditions**

d. *Evaluating flow rate:* with a baseline resolution, flow rate can be increased to 1.5 ml/min (or 0.3 ml/min for microbore columns), but if this is not enough, try to re-adjust retention times by further increasing the concentration of the counter-ion or changing the buffer type in the mobile phase.

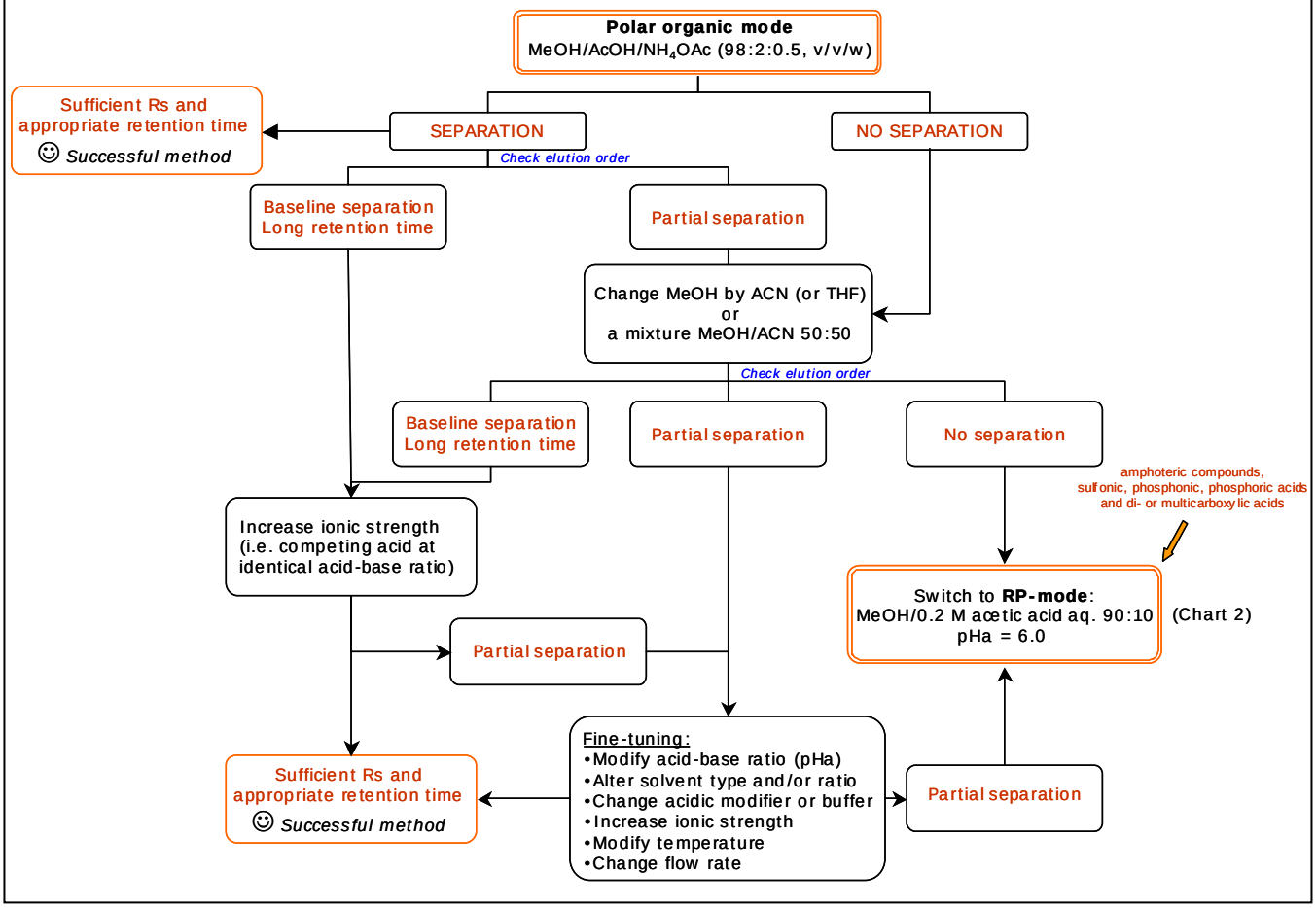
e. *Increasing temperature:* except for a few solutes, higher temperature decreases retention times, but also diminishes enantioselectivity. If retention is too high at 25°C, try 30°C (maximum 40°C).

f. *Changing the co-ion*: some minor differences may be observed between the adjustment of pH with TEA or NH_3.

4) If only a **partial separation** is achieved with the initial methanol mobile phase, check also the methanol/acetonitrile mixture (or even other solvents like THF). For near-baseline separations, type of buffer, co-ion, flow rate or temperature may be sufficient. If these are not successful, switch to RP-mode.

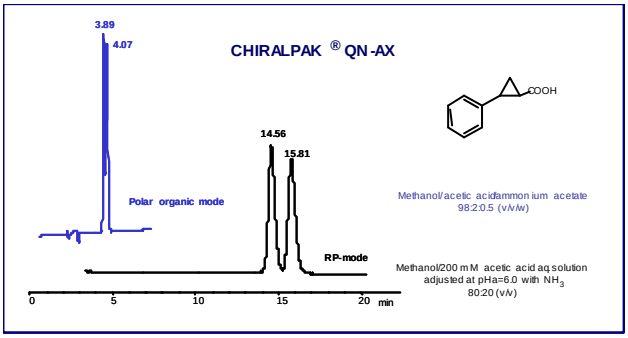
5) If **no separation** is achieved with the initial methanol mobile phase, try acetonitrile or methanol/acetonitrile mixture containing the above specified buffer or switch directly to RP-mode.

Chart 1. Acidic compounds in polar mode.



Factors affecting elution times (examples)

To *increase* elution times	To *decrease* elution times
⇩ Counter-ion concentration	⇧ Counter-ion concentration
Acid-base ratio: ⇩ base (PO)	Acid-base ratio: ⇧ base (PO)
⇩ pHa from 6 to 5 (RP) (even to 2, depending on pKa of compound)	⇧ pHa from 6 to 8 (RP)
⇩ Organic content (RP)	⇧ Organic content (RP)
⇩ Flow rate	⇧ Flow rate
Change acid type retention: acetic acid > succinic acid ≥ formic acid > glycolic acid > malonic acid (PO)	
Change buffer type retention: acetate > formate > phosphate > citrate (RP)	



General conditions: CHIRALPAK® QN-AX and QD-AX, 150 x 4.6 mm, Flow rate: 1 ml/min, 25°C

Acidic Compounds in Reversed-Phase (RP) Mode *(Chart 2)*

1) Test a mobile phase of methanol/0.2 M acetic acid aq. solution (90:10, v/v) (pHa of the mixture adjusted to 6 with concentrated aq. ammonia). Start the screening at 1 ml/min (0.1 ml/min for microbore columns) and 25 °C. If the screening was previously carried out in the polar mode and an acetonitrile mixture proved to be superior, use this organic modifier for further experiments and optimization.

   ⇨ As soon as a separation is detected, the **elution order** can be checked. If necessary, switch from CHIRALPAK® QN-AX to QD-AX or vice versa to pursue the method development, as they show inversion of elution order in most cases.

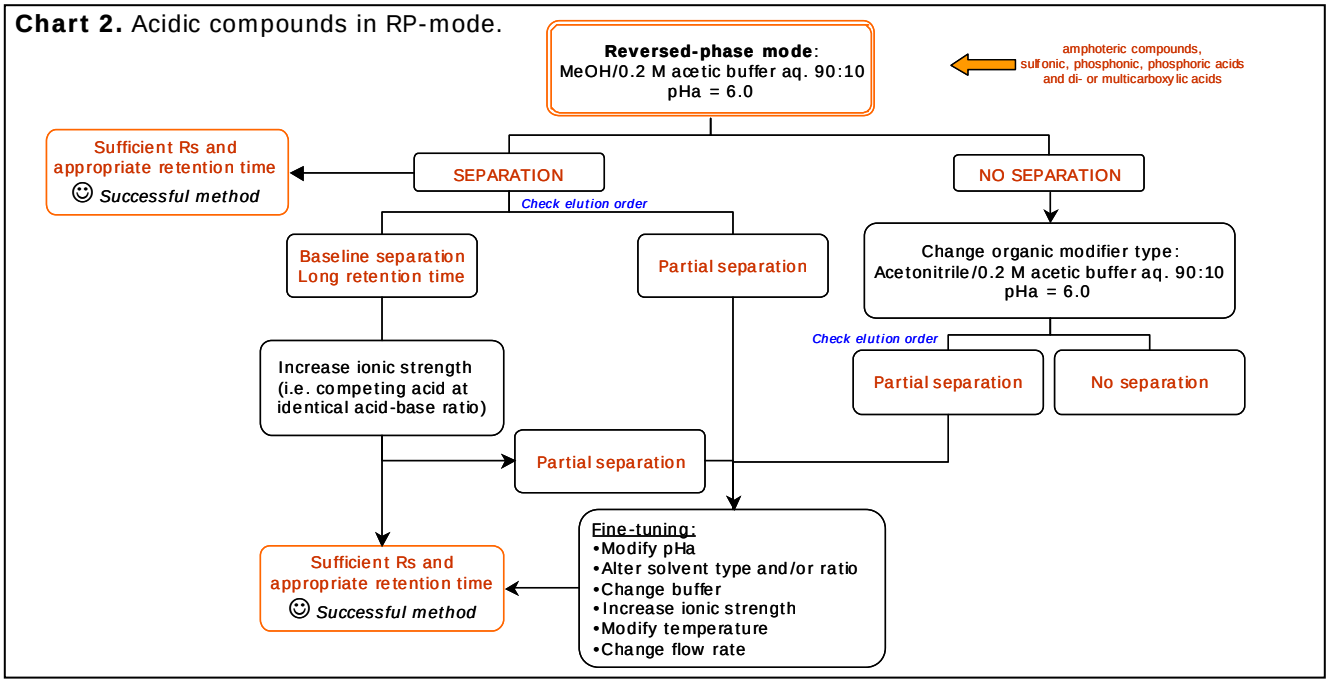
2) If a **baseline separation** is achieved but **retention times are too long**, select the mobile phase combination that gave suitable selectivity for further optimization and adjust retention factors by:

   a. *Increasing the concentration of the buffer in the eluent:* this will decrease retention. For example, a mixture of methanol/0.5 M acetic acid aq. solution (90:10, v/v) (pHa of the mixture adjusted to 6 with concentrated aq. ammonia) can be tested. For multiply charged analytes the buffer concentration should be at least 0.5 M or 1 M ammonium acetate buffer, in order to be able to elute the analytes within a reasonable time. For phosphonic and phosphoric acids, 20-50 mM phosphate buffer (pHa 6) may perform better in terms of peak shape and elution time. A higher volume percentage of aqueous component is recommended to avoid solubility problems of phosphate buffer in the hydroorganic mixture.
   b. *Changing the type of counter-ion (competing acid):* if the pH optimum is lower than pH 4, acetate could be replaced by formate. A change to phosphate or citrate, which have considerably higher elution strength, has in the majority of cases a negative effect on enantioselectivity. These buffers should solely be used if reasonable elution times cannot be achieved even with highly concentrated acetate or formate buffers. This may be the case e.g. for phosphonic acids and di- or tricarboxylic acids. If citrate or any other buffer with high affinity towards the chiral selector was used, it is recommended to regenerate the column after use by rinsing for 20 min with a solution of methanol/TEA (100:2, v/v) and then washing with methanol/water (50:50, v/v) for 30 min before switching to another mobile phase.
   c. *Increasing the organic content*: study the effect of organic modifier content (90-95%). Consider also in such a case the polar organic mode for evaluation.
   d. *Modifying pHa:* The optimal pH for carboxylic acids is usually found between pH 5 and 6, for stronger acids it is often shifted towards a lower pH, and for amphoteric compounds towards a higher pH (allowed range between 2-8). An increase of pHa between 6 and 7 will reduce retention times with small impact on selectivity; selectivity in the range of 7-8 (α) may decrease dramatically (for example, compare first the best mobile phase at pHa 5, 6 and 7 by adjusting with TEA or NH_3). For strongly acidic compounds such as phosphonic acids or sulfonic acids, as well as amphoteric compounds, the pH optimum may be shifted and the useful pH range may be extended between 2 and 8.
   e. *Evaluating flow rate:* with a baseline resolution, flow rate can be increased to accelerate the separation (depending on the viscosity of the mobile phase) until the 180 bar pressure limit is reached. If this is not enough, try to re-adjust retention times by further increasing the concentration of the counter-ion or changing the buffer type in the mobile phase. A reduction of the flow rate will increase the plate number.
   f. *Increasing temperature:* except for a few solutes, higher temperature decreases retention times, but also diminishes enantioselectivity. If retention is too high at 25°C, try 30°C (maximum 40°C).

g. *changing the co-ion*: some minor differences may be observed between the adjustment of pH with TEA or NH_3.

3) If **no separation** or only a **partial separation** is achieved with the initial methanol or acetonitrile/aqueous (90:10) mobile phase, several situations are possible:
   a. *Very short retention times*: if k < 1 reduce the concentration of counter-ion in the mobile phase by a factor of 2 to 5 and proceed with optimization of the other parameters (see above). Alternatively, reduce the organic content (e.g. to 80 and 70%).
   b. *Reasonable retention times (1≤k<10)*: study the effect of decreasing the organic content (e.g. to 80 and 70%). If a baseline separation is attained, use the guidelines to adjust retention time described in section 2. In all cases, if resolution is nearly baseline, decrease the flow rate from 1 ml/min to 0.5 ml/min and decrease the temperature. If this leads to baseline resolution, but run times are too long, try to re-adjust retention times by further increasing the concentration of the counter-ion in the mobile phase.

Chart 2. Acidic compounds in RP-mode.



Neutral and Basic Compounds in Normal-Phase (NP) Mode

Before the column can be used in this mode, it has to be washed with methanol/water exhaustively, then methanol/TEA (100:2, v/v) (30 min), and finally with 2-propanol, which is miscible with NP eluents.

1) Start the optimization with a mobile phase consisting of hexane/2-propanol (90:10, v/v). If elution is too fast, adjust the polar modifier to 1% (e.g. for arylcarbinols). If it was too slow, increase to 30% 2-propanol.
2) If no resolution of enantiomers is achieved, replace the polar modifier e.g. instead of 2-propanol use ethanol, dichloromethane, dioxane and so forth. Also the sole use of the pure polar solvent component may afford resolution.
3) If tailing is observed e.g. for basic compounds, add 0.1% of TEA or another basic additive.

Neutral and Basic Compounds in Reversed-Phase (RP) Mode

If for practical reasons hydro-organic conditions are preferred over NP-mode, reversed-phase mode can be attempted to achieve resolution of enantiomers of neutral or basic analytes. Successful examples have been reported previously.

1) Start with a linear gradient of acetonitrile from 5% to 100% in water with 30 min gradient time, to determine the suitable organic modifier content for an isocratic separation.
2) Run an isocratic separation with a mixture of acetonitrile and water having a composition that the capacity factor is between 5 and 10.
3) If no resolution or the beginnings of a resolution is observed, repeat the procedure with methanol.
4) If retention factors have been too low or if a beginning separation is identified, it is advisable to reduce the linear flow rate of the eluent.

5) For ionizable compounds such as chiral bases, a buffer must be added and the pH of the eluent needs to be optimized. At higher pH, retention will be stronger.
6) If no separation or beginning of a separation is obtained, it is suggested to continue with NP-mode.

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 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

Handbook of chiral MPLC column

DAICEL CORPORATION

CPI Company

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Chiral MPLC column

CHIRALFLASH IA　　CHIRALFLASH IE
CHIRALFLASH IC　　CHIRALFLASH IF
CHIRALFLASH ID

✓CHIRALFLASH are chiral columns for medium pressure liquid chromatography(MPLC), packed in a solvent resistant semi-transparent fluoroplastic column with immobilized-type polysaccharide-derived chiral stationary phase (CSP).

✓Immobilized CSP is achiral selector fixed in the silica gel substrate and may use all miscible chromatographic solvent combinations.
(not only n-Hexane, alcohol but also ethyl acetate, tetrahydrofuran, chloroform etc.)

✓The joint size is 1/4-28UNF. Hence they are compatible with MPLC equipment.

【Features】

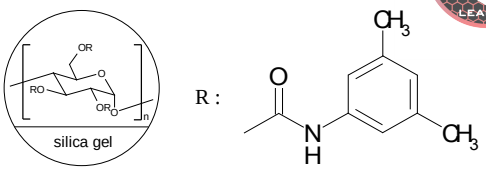
- **Load amount ca. 50~100 mg per injection.**
- **Wide variety of organic solvents can be used as a mobile phases.**
- **It is possible to be reverse cleaning and reuse.**
- **Column packing is visible. (Translucent column tube)**

1. Specifications

Structural formula of chiral selector

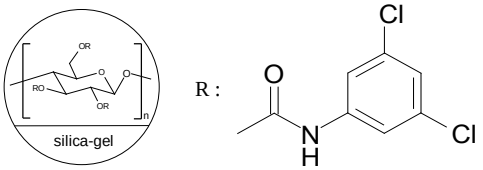
CHIRALFLASH IA

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3,5-dimethylphenylcarbamate)
Particle size : 20μm



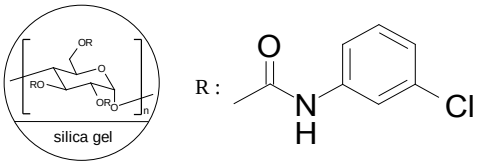
CHIRALFLASH IC

Column fitting : 1/4-28 UNF
Packing composition : Cellulose tris(3,5-dichlorophenylcarbamate)
Particle size : 20μm



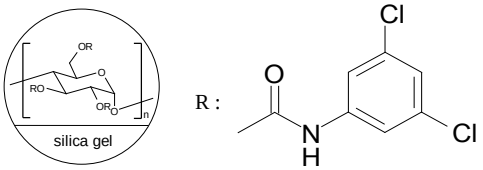
CHIRALFLASH ID

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3-chlorophenylcarbamate)
Particle size : 20μm



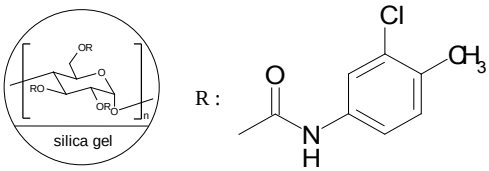
CHIRALFLASH IE

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3,5-dichlorophenylcarbamate)
Particle size : 20μm



CHIRALFLASH IF

Column fitting : 1/4-28 UNF
Packing composition : Amylose tris(3-chloro-4-methylphenylcarbamate)
Particle size : 20μm



Column size	Packing size Tube size	30 mm I.D. x 100 mmL 38 mm O.D. x 150 mmL
Column material		Fluoroplastic
CSP weight	g	ca. 40
Bed volume	mL	50
Pressure limitation	MPa	Should be maintained < 1.5 MPa (218 psi) for maximum column life
Temperature	℃	0 ~ 40
Typical flow rate	mL/min.	12
Sample loading	mg/inj.	50 ~ 100

<Important reminder>

- Do not give strong shocks to the column, or disassemble it. It may result in damage to the column and result in poor separation performance.
- When using a column, it is highly recommended to discard at least the first 300mL ~ 600mL of eluent at the beginning of a preparative work.
- When back flushing it is highly recommended to keep the flow rate below the value recommended in the operating instructions.

2. Usable solvents

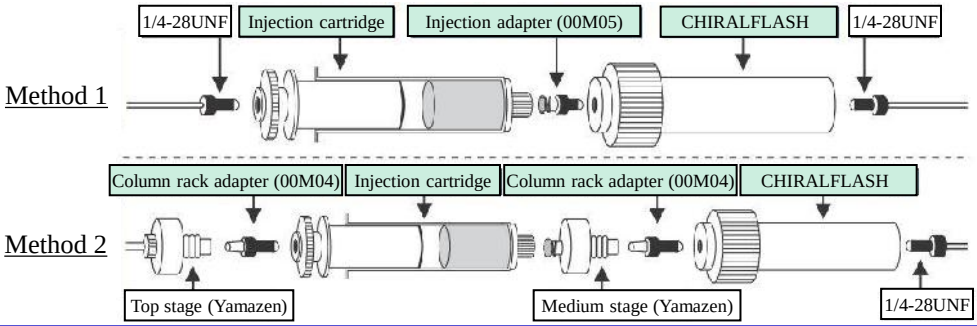
CHIRALFLASH can use a wide variety of organic solvents for a mobile phase or a sample solution.

- Alkane (n-Hexane, n-Heptane)
- Alcohol (Methanol, Ethanol, 2-Propanol)
- t-Butyl methyl ether (MTBE)
- Dichloromethane
- Chloroform
- Ethyl acetate
- Tetrahydrofuran
- Acetonitrile
- Acetone
- Toluene
- 1,4-dioxane
- Other solvent can be used for silica gel based column as a mobile phase

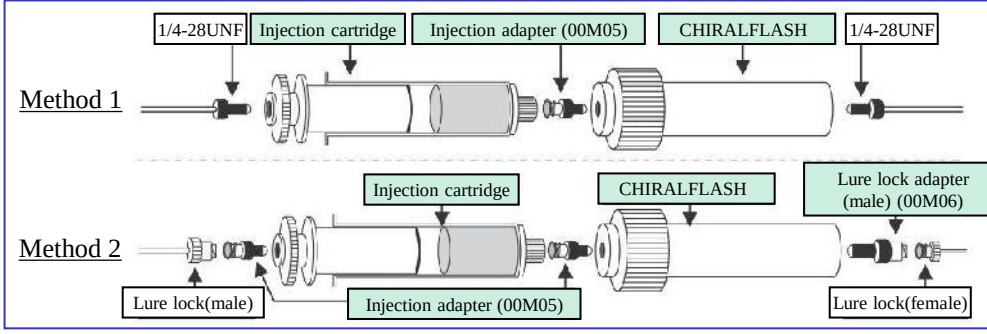
3. Installation to MPLC

CHIRALFLASH are compatible with different MPLC instruments using the following joints.
It is recommended to use the injection cartridge (details on page.4.)

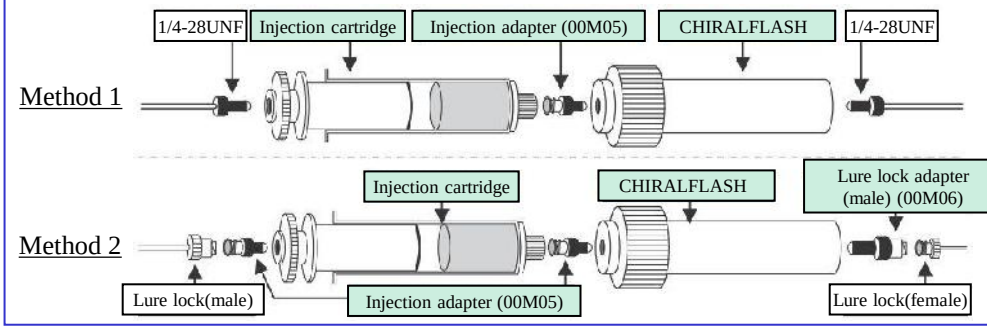
Yamazen



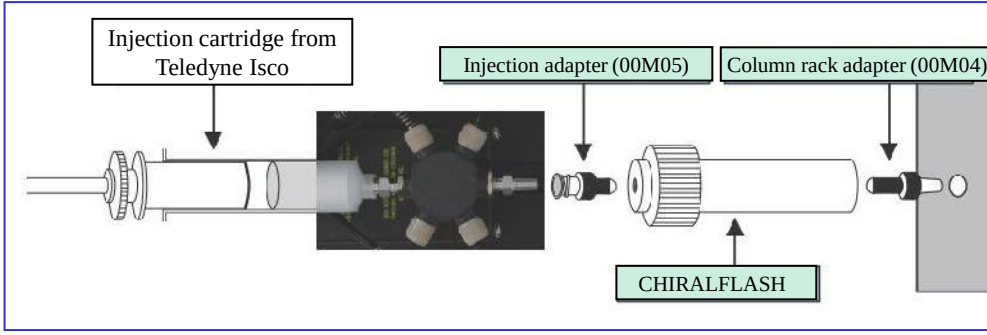
Biotage



SHOKO scientific



Teledyne Isco

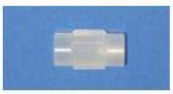


Lineup of Adapters

Column rack adapter (00M04)

Injection adapter (00M05)

Lure lock adapter (male) (00M06)



Syringe union (00M07)

LL adapter [INTRM Connector - female] (00M08)

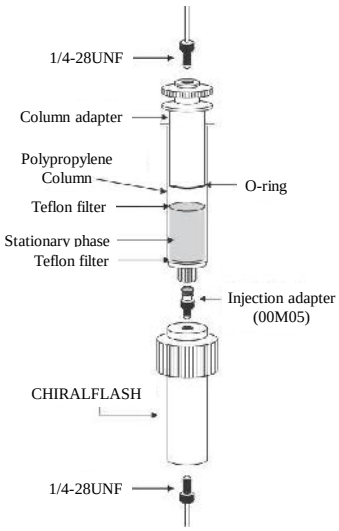
LL adapter [INTRM Connector - male] (00M09)

4. Accessories (Injection cartridge, Fitting)

Compatible accessories for CHIRALFLASH are provided such as injection cartridges and fittings.

4-1. Injection cartridge

We provide Injection cartridges for use as the guard column to prevent contamination of CHIRALFLASH, and as the injection column for loading samples.
These injections cartridges are filled with modified silica gel (C1) and are compatible with acidic, basic and neutral compounds. 3 Sizes are available. (S, M, and L)



←00M03 Injection cartridge L

←00M02 Injection cartridge M

←00M01 Injection cartridge S

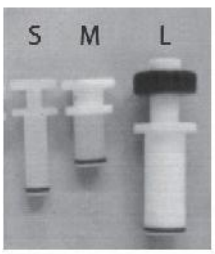
Size		Size S	Size M	Size L
Column Size (mm)	Column Length	φ 15× 44	φ 20× 75	φ 26× 80
	Tube Length	φ 15× 85	φ 20× 95	φ 26× 135
Packing Weight (g)		4.5	13	25
Max. Injection Volume (mL)		4.5	13	25

4-2. Fitting

To use the Injection cartridge a column adapter is required, dependent upon the size of the cartridge used (S, M, L)

00M13 Column holder S
00M14 Column holder M
00M15 Column holder L

00M10 Column adapter S
00M11 Column adapter M
00M12 Column adapter L



5-１．Using HPLC analytical column for method development of CHIRALFLASH

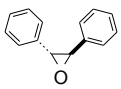
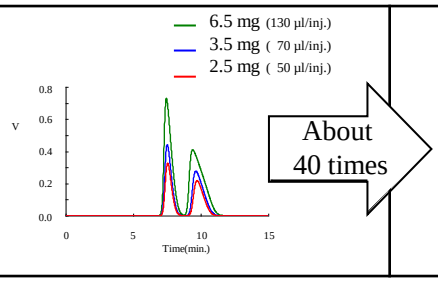
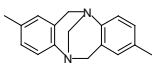
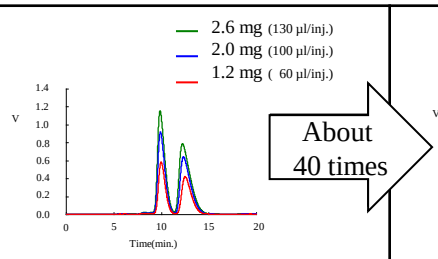
1) The optimization of separation condition and 2) the estimation of the sample loading quantity on CHIRALFLASH are possible by using HPLC analytical columns for method development of CHIRALFLASH.
There are 5 products lineup of CHIRALPAK® (IA, IC, ID, IE and IF : the particle size is 20μm) corresponding to each types of CHIRALFLASH.

The conditions established on CHIRALPAK® IA, IC, ID, IE and IF (20 μm) can be scaled up to CHIRALFLASH IA, IC, ID, IE and IF directly on the basis of column dimensions, as the same stationary phases are used in both columns. For example, approximately 40-fold sample quantity compared with 4.6 mm I.D. x 100 mm L CHIRALPAK® IA, IC, ID, IE and IF (20 μm) will be applicable on 30 mm I.D. x 100 mm L CHIRALFLASH IA, IC, ID, IE and IF.

$$\text{The quantity of sample load of } CHIRALFLASH: Y(mg) = X(mg) \times \frac{\left(\frac{30}{2}\right)^2 \times \pi}{\left(\frac{4.6}{2}\right)^2 \times \pi} \fallingdotseq X(mg) \times 40 times$$

X : The quantity of sample load of CHIRALPAK® (20 μm)

In the optimization study, all of the conditions, such as the column temperature, the sample concentration, the eluent composition, the linear flow velocity, the additives, the peak detection conditions, and so on, should preferably be representative of a preparative column.

Column size (I.D. x Length)	**< CHIRALPAK® IC(20 μm) > 4.6 x 100 mm**	**< CHIRALFLASH IC > 30 x 100 mm**
Flow rate	0.28 mL/min.	12.0 mL/min.
trans-Stilbene Oxide (t-SO)  α=1.7	Sample conc. : 50 g/L in Eluent 	
Tröger's-Base (TB)  α=1.4	Sample conc. : 20 g/L in Eluent 	

Mobile phase : n-Hexane / 2-Propanol = 90 / 10 (v/v)
Temp. : R.T.
Detect : UV 254 nm

5-2. Recommended mobile phases and additives

We recommend that the conditions shown in Table 1 are used as the basis for initial method development for CHIRALFLASH IA. After the initial evaluation the most promising methods can be optimized using the suggested ranges below. MTBE and chlorinated solvents may also be used in their pure form as the mobile phase. Moreover, in the case of solvents with strong elution intensity, such as THF and ethyl acetate, it is advised to mix them with a hydrocarbon solvent (e.g. hexane or heptane) to modulate retention and selectivity.

<The procedure of mobile phase selection>

1. For acidic or basic samples, it is necessary to incorporate an additive into the mobile phase in order to optimize the chiral separation. First choice is Acetic acid for acidic samples and Diethylamine for basic samples.

2. Please try "Typical starting condition" indicated about Table 1. sequentially from the left sides on HPLC analytical column for method development.

3. If you get separation (baseline or partial), Please optimize mobile phase composition in reference to "Advised optimization range" indicated about Table 1.

Table 1. Recommended organic miscible solvents

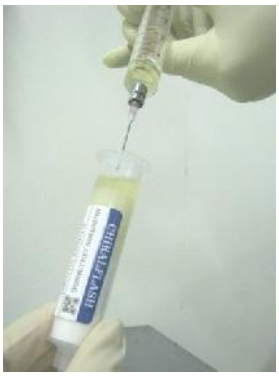
	Alkane❶/ Alcohol❷	Alkane❶/ EtOAc	Alkane❶/ $CHCl_3$	Alkane❶/ THF	MTBE / EtOH
Typical starting conditions	90:10	90:10	70:30	90:10	100:0
Advised optimization range	95:5 ~ 0:100❸	95:5 ~ 0:100	95:5 ~ 0:100	95:5 ~ 0:100	100:0 ~ 40:60

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ Methanol, ethanol, and 2-propanol are raised as typical alcohol.
Moreover, as alcohol other than the above, 1-propanol, 1-butanol, 2-butanol, etc. can be used.
Depending on a sample, separation may change greatly with kinds of alcohol.
❸As for the mixed solvent of alcohol, viscosity may become high with composition.
Please adjust the flow velocity if needed not to exceed the maximum working pressure range of a column.
Usually, retention time becomes short so that composition of alcohol becomes high, In not less than 50% of domain of alcohol, a prominent effect may not no longer be seen.
Although operating composition of methanol does not have restriction, it recommends being used mixing with the above ethanol or 2-propanol in equivalent amount with composition of methanol for compatibility with alkane.
When ethanol or 2-propanol is not mixed, if not less than 5% of methanol is used, a possibility of dissociating two layers will increase.
Moreover, even when you use it at 5% or less, if adjusted by the methanol independent, it is recommended to agitate a mobile phase continuously.

6. Methods of sample injection

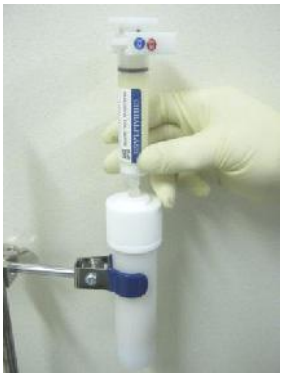
There're 2 methods of sample injection.

6-1. Using injection cartridge



1. The sample solution is added to the injection cartridge.

2. The sample solution is filtered by the injection cartridge.



3. Fix the column adapter and the column holder to the injection column, and connect with CHIRALFLASH.

6-2. Direct injection

1. Injection adapter is connected to the inlet of CHIRALFLASH.

2. The sample solution is syringed.

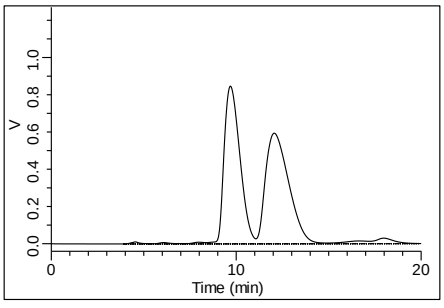
3. Attach the membrane filter (0.5μm) to the tip of a syringe.

4. The sample is poured into CHIRALFLASH.

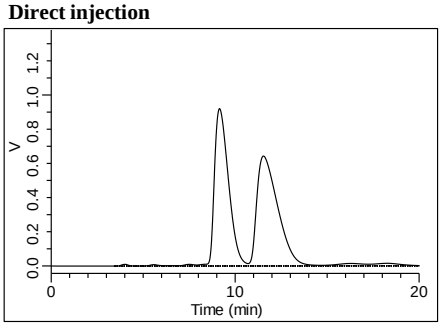
- In case of addition in dilute concentration of the sample in large quantities, the separation might get worse because of diffusion within injection cartridge. In such a case, please try the method by "Direct injection".
- For maximum column life, the sample solution should be filtered through a membrane filter of approximately 0.5μm porosity to ensure that there is no precipitate before using.

Column：CHIRALFLASH IC
Sample：Tröger's-Base, 20g/L in Eluent×4.5mL/inj.
Mobile Phase：90/10 = n-Hexane/2-Propanol
Flow-rate：12.0mL/min
Detection：UV 254nm

Using injection cartridge (Size-S)



Direct injection



7-1. Column cleaning

When acidic or basic additives are used, remove them by flushing the column with the mobile phase without the additive.
Moreover, Column cleaning (flash with ethanol at 6 mL/min for more than 30 minutes) is recommended after use of column.

7-2. Regeneration procedures

The separation characteristic of the column for polysaccharide optical resolution is dependent on the high order structure of polysaccharide. This high order structure may change depending on a mobile phase or temperature conditions, following extensive use of the column in multiple solvents for a long time, 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…). Moreover, it is able to use the following procedure also as washing conditions for a column. (However, when the solubility of the sample to the following solvent or impurities is low, please carry out the following procedure after through a mobile phase with those high solubility solvents among several hours ~ about ten hours.

< Regeneration procedures of CHIRALFLASH IA, ID, IE, IF >

1. Flush with ethanol at 6 mL/min for 120 min.
2. Flush with N,N-dimethylformamide (DMF) at 6 mL/min for 120 min.
3. Flush with ethanol at 6 mL/min for 60 min.
4. IA: Equilibrate with n-hexane/ethanol = 90/10 (v/v) at 12 mL/min for 60 min, prior to retesting the column.
   ID, IE, IF: Equilibrate with n-hexane/ethanol = 90/10 (v/v) at 12 mL/min for 60 min, prior to retesting the column.

< Regeneration procedure of CHIRALFLASH IC >

1. Flush with ethanol at 6 mL/min for 120 min.
2. Flush with ethyl acetate at 6 mL/min for 120 min.
3. Store the column at room temperature for 2 days or longer.
4. Flush with ethanol at 6 mL/min for 60 min.
5. Equilibrate with n-hexane/IPA = 90/10 (v/v) at 12 mL/min for 60 min, prior to retesting the column.

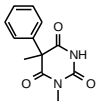
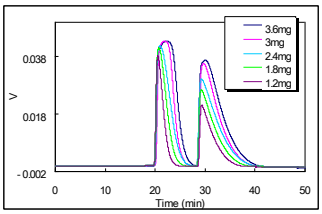
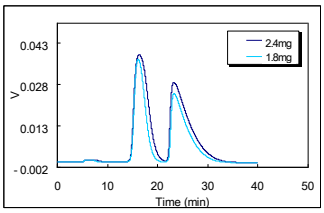
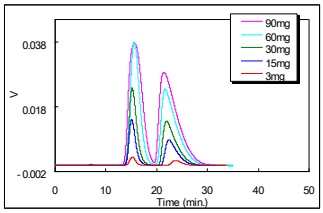
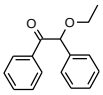
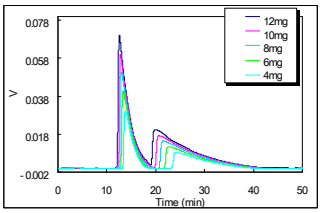
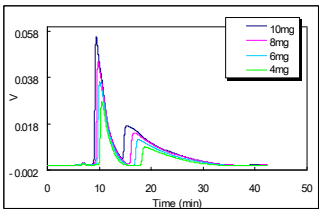
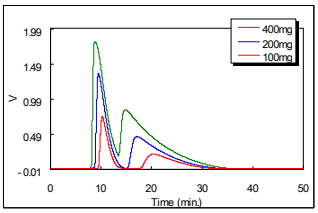
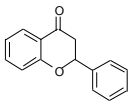
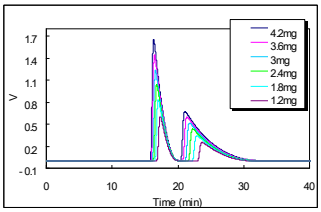
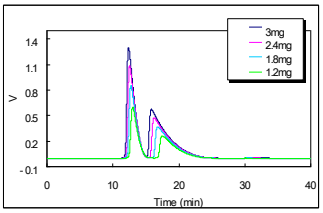
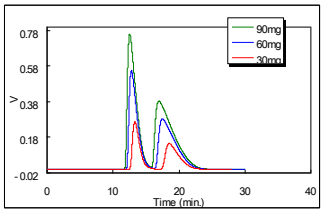
If this is not successful, then try this procedure again. However, a number of columns is affected by equipment and the column wearing method in use. Moreover, it may be subject to the influence of a temporal change of a filling state by repetition use of a column.

7-3. Column storage

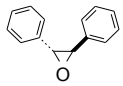
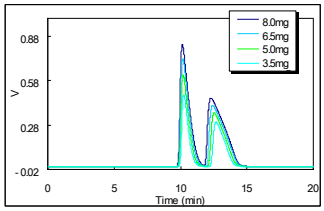
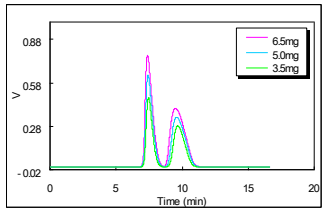
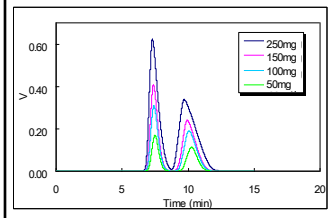
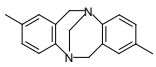
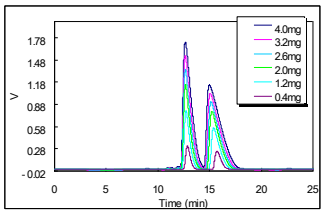
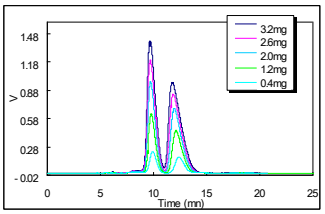
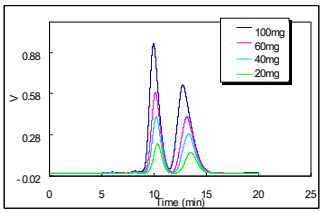
- For column storage, remove the acidic or basic additives by flushing the column with the same mobile phase without the additive. Columns can be stored end capped with additive-free mobile phases.
- Ethanol is recommended for longer column storage (longer than one week).

8. Application data

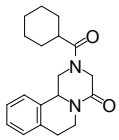
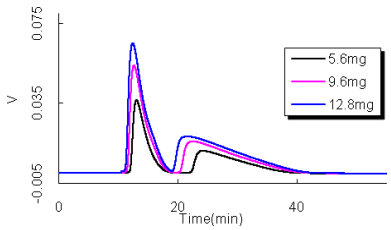
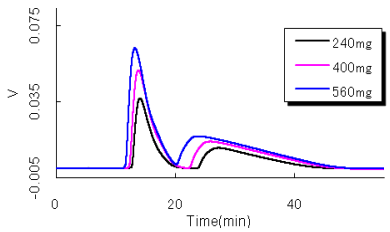
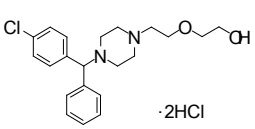
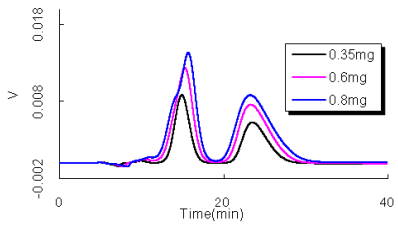
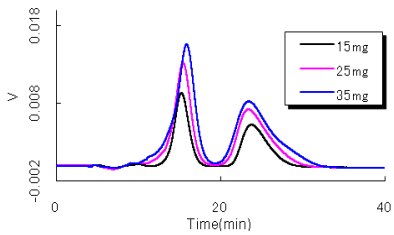
8-1. CHIRALFLASH IA

	HPLC Analytical column (CHIRALPAK® IA)		CHIRALFLASH IA
	dp=5μm 4.6mm ID×150mmL	dp=20μm 4.6mm ID×100mmL	dp=20μm 30mm ID×100mmL
1) Hexobarbital  n-Hexane/2-Propanol = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 3g/L, ~1200μL/inj. (= **~3.6mg**) Analytical Data : k'1=2.1, k'2=3.9, α=1.9	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 3g/L, ~800μL/inj. (= **~2.4mg**) Analytical Data : k'1=2.5, k'2=5.1, α=2.0	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 3g/L, ~30mL/inj. (= **~90mg**) Analytical Data : k'1=2.5, k'2=5.0, α=2.0
2) Benzoin ethyl ether  n-Hexane/EtOAc = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 100g/L, ~120μL/inj. (= **~12mg**) Analytical Data : k'1=1.4, k'2=4.4, α=3.1	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 100g/L, ~100μL/inj. (= **~10mg**) Analytical Data : k'1=1.5, k'2=4.9, α=3.3	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 100g/L, ~4mL/inj. (= **~400mg**) Analytical Data : k'1=1.6, k'2=4.9, α=3.3
3) Flavanone  n-Hexane/EtOH = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 30g/L, ~140μL/inj. (= **~4.2mg**) Analytical Data : k'1=1.8, k'2=3.3, α=1.8	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 30g/L, ~100μL/inj. (= **~3.0mg**) Analytical Data : k'1=2.1, k'2=3.8, α=1.8	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 30g/L, ~100μL/inj. (= **~90mg**) Analytical Data : k'1=2.1, k'2=3.9, α=1.9

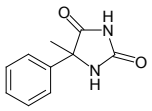
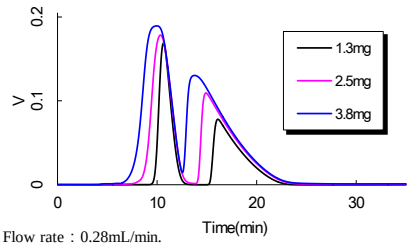
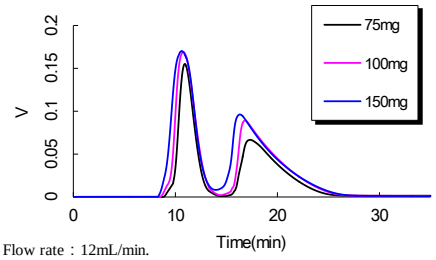
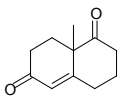
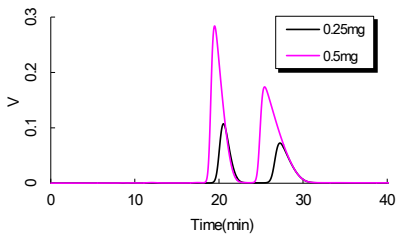
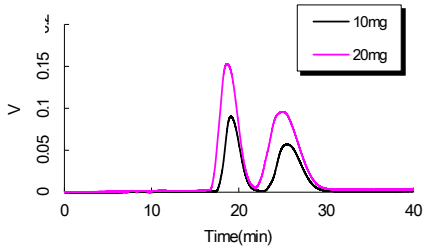
8-2. CHIRALFLASH IC

	HPLC Analytical column (CHIRALPAK® IC)		CHIRALFLASH IC
	dp=5μm 4.6mm ID×150mmL	dp=20μm 4.6mm ID×100mmL	dp=20μm 30mm ID×100mmL
1) trans-Stilbene oxide  n-Hexane/2-Propanol = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 50g/L, ～160μL/inj. (= **～8.0mg**) Analytical Data : k'1=0.5, k'2=0.9, α=1.8	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 50g/L, ～160μL/inj. (= **～6.5mg**) Analytical Data : k'1=0.7, k'2=1.3, α=1.7	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 50g/L, ～5mL/inj. (= **～250mg**) Analytical Data : k'1=0.8, k'2=1.4, α=1.8
2) Tröger's-Base  n-Hexane/2-Propanol = 90/10 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 20g/L, ～200μL/inj. (= **～4mg**) Analytical Data : k'1=0.9, k'2=1.3, α=1.5	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 20g/L, ～160μL/inj. (= **～3.2mg**) Analytical Data : k'1=1.4, k'2=1.9, α=1.4	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 20g/L, ～5mL/inj. (= **～100mg**) Analytical Data : k'1=1.4, k'2=1.9, α=1.4

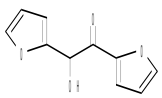
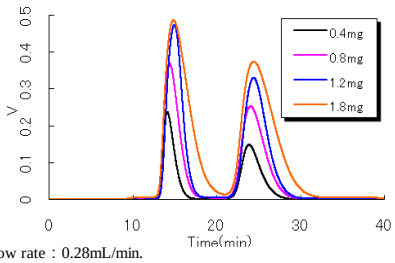
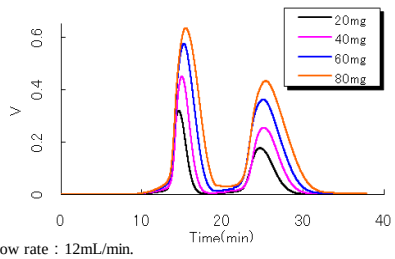
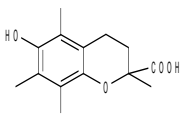
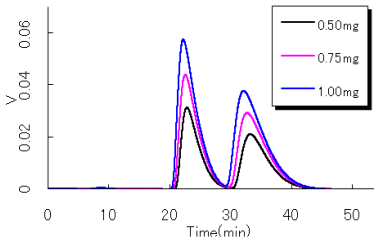
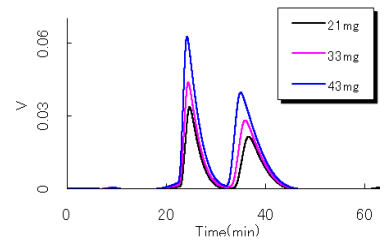
8-3. CHIRALFLASH ID

	HPLC Analytical column (CHIRALPAK® ID) dp=20μm 4.6mm ID×100mmL	CHIRALFLASH ID dp=20μm 30mm ID×100mmL
1) Praziquantel  EtOH = 100 vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 80g/L, ～160μL/inj. (= **～12.8mg**) Analytical Data : k'1=2.2, k'2=5.5, α=2.5	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 80g/L, ～7mL/inj. (= **～560mg**) Analytical Data : k'1=2.0, k'2=5.2, α=2.6
2) Hydroxyzine dihydrochloride  n-Hexane/2-Propanol/DEA = 80/20/0.1 vol/vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV254nm Sample : 5g/L, ～160μL/inj. (= **～0.8mg**) Analytical Data : k'1=2.3, k'2=4.1, α=1.8	 Flow rate : 12mL/min. Temp : R.T. Detection : UV254nm Sample : 5g/L, ～7mL/inj. (= **～35mg**) Analytical Data : k'1=2.4, k'2=4.6, α=1.9

8-4. CHIRALFLASH IE

	HPLC Analytical column (CHIRALPAK® IE) dp=20μm 4.6mm ID×100mmL	CHIRALFLASH IE dp=20μm 30mm ID×100mmL
1) 5-methyl-5-phenylhydantoin  n-Hexane/EtOH = 70/30 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV230nm Sample : 25g/L, ~150μL/inj. (= **~3.8mg**) Analytical Data : k'1=1.6, k'2=3.8, α=2.4	 Flow rate : 12mL/min. Temp : R.T. Detection : UV230nm Sample : 25g/L, ~6mL/inj. (= **~150mg**) Analytical Data : k'1=1.6, k'2=3.8, α=2.4
2) Wieland–Miescher ketone  n-Hexane/EtOH = 60/40 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV230nm Sample : 100g/L, ~5μL/inj. (= **~0.5mg**) Analytical Data : k'1=3.7, k'2=5.2, α=1.4	 Flow rate : 12mL/min. Temp : R.T. Detection : UV230nm Sample : 100g/L, ~0.2mL/inj. (= **~20mg**) Analytical Data : k'1=3.7, k'2=5.2, α=1.4

8-5. CHIRALFLASH IF

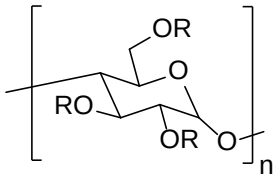
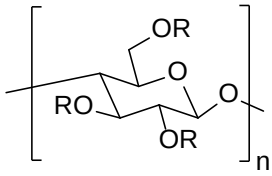
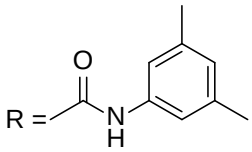
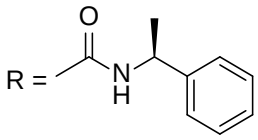
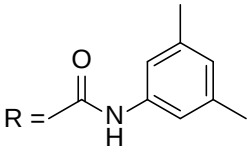
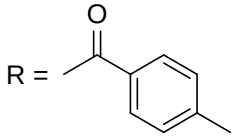
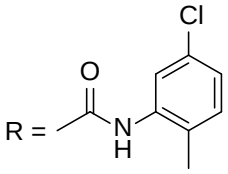
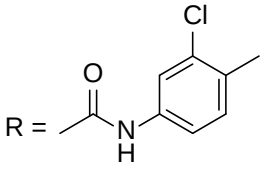
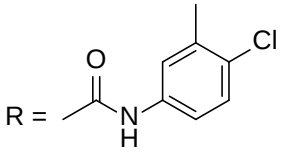
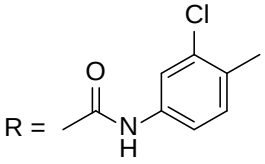
	HPLC Analytical column (CHIRALPAK® IF) dp=20μm 4.6mm ID×100mmL	CHIRALFLASH IF dp=20μm 30mm ID×100mmL
1) Furoin  n-Hexane/EtOH = 70/30 vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV273nm Sample : 2g/L, ~900μL/inj. (= **~1.8mg**) Analytical Data : k'1=2.4, k'2=5.3, α=2.2	 Flow rate : 12mL/min. Temp : R.T. Detection : UV273nm Sample : 2g/L, ~40mL/inj. (= **~80mg**) Analytical Data : k'1=2.4, k'2=5.3, α=2.2
2) 6-Hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid  n-Hexane/Chloroform/AcOH = 40/60/0.1 vol/vol/vol	 Flow rate : 0.28mL/min. Temp : R.T. Detection : UV290nm Sample : 2.5g/L, ~400μL/inj. (= **~1.0mg**) Analytical Data : k'1=4.7, k'2=8.0, α=1.7	 Flow rate : 12mL/min. Temp : R.T. Detection : UV290nm Sample : 2.5g/L, ~17mL/inj. (= **~43mg**) Analytical Data : k'1=4.7, k'2=8.0, α=1.7

INSTRUCTION MANUAL FOR Analytical Columns of CHIRALPAK® AD-3, AS-3, AY-3, AZ-3 CHIRALCEL® OD-3, OJ-3, OX-3, and OZ-3

<Normal Phase>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
 **Coated** on **3 µm silica gel**		 **Coated** on **3 µm silica gel**	
CHIRALPAK® AD-3	**CHIRALPAK® AS-3**	**CHIRALCEL® OD-3**	**CHIRALCEL® OJ-3**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY-3	**CHIRALPAK® AZ-3**	**CHIRALCEL® OX-3**	**CHIRALCEL® OZ-3**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Hexane/Isopropanol = 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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate ①	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

① Flow rates in the range of 0.5-1.0 ml/min are recommended for difficult resolution of enantiomers (search of the best resolution). Flow rates higher than 1.0 ml/min, especially 3.0-5.0 ml/min, are advised for fast analyses.

② 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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-3.

	Alkane❶/ 2-Propanol❷	Alkane❶/ Ethanol❷	Alkane❶/ MeOH❸	MeOH❹ + ❺	CH_3CN❺ + ❻ No alkane at all	Alkane❶/ MtBE
CHIRALPAK® AD-3 **CHIRALPAK® AS-3** **CHIRALPAK® AY-3** **CHIRALPAK® AZ-3** **CHIRALCEL® OD-3** **CHIRALCEL® OJ-3** **CHIRALCEL® OX-3** **CHIRALCEL® OZ-3**	100/0 to 0/100	100/0 to 0/100	100/0 to 85/15	0 to 100% EtOH or CH_3CN in MeOH	0 to 100% EtOH or MeOH in CH_3CN	100/0 to 85/15

❶ Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences have been observed when switching between these different alkanes.

❷ The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, 2-BuOH, etc. is possible, but effectiveness is not predictable.

❸ Due to the limited miscibility of MeOH in Alkane, it is necessary to add an appropriate volume of EtOH, together with MeOH, to ensure a homogenous solvent mixtures. A maximum of 5% MeOH, in n-Hexane only, may be used without adding EtOH.

❹ Ideal starting conditions: MeOH/EtOH 50:50 (v/v) when alcohol mixtures are required.

❺ The use of polar organic solvents like 100% Methanol and/or 100% Acetonitrile is possible with CHIRALPAK® AD-3/AS-3/AY-3/AZ-3 and CHIRALCEL® OD-3/OJ-3/OX-3/OZ-3 columns. Once the column is transferred to a polar organic mode, **it is recommended the column be dedicated for this specific application.**

To safely transfer the column from Hexane to Methanol or Acetonitrile, **it is strongly recommended to use 100% EtOH as a transition mobile phase** at 0.5 ml/min.

After this transition, the column needs to be thoroughly washed with Acetonitrile (~ 10 column volumes) prior to the first use in this solvent as a mobile phase.

B – Additives

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

❼ For primary amines mainly
❽ For primary amino alcohols mainly

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA Butyl amine❼ Ethanol amine❽	TFA CH_3COOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- Samples should preferably be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
- For alkane containing mobile phases, flush the column with Storage Solvent (Hexane / Isopropanol 90:10) when stored for more than one week.
- For columns dedicated to polar organic solvents, flush the column with the polar organic mobile phase,

without the additive.

- When washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours. The column can also be heated to 40°C for a more efficient cleaning.

Important Notice

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

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 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

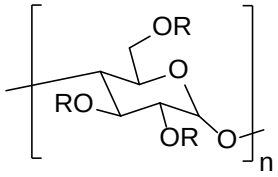
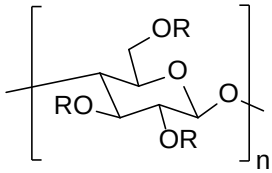
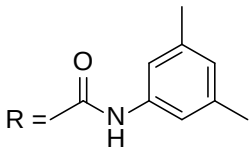
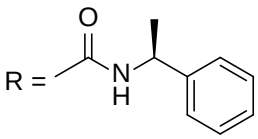
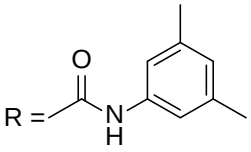
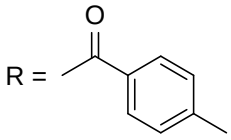
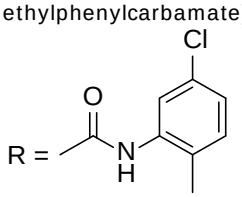
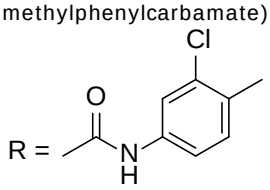
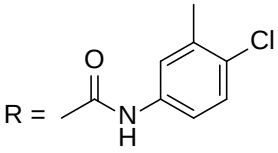
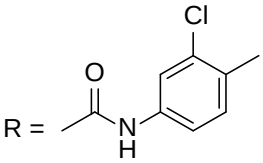
India
Daicel Chiral Technologies (India) Pvt. Ltd.
Survey No.542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR Analytical Columns of CHIRALPAK® AD-3R, AS-3R, AY-3R, AZ-3R CHIRALCEL® OD-3R, OJ-3R, OX-3R, and OZ-3R

<Reversed-Phase>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
 **Coated** on **3 µm silica gel**		 **Coated** on **3 µm silica gel**	
CHIRALPAK® AD-3R	**CHIRALPAK® AS-3R**	**CHIRALCEL® OD-3R**	**CHIRALCEL® OJ-3R**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY-3R	**CHIRALPAK® AZ-3R**	**CHIRALCEL® OX-3R**	**CHIRALCEL® OZ-3R**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Water/Acetonitrile = 60/40 (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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases / For Both UV and Mass Detections

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-3R.

	CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALPAK® AZ-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R		CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	HCOOH aq. pH 2.0	Water	20mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary mobile phases (Section B)*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

	CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALPAK® AZ-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R		CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO4 aq. pH 2.0 OR 100mM KPF_6 (or NaPF6) aq. pH 2.0 adjusted with H_3PO4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF6 (or $NaPF_6$) aq.
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 2: The concentration of all the buffering salt should be <u>less than 500mM</u>.*

❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.

❷
- ❑ It is recommended to use ACN to start the investigation
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50% ACN ≈ 65-70% EtOH ≈ 75-80% MeOH.
- ❑ The use of other organic solvents has not been investigated and could be harmful to the columns.
- ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.

❸
- ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
- ❑ Lowering the column temperature may increase the retention time and the selectivity.

- ❑ Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- ❑ To maximize column life, the use of a guard cartridge is essential when basic conditions are employed.
- ❑ The use of strongly basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
- ❑ When these columns are used at pH > 7, **the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to consequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

❻ Do not use a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

C – Buffer Preparation – Examples

➢ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50mM potassium dihydrogenphosphate
3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 9 Ammonium bicarbonate solution:*

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml with HPLC grade water

Solution B: Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.

** DEA tends to give better peak shape than other bases.*

Adjust the pH of solution A to a value of 9.0 using solution B.

➢ *Preparation of pH 8 Phosphate buffer:*

Solution A: 20mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate
1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water.

Adjust the pH of solution A to a value of 8.0 using solution B.

➢ *Preparation of pH 9 Borate buffer:*

Solution A: 20mM sodium tetraborate decahydrate
3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid
0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water

Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- 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.
- Before disconnecting the column from the HPLC, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, e.g. Water/ACN 60:40 (v/v).
- If the column is contaminated with non-eluted components, wash it with a mobile phase that does not contain any salts / buffers, then with 100% ACN for 2 hours at 0.5ml/min. Alternatively, if the non-eluting components are more soluble in methanol, this solvent may be used for the washing step.
- All salts must be flushed out from the HPLC system and column before changing to 100% ACN or 100% methanol.
- Use Water/ACN 60:40 (v/v) to store the column, at room temperature

Important Notice

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

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 or call 800-6-CHIRAL
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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

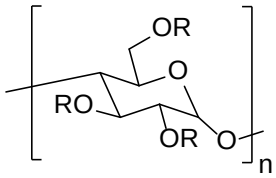
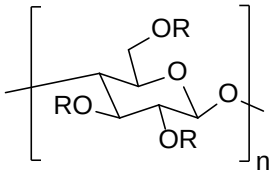
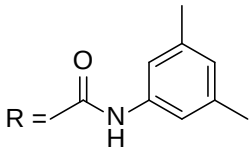
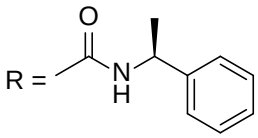
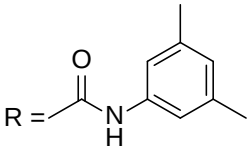
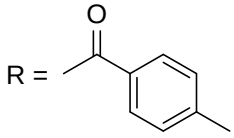
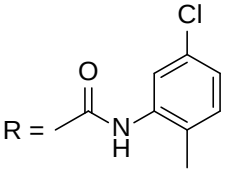
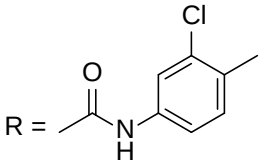
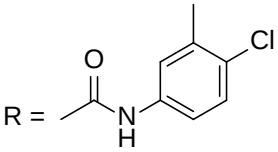
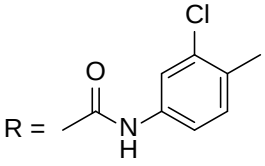
India
Daicel Chiral Technologies (India) Pvt. Ltd.
Survey No.542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR Analytical Columns of CHIRALPAK® AD-3, AS-3, AY-3, AZ-3 CHIRALCEL® OD-3, OJ-3, OX-3, and OZ-3

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		**CELLULOSE-BASED**	
 **Coated** on **3 µm silica gel**		 **Coated** on **3 µm silica gel**	
CHIRALPAK® AD-3	**CHIRALPAK® AS-3**	**CHIRALCEL® OD-3**	**CHIRALCEL® OJ-3**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY-3	**CHIRALPAK® AZ-3**	**CHIRALCEL® OX-3**	**CHIRALCEL® OZ-3**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Because different columns are shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in SFC to avoid any damage (see column transfer conditions between LC and SFC on page 4).

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 the column. Many of the solvents commonly used as SFC modifiers including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

① The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-3.

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN ❶
Typical Starting Conditions	80:20	80:20	80:20	70:30 ❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60 ❶

❶ For strongly retained compounds, an alcohol can be added into CH_3CN to enhance the eluting strength.

Note: The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, 2-BuOH, etc. is possible, but effectiveness is not predictable.

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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ❶	Acidic Samples require Acidic additives ❶
Isopropylamine (IPAm) Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid 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*

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_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).

Column Care / Maintenance

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

☞ ***Column transfer between modes:***

From LC to SFC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with 100% CO_2 or CO_2+modifier at 0.25 ml/min(*) for 45 min

From SFC to LC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with the mobile phase at 0.25 ml/min(*) for 45 min

(*) *This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

Column Storage

- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% EtOH 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.

Important Notice

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

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 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 CS80140
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

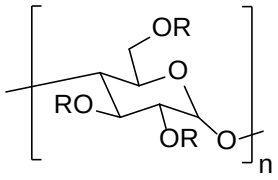
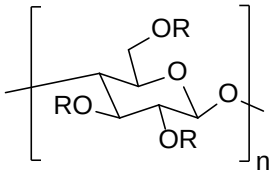
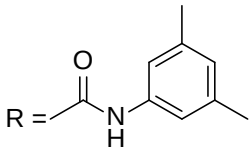
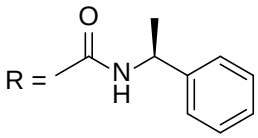
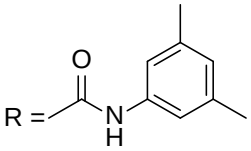
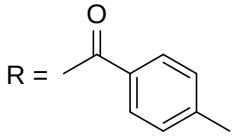
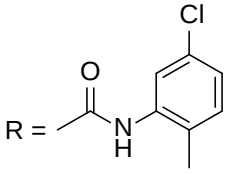
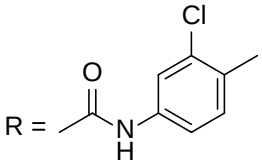
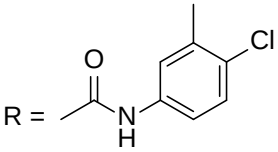
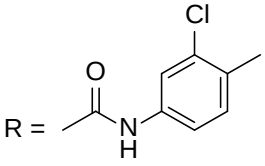
India
Daicel Chiral Technologies (India) Pvt. Ltd. Survey No.542/2, IKP Knowledge Park, Turkapally, Shamirpet Mandal, Medchal-Malkajgiri District, Hyderabad-500101, Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR SFC OPTIMIZED (3.0 mm i.d.)
CHIRALPAK® AD-3, AS-3, AY-3, AZ-3
CHIRALCEL® OD-3, OJ-3, OX-3, and OZ-3

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
 **Coated** on **3 µm silica gel**		 **Coated** on **3 µm silica gel**	
CHIRALPAK® AD-3	**CHIRALPAK® AS-3**	**CHIRALCEL® OD-3**	**CHIRALCEL® OJ-3**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY-3	**CHIRALPAK® AZ-3**	**CHIRALCEL® OX-3**	**CHIRALCEL® OZ-3**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Methanol = 100%**

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.

THIS INSTRUCTION MANUAL 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 the column. Many of the solvents commonly used as SFC modifiers including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	50 x 3.0 mm i.d. **100 x 3.0 mm i.d.** **150 x 3.0 mm i.d.** Analytical Columns
Flow Rate Direction	As indicated on the column label
Typical Flow Rate **in SFC**	~ 0.5 - 4.0 ml/min
Pressure Limitation①	< 300 bar (4350 psi) for maximum column life ***Typical CO_2 backpressure (BPr) 110-150 bar***
Temperature	0 to 40°C
Column Fitting	Please contact Technical Support for details

① The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-3.

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN ❶
Typical Starting Conditions	80:20	80:20	80:20	70:30 ❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60 ❶

❶ For strongly retained compounds, an alcohol can be added into CH3CN to enhance the eluting strength.

Note: The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, 2-BuOH, etc. is possible, but effectiveness is not predictable.

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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ❶	Acidic Samples require Acidic additives ❶
Isopropylamine (IPAm) Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid 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*

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_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).

Column Care / Maintenance

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

☞ ***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

(*) *Recommended flow rate for analytical columns (3.0mm i.d.).*

Column Storage

- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% EtOH 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.

Important Notice

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

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 or call 800-6-CHIRAL
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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
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

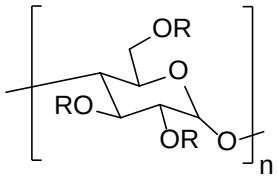
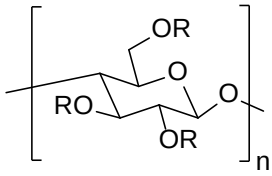
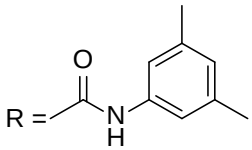
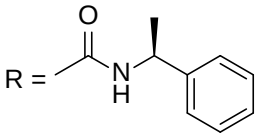
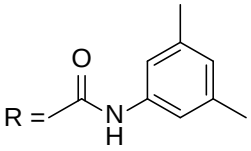
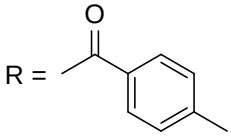
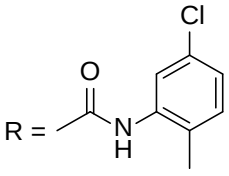
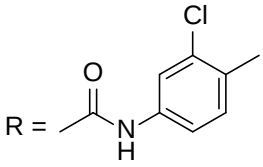
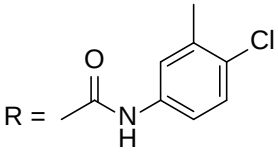
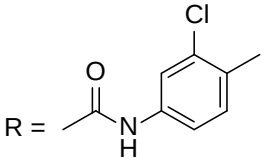
India
Daicel Chiral Technologies (India) Pvt. Ltd.
Survey No.542/2, IKP Knowledge Park, Turkapally
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91-84-1866-0700 & 703
Fax: +91-84-1866-0755
chiral@chiral.daicel.com
www.chiraltech.com

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

<Normal Phase>

Please read this instruction sheet completely before using these columns

Column Description

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

Shipping Solvent: **Hexane/Isopropanol = 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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 20 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**20 x 10 mm i.d.** **50 x 21 mm i.d.** **50 x 30 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate②	0.1-0.5 ml/min	0.5-2.5 ml/min	5 ml/min (10 mm i.d.) 20 ml/min (20 mm i.d.) 42 ml/min (30 mm i.d.) 118 ml/min (50 mm i.d.)
Pressure Limitation③	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

③ 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.

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-H.

	Alkane❶/ 2-Propanol❷	Alkane❶/ Ethanol❷	Alkane❶/ MeOH❸	MeOH❹+❺	ACN❺+❻ No alkane at all	Alkane❶/ MtBE
CHIRALPAK® AD-H **CHIRALPAK® AS-H** **CHIRALPAK® AY-H** **CHIRALPAK® AZ-H** **CHIRALCEL® OD-H** **CHIRALCEL® OJ-H** **CHIRALCEL® OX-H** **CHIRALCEL® OZ-H**	100/0 to 0/100	100/0 to 0/100	100/0 to 85/15	0 to 100% EtOH or 2-PrOH in MeOH	0 to 100% 2-PrOH in ACN	100/0 to 85/15
				0 to 15% ACN in MeOH ❻	0 to 15% MeOH or EtOH in ACN ❻	

❶ Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences have been observed when switching between these different alkanes.

❷ The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, etc. is possible, but effectiveness is not predictable.

❸ Due to the limited miscibility of MeOH in Alkane, it is necessary to add an appropriate volume of EtOH, together with MeOH, to ensure a homogenous solvent mixtures. A maximum of 5% MeOH, in n-Hexane only, may be used without adding EtOH.

❹ Ideal starting conditions: MeOH/EtOH 50:50 (v/v) when alcohol mixtures are required.

❺ The use of polar organic solvents like 100% Methanol and/or 100% ACN is possible with CHIRALPAK® AD-H/AS-H/AY-H/AZ-H and CHIRALCEL® OD-H/OJ-H/OX-H/OZ-H columns. Once the column is transferred to a polar organic mode, **it is recommended the column be dedicated for this specific application**.

❻ The column is particularly efficient in this range when such solvent mixtures are employed. Other alcohols such as 1-Propanol, 1-BuOH, etc. can also be used, but should not exceed 15% by volume.

To safely transfer the column from Hexane to Methanol or ACN, **it is strongly recommended to use 100% EtOH or 2-PrOH as a transition mobile phase** at 0.5 ml/min.

After this transition, the column needs to be thoroughly washed with ACN (~ 10 column volumes) prior to the first use in this solvent as a mobile phase.

B – Additives

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

❼ For primary amines mainly
❽ For primary amino alcohols mainly

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA Butyl amine❼ Ethanol amine❽	TFA CH_3COOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

Column Care / Maintenance

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ Samples should preferably be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
- ❑ For alkane containing mobile phases, flush the column with Storage Solvent (Hexane / Isopropanol 90:10) when stored for more than one week.
- ❑ For columns dedicated to polar organic solvents, flush the column with the polar organic mobile phase, without the additive.
- ❑ When washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours. The column can also be heated to 40°C for a more efficient cleaning.

Important Notice

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

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 or call 800-6-CHIRAL
In the EU: cte@cte.daicel.com or call +33 (0) 3 88 79 52 00
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Locations:

North/Latin America
Chiral Technologies. Inc.
1475 Dunwoody Dr., Ste 310
West Chester, PA 19380
800 6 CHIRAL
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Europe
Chiral Technologies Europe SAS
Parc d'Innovation
160, Bd Gonthier d'Andernach CS80140
67404 Illkirch Cedex, France
Tel: +33 (0) 3 88 79 52 00
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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

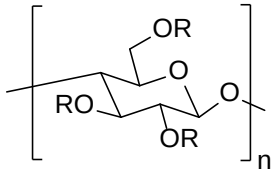
INSTRUCTION MANUAL FOR CHIRALCEL® OB-H

<Normal Phase>

Please read this instruction sheet completely before using these columns

Column Description

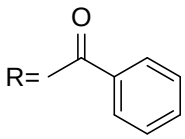
CELLULOSE-BASED



Coated on **5 µm silica gel**

CHIRALCEL® OB-H

Cellulose tribenzoate



THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 4.6. mm i.d. **250 x 4.6. mm i.d.** Analytical Columns
Guard	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label
Typical Flow Rate①	1.0 ml/min
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.
Temperature	0 to 40°C
Column Fitting	Please contact Technical Support for details

① The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

② 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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

	Alkane❶/ 2-Propanol❷	Alkane❶/ Ethanol❷
CHIRALCEL® OB-H	100/0 to 0/100	100/0 to 0/100

❶ Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences have been observed when switching between these different alkanes.

❷ The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, etc. is possible, but effectiveness is not predictable.

B – Additives

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

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA n-Butylamine❼ Ethanolamine❽	TFA CH_3COOH HCOOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

❼ For primary amines mainly
❽ For primary amino alcohols mainly

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- Samples should preferably be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
- For CHIRALCEL® OB-H, the column should be flushed with Hexane/2-Propanol (90:10 v/v) when stored for more than one week.
- For CHIRALCEL® OB-H, when washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours.

Important Notice

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

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 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 CS80140
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

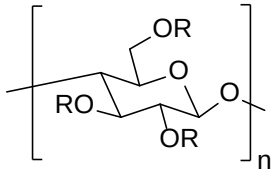
INSTRUCTION MANUAL FOR CHIRALCEL® OC-H

<Normal Phase>

Please read this instruction sheet completely before using these columns

Column Description

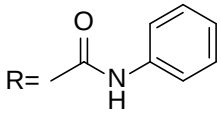
CELLULOSE-BASED



Coated on **5 µm silica gel**

CHIRALCEL® OC-H

Cellulose triphenylcarbamate



THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	250 x 4.6. mm i.d. Analytical Columns
Guard	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label
Typical Flow Rate①	1.0 ml/min
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.
Temperature	0 to 40°C
Column Fitting	Please contact Technical Support for details

① The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

② 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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

	Alkane❶/ 2-Propanol❷	Alkane❶/ Ethanol❷	Methanol/Ethanol	Methanol/2-Propanol
CHIRALCEL® OC-H	100/0 to 0/100	100/0 to 0/100	100/0 to 0/100	100/0 to 0/100

❶ Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences have been observed when switching between these different alkanes.

❷ The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, etc. is possible, but effectiveness is not predictable.

B – Additives

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

❼ For primary amines mainly
❽ For primary amino alcohols mainly

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA n-Butylamine❼ Ethanolamine❽	TFA CH_3COOH HCOOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- Samples should preferably be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
- For CHIRALCEL® OC-H, the column should be flushed with Hexane/2-Propanol (90:10 v/v) when stored for more than one week.
- For CHIRALCEL® OC-H, when washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours.

Important Notice

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

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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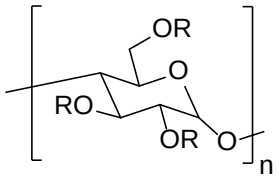
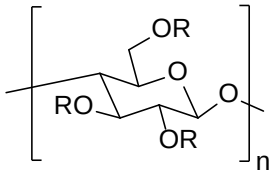
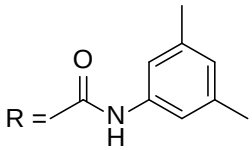
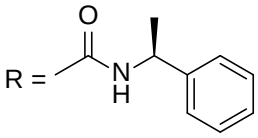
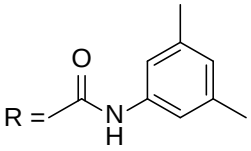
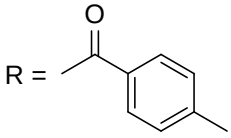
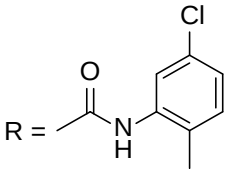
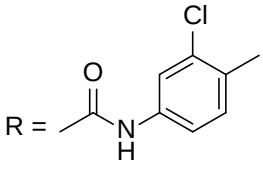
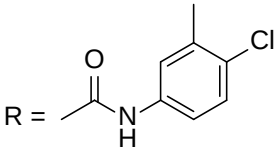
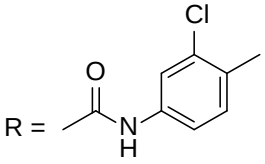
www.chiraltech.com

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

<Reversed-Phase>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
 **Coated** on **5 µm silica gel**		 **Coated** on **5 µm silica gel**	
CHIRALPAK® AD-RH	**CHIRALPAK® AS-RH**	**CHIRALCEL® OD-RH**	**CHIRALCEL® OJ-RH**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY-RH	**CHIRALPAK® AZ-RH**	**CHIRALCEL® OX-RH**	**CHIRALCEL® OZ-RH**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Water/ACN = 60/40 (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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** Analytical Columns	**250 x 21 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**/ /**
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate②	0.1-0.5 ml/min	0.5-2.5 ml/min	21 ml/min (21 mm i.d.)
Pressure Limitation③	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 500 ml (for 250 x 21 mm i.d) of eluent at the beginning of each preparative work.

② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

③ 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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-RH.

	CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH CHIRALPAK® AZ-RH CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH		CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	HCOOH aq. pH 2.0	Water	20mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary mobile phases (Section B)*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

	CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH CHIRALPAK® AZ-RH CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH		CHIRALPAK® AD-RH CHIRALPAK® AS-RH CHIRALPAK® AY-RH CHIRALCEL® OD-RH CHIRALCEL® OJ-RH CHIRALCEL® OX-RH CHIRALCEL® OZ-RH
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO4 aq. pH 2.0 OR 100mM KPF_6 (or NaPF6) aq. pH 2.0 adjusted with H_3PO4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF6 (or $NaPF_6$) aq.
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 2: The concentration of all the buffering salt should be <u>less than 500mM</u>.*

❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.

❷
- ❑ It is recommended to use ACN to start the investigation
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50% ACN ≈ 65-70% EtOH ≈ 75-80% MeOH.
- ❑ The use of other organic solvents has not been investigated and could be harmful to the columns.
- ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.

❸
- ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
- ❑ Lowering the column temperature may increase the retention time and the selectivity.

- Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- To maximize column life, the use of a guard cartridge is essential when basic conditions are employed.
- The use of strongly basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
- When these columns are used at pH > 7, **the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to consequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

❻ Do not use a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

C – Buffer Preparation – Examples

➢ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50mM potassium dihydrogenphosphate
3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 9 Ammonium bicarbonate solution:*

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml with HPLC grade water

Solution B: Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
** DEA tends to give better peak shape than other bases.*

Adjust the pH of solution A to a value of 9.0 using solution B.

➢ *Preparation of pH 8 Phosphate buffer:*

Solution A: 20mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate
1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water.

Adjust the pH of solution A to a value of 8.0 using solution B.

➢ *Preparation of pH 9 Borate buffer:*

Solution A: 20mM sodium tetraborate decahydrate
3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid
0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water

Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- 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.
- Before disconnecting the column from the HPLC, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, e.g. Water/ACN 60:40 (v/v).
- If the column is contaminated with non-eluted components, wash it with a mobile phase that does not contain any salts / buffers, then with 100% ACN for 2 hours at 0.5ml/min. Alternatively, if the non-eluting components are more soluble in methanol, this solvent may be used for the washing step.
- All salts must be flushed out from the HPLC system and column before changing to 100% ACN or 100% methanol.
- Use Water/ACN 60:40 (v/v) to store the column, at room temperature

Important Notice

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

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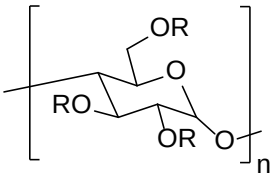
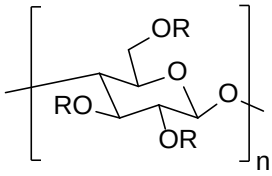
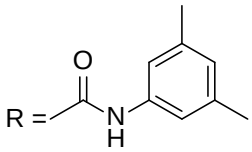
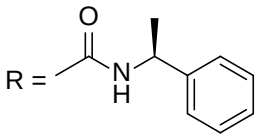
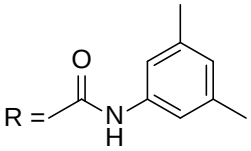
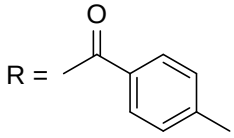
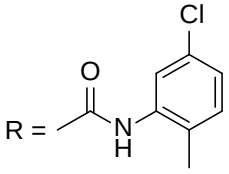
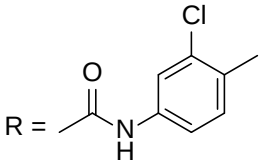
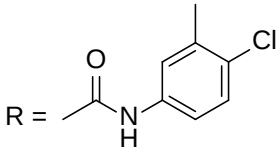
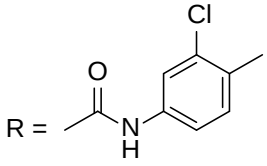
INSTRUCTION MANUAL FOR
CHIRALPAK® AD-H, AS-H, AY-H, AZ-H
CHIRALCEL® OD-H, OJ-H, OX-H, and OZ-H

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns

Column Description

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

Shipping Solvent: **Hexane/2-PrOH = 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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Because different columns are shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in SFC to avoid any damage (see column transfer conditions between LC and SFC on page 4).

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 the column. Many of the solvents commonly used as SFC modifers including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 21 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**50 x 10 mm i.d.** **50 x 21 mm i.d.** **50 x 30 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate **in SFC**	0.5-1.0 ml/min	1.0-5.0 ml/min	15 ml/min (10 mm i.d.) 60 ml/min (20 mm i.d.) 120 ml/min (30 mm i.d.) 350 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-H.

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ For strongly retained compounds, an alcohol can be added into ACN to enhance the eluting strength.

Note: The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, 2-BuOH, etc. is possible, but effectiveness is not predictable.

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

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

Acidic samples **<u>do not always</u>** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

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

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

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_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.

Column Care / Maintenance

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ Samples should preferably be dissolved in the modifier.
- ❑ Sample solutions should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before use.

☞ ***Column transfer between modes:***

From LC to SFC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with 100% CO_2 or CO_2+modifier at 0.25 ml/min(*) for 45 min

From SFC to LC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with the mobile phase at 0.25 ml/min(*) for 45 min

(*) *This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

Column Storage

- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% EtOH 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.

Important Notice

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

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 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

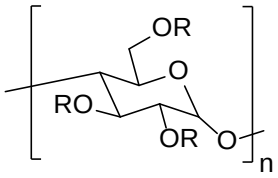
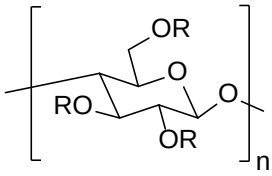
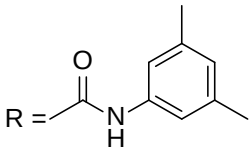
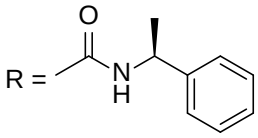
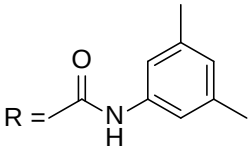
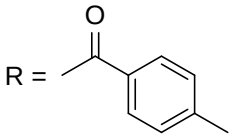
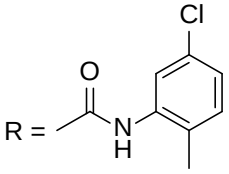
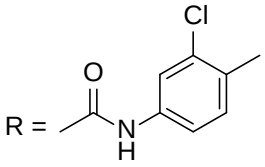
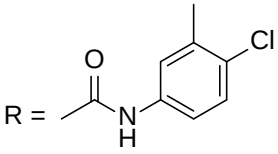
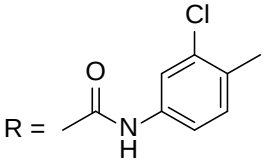
India
Daicel Chiral Technologies (India) Pvt Ltd.
Survey No.542/2, IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

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

<Normal Phase>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
 **Coated** on **10 µm silica gel**		 **Coated** on **10 µm silica gel**	
CHIRALPAK® AD	**CHIRALPAK® AS**	**CHIRALCEL® OD**	**CHIRALCEL® OJ**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY	**CHIRALPAK® AZ**	**CHIRALCEL® OX**	**CHIRALCEL® OZ**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Hexane/Isopropanol = 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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 20 mm i.d.**① **250 x 30 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**50 x 4.6 mm i.d.** Guard Column	**50 x 10 mm i.d.** **50 x 20 mm i.d.** **50 x 30 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate②	0.1-0.5 ml/min	0.5-2.5 ml/min	5 ml/min (10 mm i.d.) 20 ml/min (20 mm i.d.) 42 ml/min (30 mm i.d.) 118 ml/min (50 mm i.d.)
Pressure Limitation③	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

③ 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.

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-10.

	Alkane❶/ 2-Propanol❷	Alkane❶/ Ethanol❷	Alkane❶/ MeOH❸	MeOH❹+❺	ACN❺+❻ No alkane at all	Alkane❶/ MtBE
CHIRALPAK® AD **CHIRALPAK® AS** **CHIRALPAK® AY** **CHIRALPAK® AZ** **CHIRALCEL® OD** **CHIRALCEL® OJ** **CHIRALCEL® OX** **CHIRALCEL® OZ**	100/0 to 0/100	100/0 to 0/100	100/0 to 85/15	0 to 100% EtOH or 2-PrOH in MeOH	0 to 100% 2-PrOH in ACN	100/0 to 85/15
				0 to 15% ACN in MeOH ❻	0 to 15% MeOH or EtOH in ACN ❻	

❶ Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences have been observed when switching between these different alkanes.

❷ The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, etc. is possible, but effectiveness is not predictable.

❸ Due to the limited miscibility of MeOH in Alkane, it is necessary to add an appropriate volume of EtOH, together with MeOH, to ensure a homogenous solvent mixtures. A maximum of 5% MeOH, in n-Hexane only, may be used without adding EtOH.

❹ Ideal starting conditions: MeOH/EtOH 50:50 (v/v) when alcohol mixtures are required.

❺ The use of polar organic solvents like 100% Methanol and/or 100% ACN is possible with CHIRALPAK® AD/AS/AY/AZ and CHIRALCEL® OD/OJ/OX/OZ columns. Once the column is transferred to a polar organic mode, **it is recommended the column be dedicated for this specific application.**

❻ The column is particularly efficient in this range when such solvent mixtures are employed. Other alcohols such as 1-Propanol, 1-BuOH, etc. can also be used, but should not exceed 15% by volume.

To safely transfer the column from Hexane to Methanol or ACN, **it is strongly recommended to use 100% EtOH or 2-PrOH as a transition mobile phase** at 0.5 ml/min.

After this transition, the column needs to be thoroughly washed with ACN (~ 10 column volumes) prior to the first use in this solvent as a mobile phase.

B – Additives

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

❼ For primary amines mainly
❽ For primary amino alcohols mainly

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA Butyl amine❼ Ethanol amine❽	TFA CH_3COOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

Column Care / Maintenance

- ❑ The use of a guard column is highly recommended for maximum column life.
- ❑ Samples should preferably be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
- ❑ For alkane containing mobile phases, flush the column with Storage Solvent (Hexane / Isopropanol 90:10) when stored for more than one week.
- ❑ For columns dedicated to polar organic solvents, flush the column with the polar organic mobile phase, without the additive.
- ❑ When washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours. The column can also be heated to 40°C for a more efficient cleaning.

Important Notice

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

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 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 CS80140
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

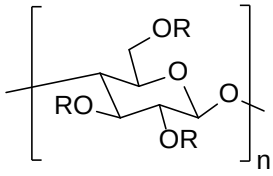
INSTRUCTION MANUAL FOR CHIRALCEL® OA, OB, OC, OF, OG, OK

<Normal Phase>

Please read this instruction sheet completely before using these columns

Column Description

CELLULOSE-BASED



Coated on **10 µm silica gel**

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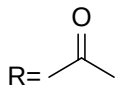
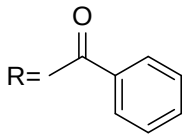
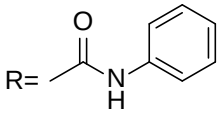
Cellulose triacetate 	Cellulose tribenzoate 	Cellulose triphenylcarbamate 
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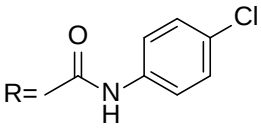
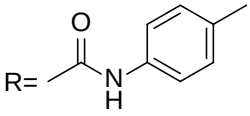
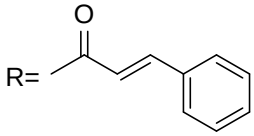
Cellulose tris(4-chlorophenylcarbamate) 	Cellulose tris(4-methylphenylcarbamate) 	Cellulose tricinnamate 

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 4.6 mm i.d. **250 x 4.6 mm i.d.** Analytical Columns
Guard	**50 x 4.6 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label
Typical Flow Rate①	1.0 ml/min
Pressure Limitation②	Should be maintained < 100 Bar (1450 psi) for maximum column life. Adapt flow rates to column size.
Temperature	0 to 40°C
Column Fitting	Please contact Technical Support for details

① The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 100 Bar).

② 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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

	Alkane❶/ 2-Propanol❷	Alkane❶/ Ethanol❷
CHIRALCEL® OA **CHIRALCEL® OB** **CHIRALCEL® OC** **CHIRALCEL® OK**	100/0 to 0/100	100/0 to 0/100
CHIRALCEL® OF	100/0 to 50/50	**NOT ALLOWED**
CHIRALCEL® OG	100/0 to 50/50	100/0 to 80/20

❶ Alkane: n-Hexane or iso-Hexane or n-Heptane. Some small selectivity differences have been observed when switching between these different alkanes.

❷ The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents.

B – Additives

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

❼ For primary amines mainly
❽ For primary amino alcohols mainly

Basic Samples require Basic additives	Acidic Samples require Acidic additives
DEA n-Butylamine❼ Ethanolamine❽	TFA CH_3COOH HCOOH
< 0.5% Typically 0.1%	< 0.5% Typically 0.1%

Column Care / Maintenance

- The use of a guard column is highly recommended for maximum column life.
- Samples should preferably be dissolved in the mobile phase and should be filtered through a membrane filter of approximately 0.5µm porosity.
- For CHIRALCEL® OA, OB, OC, OF, OG, and OK, the column should be flushed with Hexane/2-Propanol (90:10 v/v) when stored for more than one week.
- For CHIRALCEL® OA, OB, OC, and OK, when washing is required, use pure Ethanol at 0.5 ml/min for 1 to 3 hours.
- For CHIRALCEL® OF, when washing is required, use Hexane/2-Propanol (50:50 v/v) at 0.5 ml/min for 1 to 3 hours.
- For CHIRALCEL® OG, when washing is required, use Hexane/Ethanol (80:20 v/v) at 0.5 ml/min for 1 to 3 hours.

Important Notice

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

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 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 CS80140
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

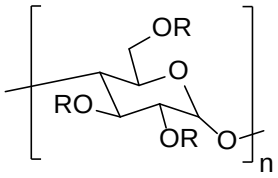
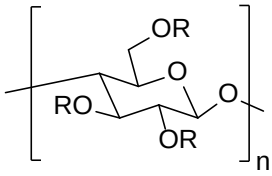
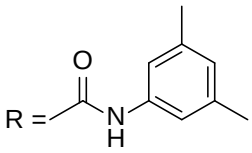
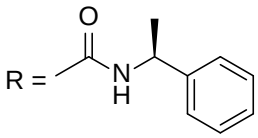
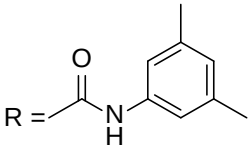
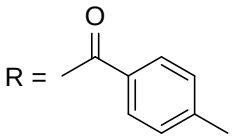
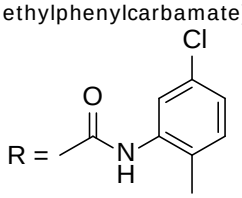
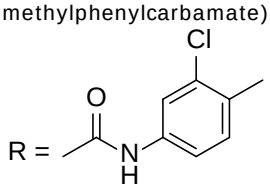
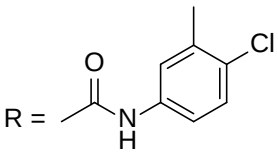
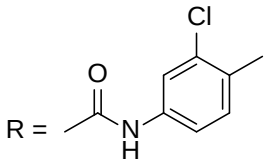
India
Daicel Chiral Technologies (India) Pvt. Ltd. Survey No.542/2, IKP Knowledge Park, Turkapally, Shamirpet Mandal, Medchal-Malkajgiri District, Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

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

<Reversed-Phase>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
 **Coated** on **10 µm silica gel**		 **Coated** on **10 µm silica gel**	
CHIRALPAK® AD	**CHIRALPAK® AS**	**CHIRALCEL® OD** **CHIRALCEL® OD-R**	**CHIRALCEL® OJ**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY	**CHIRALPAK® AZ**	**CHIRALCEL® OX**	**CHIRALCEL® OZ**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Water/ACN = 60/40 (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.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

CAUTION

The entire HPLC 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 the column. Many of the solvents commonly used as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**10 x 4.0 mm i.d. for CHIRALCEL OD-R (10)** Guard Cartridge **50 x 4.6 mm i.d. for others** Guard Column
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate②	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation③	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

① When using a semi-preparative column, it is highly recommended to discard at least the first 500 ml (for 250 x 21 mm i.d) of eluent at the beginning of each preparative work.

② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 300 Bar).

③ 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.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ.

	CHIRALPAK® AD CHIRALPAK® AS CHIRALPAK® AY CHIRALPAK® AZ CHIRALCEL® OD CHIRALCEL® OD-R CHIRALCEL® OJ CHIRALCEL® OX CHIRALCEL® OZ		CHIRALPAK® AD CHIRALPAK® AS CHIRALPAK® AY CHIRALCEL® OD CHIRALCEL® OD-R CHIRALCEL® OJ CHIRALCEL® OX CHIRALCEL® OZ
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	HCOOH aq. pH 2.0	Water	20mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary mobile phases (Section B)*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

	CHIRALPAK® AD CHIRALPAK® AS CHIRALPAK® AY CHIRALPAK® AZ CHIRALCEL® OD CHIRALCEL® OD-R CHIRALCEL® OJ CHIRALCEL® OX CHIRALCEL® OZ		CHIRALPAK® AD CHIRALPAK® AS CHIRALPAK® AY CHIRALCEL® OD CHIRALCEL® OD-R CHIRALCEL® OJ CHIRALCEL® OX CHIRALCEL® OZ
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO4 aq. pH 2.0 OR 100mM KPF_6 (or NaPF6) aq. pH 2.0 adjusted with H_3PO4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF6 (or $NaPF_6$) aq.
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 2: The concentration of all the buffering salt should be <u>less than 500mM</u>.*

❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.

❷
- ❑ It is recommended to use ACN to start the investigation
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50% ACN ≈ 65-70% EtOH ≈ 75-80% MeOH.
- ❑ The use of other organic solvents has not been investigated and could be harmful to the columns.
- ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.

❸
- Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
- Lowering the column temperature may increase the retention time and the selectivity.
- Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- To maximize column life, the use of a guard cartridge is essential when basic conditions are employed.
- The use of strongly basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
- When these columns are used at pH > 7, **the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to consequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

❻ Do not use a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

C – Buffer Preparation – Examples

➢ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50mM potassium dihydrogenphosphate
3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 9 Ammonium bicarbonate solution:*

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml with HPLC grade water

Solution B: Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
** DEA tends to give better peak shape than other bases.*

Adjust the pH of solution A to a value of 9.0 using solution B.

➢ *Preparation of pH 8 Phosphate buffer:*

Solution A: 20mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate
1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water.

Adjust the pH of solution A to a value of 8.0 using solution B.

➢ *Preparation of pH 9 Borate buffer:*

Solution A: 20mM sodium tetraborate decahydrate
3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid
0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water

Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- The use of a guard cartridge or guard column is highly recommended for maximum column life.
- 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.
- Before disconnecting the column from the HPLC, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, e.g. Water/ACN 60:40 (v/v).
- If the column is contaminated with non-eluted components, wash it with a mobile phase that does not contain any salts / buffers, then with 100% ACN for 2 hours at 0.5ml/min. Alternatively, if the non-eluting components are more soluble in methanol, this solvent may be used for the washing step.
- All salts must be flushed out from the HPLC system and column before changing to 100% ACN or 100% methanol.
- Use Water/ACN 60:40 (v/v) to store the column, at room temperature

Important Notice

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

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 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

INSTRUCTION MANUAL FOR
CHIRALPAK® AD-10, AS-10, AY-10, AZ-10
CHIRALCEL® OD-10, OJ-10, OX-10, and OZ-10

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns

Column Description

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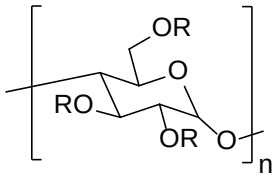
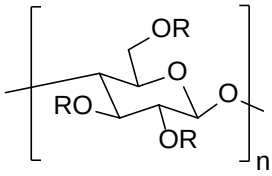
<tr><td colspan="2"> 
Coated on 10 µm silica gel</td><td colspan="2"> 
Coated on 10 µm silica gel</td></tr>
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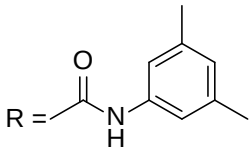
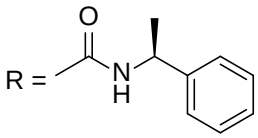
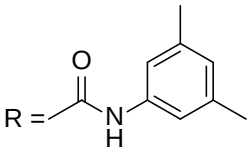
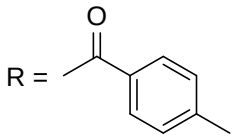
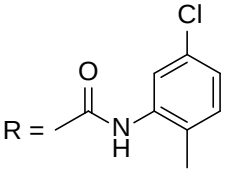
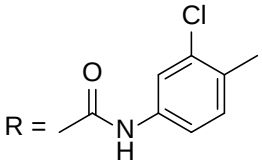
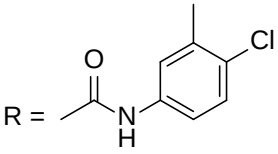
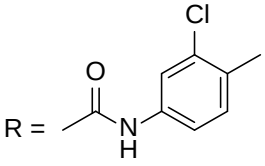
  </td><td>Amylose tris[(S)- α-methylbenzylcarbamate]
  </td><td>Cellulose tris(3,5-dimethylphenylcarbamate)
  </td><td>Cellulose tris (4-methylbenzoate)
  </td></tr>
<tr><th>CHIRALPAK® AY-10</th><th>CHIRALPAK® AZ-10</th><th>CHIRALCEL® OX-10</th><th>CHIRALCEL® OZ-10</th></tr>
<tr><td>Amylose tris(5-chloro-2-methylphenylcarbamate)
  </td><td>Amylose tris(3-chloro-4-methylphenylcarbamate)
  </td><td>Cellulose tris(4-chloro-3-methylphenylcarbamate)
  </td><td>Cellulose tris(3-chloro-4-methylphenylcarbamate)
  </td></tr>
<tr><td colspan="4">Shipping Solvent: Hexane/2-PrOH = 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

Because different columns are shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in SFC to avoid any damage (see column transfer conditions between LC and SFC on page 4).

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 the column. Many of the solvents commonly used as SFC modifers including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 21 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**50 x 4.6 mm i.d.** Guard Column	**/ /**
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate **in SFC**	0.5-1.0 ml/min	1.0-5.0 ml/min	15 ml/min (10 mm i.d.) 60 ml/min (21 mm i.d.) 120 ml/min (30 mm i.d.) 350 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 21 mm i.d) of eluent at the beginning of each preparative work.

② The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

A - Mobile Phases

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-10.

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ For strongly retained compounds, an alcohol can be added into ACN to enhance the eluting strength.

Note: The retention is generally shorter with Ethanol than with 2-Propanol, and the retention is generally shorter with higher alcohol contents. The use of other alcohols such as 1-Propanol, 1-BuOH, 2-BuOH, etc. is possible, but effectiveness is not predictable.

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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

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

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_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.

Column Care / Maintenance

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

☞ ***Column transfer between modes:***

From LC to SFC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with 100% CO_2 or CO_2+modifier at 0.25 ml/min(*) for 45 min

From SFC to LC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with the mobile phase at 0.25 ml/min(*) for 45 min

(*) *This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

Column Storage

- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% EtOH 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.

Important Notice

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

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 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

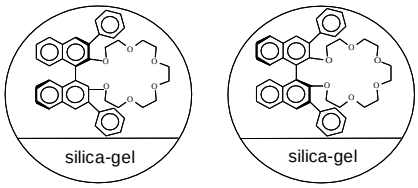
India
Daicel Chiral Technologies (India) Pvt Ltd.
Survey No.542/2, IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR CROWNPAK® CR(+) / CR(-)

Please read this instruction sheet completely before using these columns

Column Description

Packing composition: Chiral Crown Ether coated on **5µm silica-gel**.



CROWNPAK® CR(+) CROWNPAK® CR(-)

CROWNPAK® CR(-) CROWNPAK® CR(+)

Shipping solvent: H_2O/MeOH 95:5 (v/v)

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.

CAUTION

CROWNPAK® CR(+) / CR(-) require special care as limitations exist with solvents.
Exposing the column to inappropriate conditions will result in a rapid degradation of the stationary phase or to a loss in the column performance. Any traces of pure solvents must be removed.

BEFORE CONNECTING THE COLUMN, the entire HPLC system including the injector and the injection loop must be flushed with ethanol followed by 100 % distilled water.

Chiral Recognition

Chiral recognition can be achieved with CROWNPAK® CR(+) / CR(-) columns when a complex is formed between the crown ether and an ammonium ion ($-NH_3^+$) derived from a sample, under acidic conditions.
These columns can resolve not only amino acids but also compounds bearing a primary amino group near the chiral center.

With CROWNPAK® CR(+), the D-form of amino acids always elutes in first position.
Using CROWNPAK® CR(-) will result in an inversion of the elution order.

Operating Restrictions

	150 x 4.0 mm i.d. Analytical column
Flow rate direction	As indicated on the column label
Typical Flow rate ①	~ 0.5mL/min Do not exceed 1.5mL/min
Pressure limitation ②	Should be maintained < 150 Bar (~2100 psi)③ for maximum column life Do not exceed 200 Bar (~2900 psi)
Temperature ④	-5°C to 50°C

①+② The maximum flow rate depends on the mobile phase viscosity (mobile phase composition and temperature). Flow rate should be adjusted in accordance with pressure limitations.

Example	25°C	0°C
pH 2 $HClO_4$ 0.5mL/min	~ 50 Bar	~ 100 Bar

The back pressure value that should be taken into account is the one generated by the column itself. This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

③ Ideal value for maximum column life, but stable up to 200 Bar.

④ Generally, the lower the temperature is, the better the resolution becomes, especially for hydrophilic samples. Note that some hydrophobic samples may be strongly retained on the stationary phase at low temperatures.

Operating Procedure

Please contact Chiral Technologies for further assistance before trying any solvents not mentioned below.

This column should be operated under acidic condition as stated above.

	Aqueous solution of $HClO_4$❶	Aqueous solution of $HClO_4$❶ / Methanol❷
CROWNPAK® CR(+) / CR(-) 150 x 4.0 mm i.d.	100%	100/0 to 85/15 **(15% MeOH Max.)**

❶ ⇨ Typical pH range of the mobile phase: from pH 1 to pH 2 (column stable up to pH 9).
⇨ Lower pH will result in a good resolution but in a shorter column life. Choose the highest pH giving a satisfactory separation to prolong column life time.
⇨ Decreasing the temperature is also effective to increase the selectivity.
⇨ Other acids such as nitric acid and TFA can also be used. However, we recommend to use perchloric acid preferably which gives, in most cases, better resolutions and also for its low UV-absorption.

❷ ⇨ To shorten the retention time of hydrophobic compounds, the addition of methanol (15% max.) has been shown to be efficient.
Exceeding 15% of Methanol or using a different organic modifier is likely to damage the stationary phase contained in the column.

Examples of mobile phase preparation:

a) pH = 1.0

Weigh out 16.3 grams of commercially available perchloric acid (70%) and diluted to 1 L with distilled water.

b) pH = 2.0

100 mL of pH 1.0 solution is diluted to 1 L with distilled water.

c) pH = 1.5

316 mL of pH 1.0 solution is diluted to 1 L distilled water.

d) pH = 1.3

500 mL of pH 1.0 solution is diluted to 1 L distilled water.

Notes: *- Mobile phases should be completely degassed or thoroughly purged with helium.*

- Capacity factors on these columns depend on the hydrophobic nature of samples. Hydrophobic compounds are more retained compared to the hydrophilic ones. When your sample shows a little retention and a poor resolution the separation may be improved by decreasing the pH of the mobile phase (and column temperature).

- Although this column is not damaged by K^+ ions, the chiral recognition may be disturbed when present. The use of mobile phases containing K^+ ions should be avoided.

Column Care / Maintenance

- The use of a guard column is highly recommended for maximum column life.
- Samples should be dissolved in H_2O and filtered through a membrane filter of approximately 0.5µm porosity. Even for sample preparation, using a solution that contains more than 15% Methanol may cause irreversible damages.
- Injecting a too concentrated sample solution will lead to a low column efficiency. Injections of 10^{-8} to 10^{-7} mol of compound is usually enough to see a signal by UV.
- The column should be flushed with distilled water when your analysis is finished. For a long term storage (more than 1 week), keep the column end capped in the refrigerator (3-6°C) to avoid microbial contamination.
- If peaks start to split (after a long time of use), back-flushing the column with 100% H_2O may resolve the problem. Back-flushing could be a method to rescue the column but is usually not recommended.

Examples of Amino Acids

DL-Amino acid	$HClO_4$ (pH)	F.R. (mL/min)	Temp. (℃)	k_D'	k_L'	α	Rs
Alanine	1.5	0.4	25	0.38	0.70	1.86	3.17
Valine	1.5	0.4	0	1.09	1.64	1.51	3.47
Norvaline	2.0	0.8	25	0.69	1.17	1.69	2.74
Leucine	2.0	0.8	25	1.44	2.39	1.67	3.73
Norleucine	2.0	0.8	25	1.76	2.91	1.66	3.38
Isoleucine	2.0	0.4	0	1.76	2.79	1.58	4.29
tert-leucine	2.0	0.4	0	2.06	2.26	1.10	0.7
Phenylalanine	2.0	0.8	25	3.88	4.93	1.27	2.80
DOPA	2.0	0.8	25	2.88	3.67	1.28	2.47
Methionine	2.0	0.8	25	1.05	2.10	2.00	5.87
Ethionine	2.0	0.8	25	2.43	4.68	1.93	6.03
Phenylglycine	2.0	1.0	40	1.06	2.49	2.35	7.14
Serine	1.5	0.4	0	0.48	0.85	1.75	3.04
Threonine	2.0	0.4	0	0.39	1.00	2.58	4.20
Cysteine	1.5	0.4	25	0.44	0.74	1.67	3.31
Tyrosine	2.0	0.8	25	2.88	3.67	1.28	2.47
Asparagine	1.5	0.4	0	0.53	0.90	1.69	3.15
Glutamine	2.0	0.4	25	0.25	0.53	2.13	3.11
Aspartic acid	2.0	0.4	0	0.61	1.23	2.01	4.07
Glutamic acid	2.0	0.4	25	0.33	0.92	2.81	5.32
Ornithine	1.5	0.4	25	0.65	0.97	1.49	2.82
Lysine	1.5	0.4	25	1.18	1.50	1.26	2.20
Arginine	1.5	0.8	25	0.65	1.43	2.21	5.18
Citrulline	1.5	0.4	25	0.43	0.94	2.18	3.97
Proline	1.5	0.4	0	0.73	0.73	1.00	----
Histidine	1.5	0.4	0	0.90	1.64	1.82	5.28
Tryptophane	2.0	1.2	25	18.45	21.94	1.19	2.22

Sample Loda: 10^{-7}~10^{-8}mol Detection: UV200nm

These data are not guaranteed.

Analytical Conditions of Amino Acids (aq. $HClO_4$)

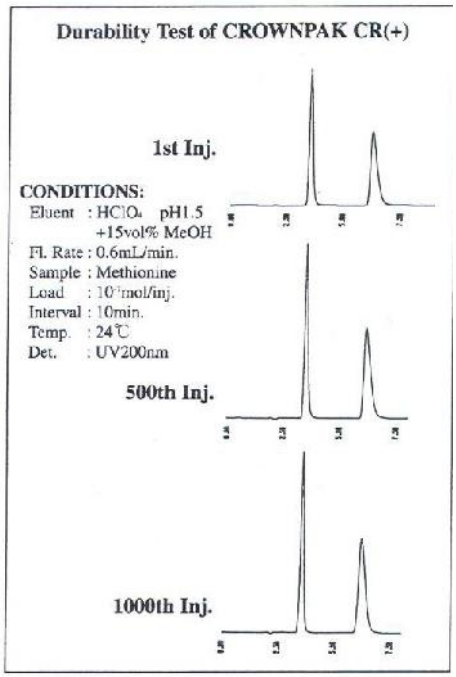
DL-Amino acid	pH2.0			pH1.5		pH1.0
	25℃	15℃	0℃	25℃	0℃	25℃
Alanine	P	C	C	C	C	
Valine	P	P	C	P	C	C
Norvaline	C	C	C	C	C	
Leucine	C	C	C	C	C	
Norleucine	C	C	C	C	C	
Isoleucine	A	C	C	C	C	
Phenylalanine	C	C	C	C	C	
DOPA	C	C	C	C	C	
Methionine	C	C	C	C	C	
Ethionine	C	C	C	C	C	
Phenylglycine	C	C	C	C	C	
Serine	U	U	A	U	C	
Threonine	P	A	C	A	C	
Cysteine	A	C	C	C	C	
Tyrosine	C	C	C	C	C	
Asparagine	U	U	P	P	C	A
Glutamine	C	C	C	C	C	
Aspartic acid	P	A	C	P	C	
Glutamic acid	C	C	C	C	C	
Ornithine	P	A	C	C	C	
Lysine	P	A	C	C	C	
Arginine	A	C	C	C	C	
Citrulline	A	C	C	C	C	
Proline	U	U	U	U	U	
Histidine	U	P	P	P	C	A
Tryptophane	C	C	C	C	C	

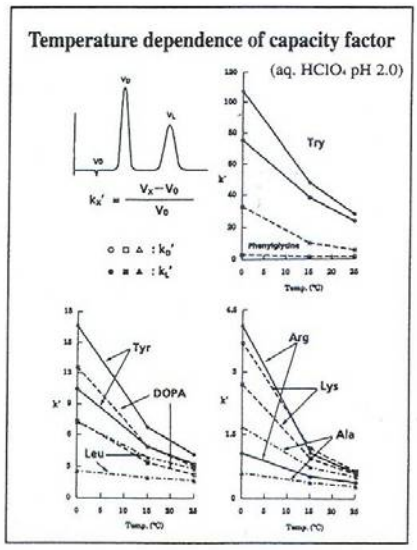
C : Completely resolved. ($2 \leqq Rs$)
A : Almost completely resolved. ($1.5 \leqq Rs < 2$)
P : Partially resolved. ($Rs < 1.5$)
U : Unresolved.

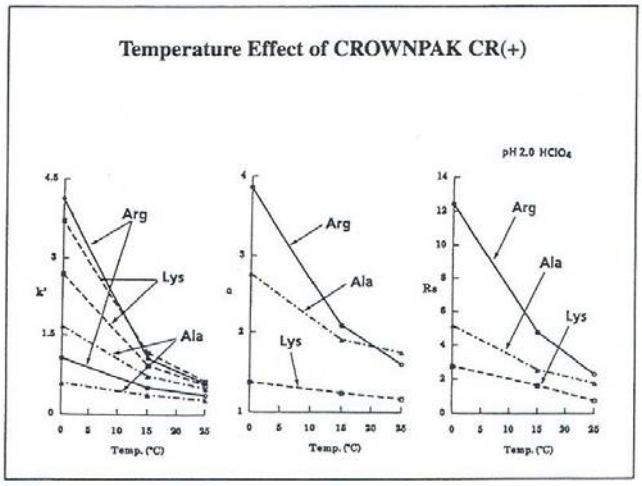
Examples of Amines and Aminoalcohols

Amines and Aminoalcohols	Eluent	Temp (℃)	Det.	k1'	α
	$HClO_4$ pH 1	1	OPA	2.57	1.10
	$HClO_4$ pH 1	1	OPA	1.84	1.15
	$HClO_4$ pH 2	25	UV	8.44	1.31
	$HClO_4$ pH 1.5	9	UV	21.8	1.17
	$HClO_4$ pH 1	1	OPA	0.67	1.58
	$HClO_4$ pH 1	1	OPA	1.67	1.42
	$HClO_4$ pH 1	1	OPA	1.54	1.30
	$HClO_4$ pH 1	1	OPA	4.19	1.43
	$HClO_4$ pH 2	10	UV	4.19	1.23
	$HClO_4$ pH 2	10	UV	9.07	1.18
	$HClO_4$ pH 1.5	40	UV	18.8	1.39
	$HClO_4$ pH 2	25	UV	0.54	1.83

Durability Test of CROWNPAK CR(+)







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@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

INSTRUCTION MANUAL FOR CROWNPAK® CR-I(+) and CROWNPAK® CR-I(-)

<Normal Phase>

Please read this instruction sheet completely before using these columns

These columns can also be used in reversed phase mode.
Please refer to the corresponding instruction sheet for details.

Switching Between RP and NP Mode

Shipping solvent of CROWNPAK CR-I(+)/CR-I(-) columns are H_2O/MeOH=95/5.

To switch from reversed phase mode to normal phase mode, and vice versa, column should be carefully flushed with miscible solvent (ethanol and 2-propanol).

Sufficient equilibration time is necessary for the stabilization of retention times when the column is switched from reversed phase mode to normal phase mode.

Operating Procedure / Normal Phase

A. Mobile phase

When developing methods, we would recommend reversed phase mode as a first choice. Normal phase mode is a second choice.

Primary solvent	Alkane❶/EtOH❷/TFA❸/H_2O❹
Typical starting conditions (v/v/v/v)	50 / 50 / 0.5 / 0.5
Advised optimisation range (v/v/v/v)	70 / 30 / 0.5 / 0.5 ~ 30 / 70 / 0.5 / 0.5

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ The retention is generally quite shorter with Ethanol than with 2-Propanol.
❸ Use TFA at less than 1.0% to prolong column life time.
❹ By the addition of H_2O, the peak shapes can be improved. When additive amount of H_2O is so high, the mobile phase is not miscible. Maximum additive amount allowed of H_2O is depending on the kinds and proportion of alcohol. In the case of n-Hexane / EtOH = 50 / 50 (v/v), the additive amount of H_2O is up to 3.0%.

Column Care / Maintenance

- When washing is required, use the solvent which can dissolve the sample such as pure methanol or ethanol at 0.2 mL/min for about 2 hours (room temperature).
- The column should be immediately flushed with a mobile phase without the TFA and H_2O after the use.
- n-Hexane / ethanol = 50 / 50 can be used as a storage solvent when used continuously under normal phase.

Refer to instruction sheet for reverse phase and column care / maintenance.

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 this column, 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

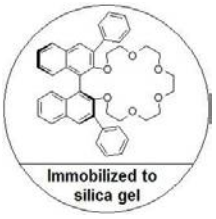
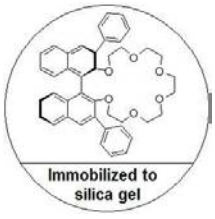
INSTRUCTION MANUAL FOR CROWNPAK® CR-I(+) and CROWNPAK® CR-I(-)

<Reversed Phase>

Please read this instruction sheet completely before using these columns

These columns can also be used in normal phase mode.
Please refer to the corresponding instruction sheet for details.

Column Description

CROWNPAK® CR-I(+)	CROWNPAK® CR-I(-)
	

Chiral Crown Ether **immobilized** to **5 µm silica-gel**

Shipping solvent: H_2O/MeOH 95:5 (v/v)

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.

Chiral Recognition

Chiral recognition can be achieved with CROWNPAK® CR-I(+) / CR-I(-) columns when a complex is formed between the crown ether and an ammonium ion ($-NH_3^+$) derived from a sample, under acidic conditions.
These columns can resolve not only amino acids but also compounds bearing a primary amino group near the chiral center.

With CROWNPAK® CR-I(+), the D-form of amino acids always elutes in first position.
Using CROWNPAK® CR-I(-) will result in an inversion of the elution order.

Operating Restrictions

	3.0 x 150 mm Analytical column
Flow rate direction	As indicated on the column label
Typical flow rate	0.2 ~ 0.4 mL / min
Pressure limitation❶	Should be maintained < 300 Bar (4350 psi) for maximum column life
Temperature	-5 to 40°C

❶The back pressure value that should be taken into account is the one generated by the column itself.
This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

Operating Procedure / Reversed Phase

A. Mobile phase

CROWNPAK CR-I(+) and CR-I(-) columns allow *free choice* of any miscible solvents, which are commonly used for HPLC analyses, to compose the mobile phase. When developing methods, we would recommend reversed phase mode as a first choice. Normal phase mode is a second choice.

Aqueous solution❶	$HClO_4$ aqueous solution
Organic solvent❷	CH_3CN, MeOH, EtOH, IPA, THF
Typical starting conditions❸	Aqueous solution of $HClO_4$ (pH=1.5) / CH_3CN = 80 / 20 (v/v)

CH_3CN: Acetonitrile, MeOH: Methanol, EtOH: Ethanol, IPA: 2-Propanol, THF: Tetrahydrofuran

❶ ⇨ Typical pH range of the mobile phase: from pH 1 to pH 2 (column stable up to pH 7).
⇨ Lower pH will result in longer retention times but a shorter column life. Choose the highest pH giving a satisfactory separation to prolong column life time.
⇨ Decreasing the temperature is also effective to increase the selectivity.
⇨ Other acids such as nitric acid and TFA can also be used. However, we recommend to use perchloric acid preferably which gives, in most cases, better resolutions and also for its low UV-absorption.

❷ ⇨ The elution power of organic modifiers for these columns is in the descending order of
THF > CH_3CN > IPA > EtOH > MeOH.
⇨The use of alcohols causes the back pressure to be significantly higher compared to CH_3CN due to their high viscosity in mixtures with water.

❸ ⇨ The aqueous solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.
⇨Retention can be adjusted by changing the proportion of organic solvent.

B. Examples of mobile phase preparation:

Hereafter are some indications to prepare the aqueous solution. The resulting pH value of the solution must be measured and adjusted for accuracy.

a) pH = 1.0

Weigh out 16.3 grams of commercially available perchloric acid (70%) and diluted to 1 L with distilled water.

b) pH = 2.0

100 mL of pH 1.0 solution is diluted to 1 L with distilled water.

c) pH = 1.5

316 mL of pH 1.0 solution is diluted to 1 L distilled water.

d) pH = 1.3

500 mL of pH 1.0 solution is diluted to 1 L distilled water.

Notes: *- Mobile phases should be completely degassed or thoroughly purged with helium.*

- Capacity factors on these columns depend on the hydrophobic nature of samples. Hydrophobic compounds are more retained compared to the hydrophilic ones. When your sample shows a little retention and a poor resolution the separation may be improved by decreasing the pH of the mobile phase (and column temperature).

- Although this column is not damaged by K^+ ions, the chiral recognition may be disturbed when present. The use of mobile phases containing K^+ ions should be avoided.

Column Care / Maintenance

- The use of a guard filter is highly recommended for maximum column life. The guard filter is in common use between CROWNPAK® CR-I(+) and CR-I(-).
- 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 0.5 µm porosity to ensure that there is no precipitate before using.
- Injecting a too concentrated sample solution will lead to a low column efficiency.
- When washing is required, use the solvent which can dissolve the sample such as pure methanol or acetonitrile at 0.2 mL/min for about 2 hours (room temperature).
- The column and the guard filter should be immediately flushed with a mobile phase with no acidic additive after use.
- H_2O / MeOH = 95 / 5 (v/v) can be used as a storage solvent when used continuously under reversed phase.

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 this column, 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

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

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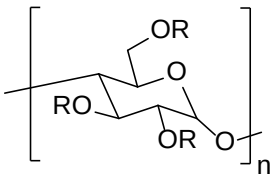
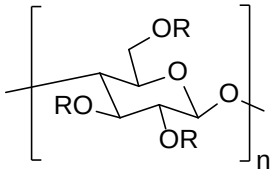
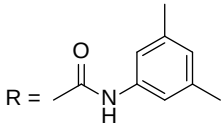
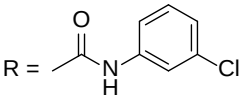
 

Immobilized on 1.6 µm silica gel</th><th>CELLULOSE-BASED
 

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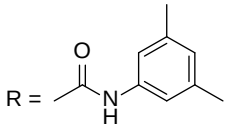
</td></tr>
<tr><td>CHIRALPAK® IG-U</td><td>CHIRALPAK® IH-U</td><td>CHIRALPAK® IC-U</td></tr>
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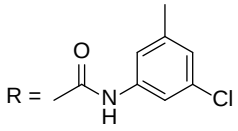
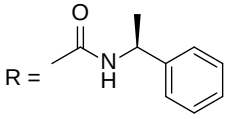
 
</td><td>Amylose tris[(S)- α-methylbenzylcarbamate]
 
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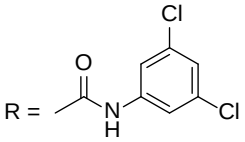
 
</td></tr>
<tr><td colspan="3">Shipping solvent: Hexane/IPA = 90:10 (v/v)

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.

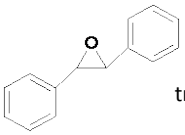
For detailed Regeneration Procedures, please click here

Column Storage

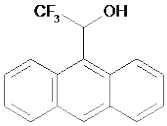
- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% 2-PrOH or 100% methanol, without additives.
- ❑ Columns can be stored with ends capped in the additive-free mobile phase, or the shipping solvent, at room temperature.

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

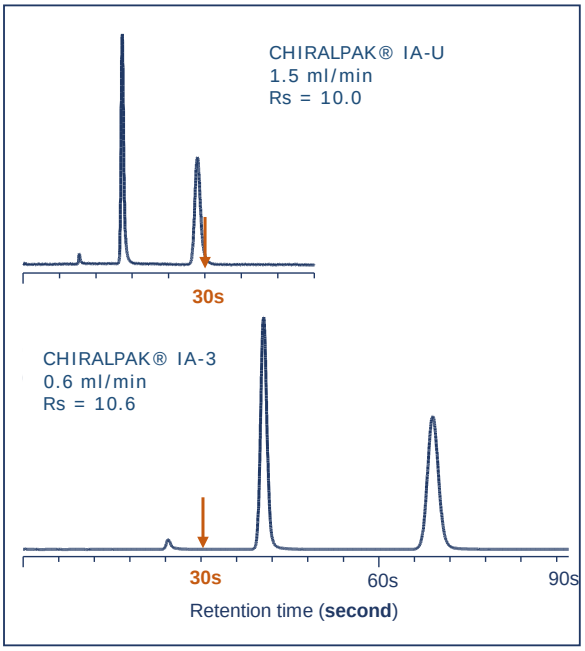


Figure 1

Common conditions

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

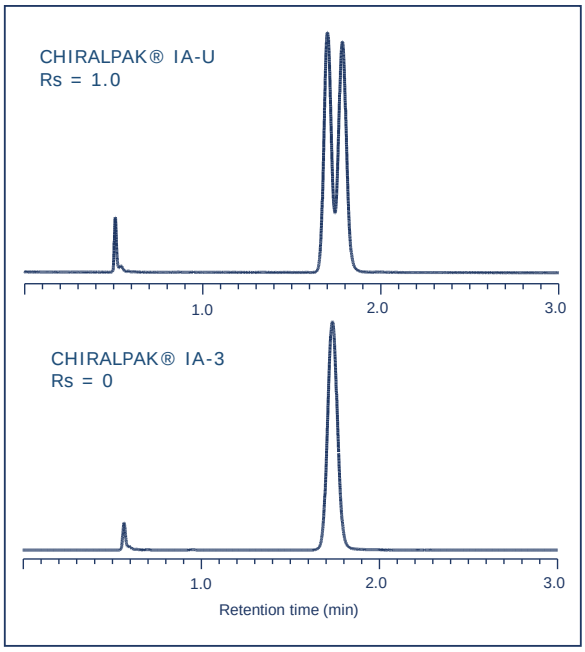


Figure 2

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

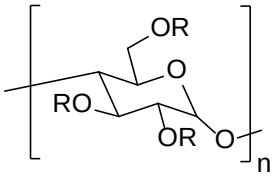
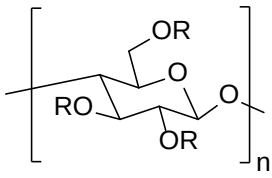
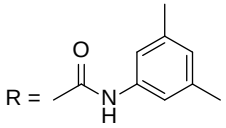
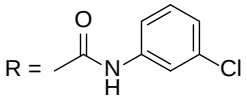
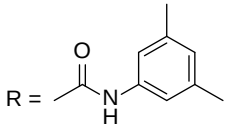
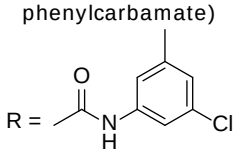
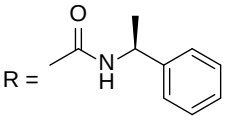
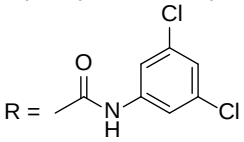
India
Daicel Chiral Technologies (India) Pvt Ltd
Survey No. 542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

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

<Reversed-Phase>

Please read this instruction manual completely before using these columns.
These columns can also be used in normal phase and SFC. 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**
CHIRALPAK® IA-U	**CHIRALPAK® ID-U**	**CHIRALPAK® IB-U**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethyl phenylcarbamate) 
CHIRALPAK® IG-U	**CHIRALPAK® IH-U**	**CHIRALPAK® IC-U**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dichloro phenylcarbamate) 
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.		

Because these columns are shipped in hexane/IPA, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in reversed-phase to avoid any damage.

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.

General Recommendations

To switch from reversed-phase to normal phase or SFC, and vice versa, the column should be carefully flushed with a miscible solvent, such as ethanol or isopropanol. The column should also be flushed with such a solvent when initially received after purchase, before first used in reversed-phase, as it could contain a hexane/alcohol mixture.

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.

It is also highly recommended:
- to apply the **regeneration procedure** described in the Regeneration Procedure manual for switching back to normal phase or SFC. Before applying this protocol, any trace salts should be removed by flushing the column with a mobile phase that does not contain any salts / buffers, for example Water/ACN = 60/40, and then flushing with ethanol or isopropanol.
- to adjust the flow rate to ensure the column pressure < 700 bar.

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 **<u>It is highly recommended the matching fitting be used.</u>** 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 / Reversed-phase

A - Mobile Phases / For Both UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds ❹	**NEUTRAL** Compounds ❹	**BASIC** Compounds ❹
CHIRALPAK® IA-U **CHIRALPAK® IB-U** **CHIRALPAK® IC-U** **CHIRALPAK® ID-U** **CHIRALPAK® IG-U** **CHIRALPAK® IH-U**	Aqueous Solution ❶	HCOOH aq. pH 2.0	Water	20mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
	Organic Modifier ❷	ACN or MeOH or EtOH or 2-PrOH or THF		
	Typical Starting Conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary aqueous solutions*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

		ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
CHIRALPAK® IA-U **CHIRALPAK® IB-U** **CHIRALPAK® IC-U** **CHIRALPAK® ID-U** **CHIRALPAK® IG-U** **CHIRALPAK® IH-U**	Aqueous Solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF_6 (or $NaPF_6$) aq.

☞ *NOTE 2: The concentration of all the buffering salt should be less than 500mM.*

❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.

❷
- ❑ It is recommended to use ACN to start the investigation
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50%ACN ≈ 65-70%EtOH ≈ 75-80%MeOH.
  The use of other organic solvents, **except THF**, has not been investigated and could be harmful to the columns.
- ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their high viscosity in mixtures with water.

❸
- ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present into the mobile phase.
- ❑ Lowering the column temperature may increase the retention time and the selectivity.
- ❑ Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- ❑ **To maximize the column life, it is essential to inject filtered clean sample solutions.** It is recommended to use at least a filter with a porosity of 0.5 µm.
- ❑ The use of strong basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
- ❑ When these columns are used at pH > 7**, the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to consequent clogging of the column (refer to the table below).

Water / Organic Modifier	**Buffer solution / Organic Modifier**
90 / 10 to **0 / 100**	**90 / 10** to **15 / 85**

❻ Do not use the phosphate buffer for pH > 8. When pH 9 is necessary, use the ammonium bicarbonate solution or borate buffer for maximum column life.

C – Buffer Preparation – Examples

➢ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50mM potassium dihydrogenphosphate
3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water
Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500ml with HPLC grade water
Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 9 Ammonium bicarbonate solution:*

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml with HPLC grade water
Solution B Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
** DEA tends to give better peak shape than other bases.*
Adjust the pH of solution A to a value of 9.0 using solution B.

➢ *Preparation of pH 8 Phosphate buffer:*

Solution A: 20mM potassium hydrogenophosphate

1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate

1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water.

Adjust the pH of solution A to a value of 8.0 using solution B.

- *Preparation of pH 9 Borate buffer:*

Solution A: 20mM sodium tetraborate decahydrate

3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid

0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water

Adjust the pH of solution A to a value of 9.0 using solution B.

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 and/or switching to 100% organic solvent, any traces of salts should be removed by flushing the column with a mobile phase which doesn't contain any salts or buffers, for instance Water/ACN = 60/40 (v/v).
- 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 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

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

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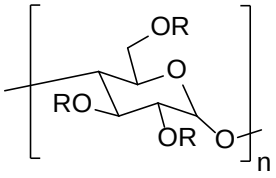
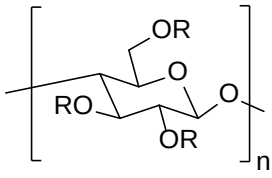
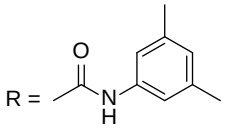
 

Immobilized on 1.6 µm silica gel</th><th>CELLULOSE-BASED
 

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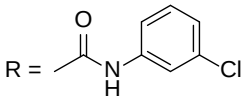
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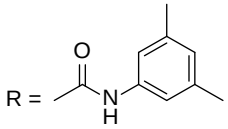
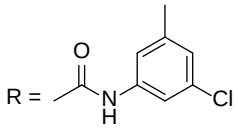
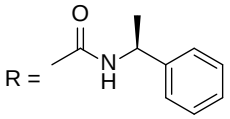
 
</td></tr>
<tr><td>CHIRALPAK® IG-U</td><td>CHIRALPAK® IH-U</td><td>CHIRALPAK® IC-U</td></tr>
<tr><td>Amylose tris(3-chloro-5-methyl-phenylcarbamate)
 
</td><td>Amylose tris[(S)- α-methylbenzylcarbamate]
 
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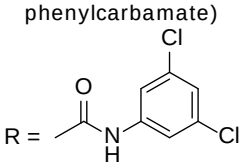
 
</td></tr>
<tr><td colspan="3">Shipping solvent: Hexane/IPA = 90:10 (v/v)

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>

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 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 / 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_2),* progressing from the traditional solvents used with other DAICEL columns (mixtures of CO_2 with alcohols or acetonitrile (CH_3CN)) to mobile phases containing CO_2 with 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 trying the Primary Screening with the product dissolved in a stronger solvent (DCM/alcohol...).

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	**CO_2** / MeOH	**CO_2** / EtOH	**CO_2** /2-PrOH	**CO_2** / CH_3CN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ Alcohols can be added into CH_3CN 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_2 / THF	CO_2 / (DCM+MeOH 90:10)	CO_2 / (EtOAc+MeOH 90:10)	CO_2 / (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

ⓘ 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 $CHCl_3$, 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 ⓘ	Acidic Samples require Acidic additives ⓘ
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_2) are sometimes enough to elute the product properly.

ⓘ 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_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).

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_2 or CO_2+ 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 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

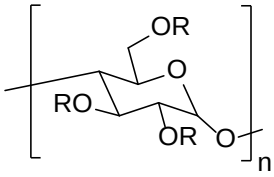
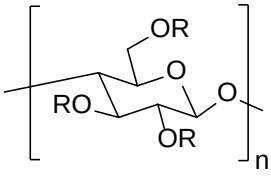
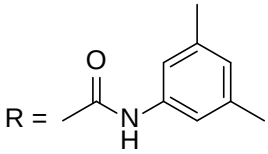
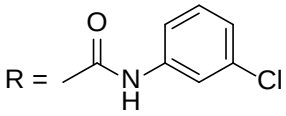
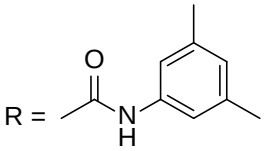
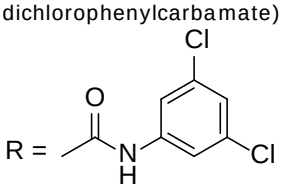
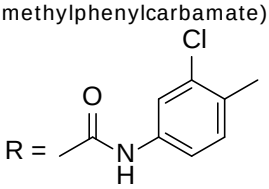
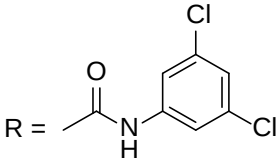
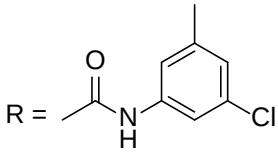
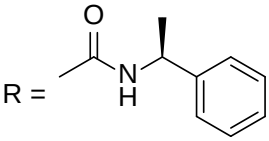
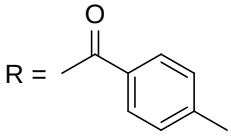
India
Daicel Chiral Technologies (India) Pvt Ltd
Survey No. 542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3

<Normal Phase>

Please read this instruction sheet completely before using these columns.
These columns can also be used in reversed-phase and SFC. Please refer to the corresponding instruction sheet for details.

Column Description

AMYLOSE-BASED		CELLULOSE-BASED
 **Immobilized** on **3 µm silica gel**		 **Immobilized** on **3 µm silica gel**
CHIRALPAK® IA-3	**CHIRALPAK® ID-3**	**CHIRALPAK® IB-3** **CHIRALPAK® IB N-3**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
CHIRALPAK® IE-3	**CHIRALPAK® IF-3**	**CHIRALPAK® IC-3**
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG-3	**CHIRALPAK® IH-3**	**CHIRALPAK® IJ-3**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

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.

Because different columns, including custom columns, can be shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

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.

Method Development / Normal Phase

A - Mobile Phases

CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 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 ($CHCl_3$), 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

- ⇨ Only immobilized CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 are suitable for the Secondary Screening
- ⇨ Additional solvent combinations such as $CHCl_3$/Alkane, 1,4-Dioxane/Alkane, Toluene/Alkane, or Acetone/Alkane can also be investigated with CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 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 adding 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 RI detector or 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 behavior 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 additives❶	Acidic Samples require Acidic additives
Diethylamine (DEA) 2-Aminoethanol (AE) Ethylenediamine (EDA) Butyl amine (BA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid
< 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

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ 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…).

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 the same mobile phase, but without the additive.
- 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 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 & 0703

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

INSTRUCTION MANUAL FOR CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3

<Reversed-Phase>

Please read this instruction sheet completely before using these columns.

These columns can also be used in normal phase mode. Please refer to the corresponding instruction sheet for details.

Column Description

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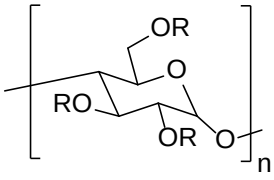
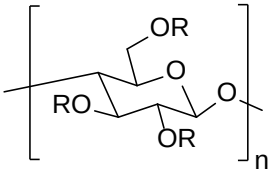
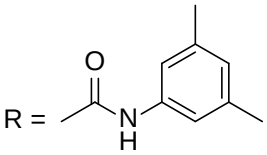
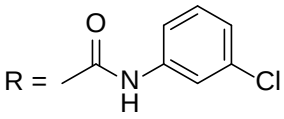
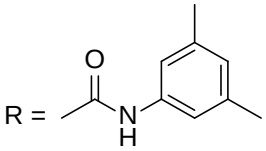
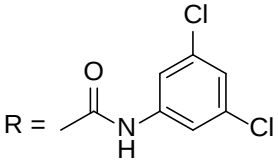
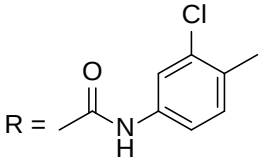
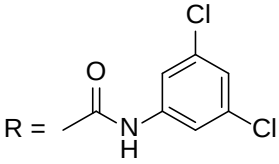
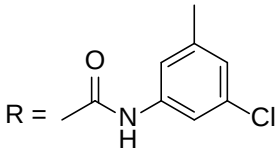
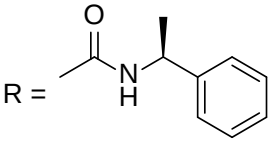
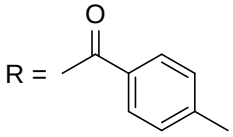
		
Immobilized on **3 µm silica gel**		**Immobilized** on **3 µm silica gel**
CHIRALPAK® IA-3	**CHIRALPAK® ID-3**	**CHIRALPAK® IB-3** **CHIRALPAK® IB N-3**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
CHIRALPAK® IE-3	**CHIRALPAK® IF-3**	**CHIRALPAK® IC-3**
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG-3	**CHIRALPAK® IH-3**	**CHIRALPAK® IJ-3**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

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.

Because different columns, including custom columns, can be shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.

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Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

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.

Switching between RP and NP or SFC

To switch from reversed-phase to normal phase or SFC, and vice versa, the column should be carefully flushed with miscible solvent, such as ethanol or isopropanol. The column should be flushed in a similar manner with ethanol or isopropanol when initially received after purchase, before first used in reversed-phase, as it could contain a hexane/alcohol mixture.

It is highly recommended that the **regeneration procedure** (link below in Column Care section) be used when switching from reversed-phase to normal phase or SFC. Before applying this procedure, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, for example Water/ACN = 60/40, and then flushing with ethanol or isopropanol.

A - Mobile Phases / For Both UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
CHIRALPAK® IA-3 CHIRALPAK® ID-3 CHIRALPAK® IE-3 CHIRALPAK® IF-3 CHIRALPAK® IG-3 CHIRALPAK® IH-3 CHIRALPAK® IB-3 CHIRALPAK® IB N-3 CHIRALPAK® IC-3 CHIRALPAK® IJ-3	Aqueous Solution ❶	HCOOH aq. pH 2.0	Water	20 mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
	Organic Modifier ❷	ACN or MeOH or EtOH or IPA or THF		
	Typical Starting Conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary aqueous solutions*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
CHIRALPAK® IA-3 CHIRALPAK® ID-3 CHIRALPAK® IE-3 CHIRALPAK® IF-3 CHIRALPAK® IG-3 CHIRALPAK® IH-3 CHIRALPAK® IB-3 CHIRALPAK® IB N-3 CHIRALPAK® IC-3 CHIRALPAK® IJ-3	Aqueous Solution ❶	50 mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100 mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20 mM Borate Buffer pH 9.0 OR 20 mM Phosphate Buffer pH 8.0 ❻ OR 100 mM KPF_6 (or $NaPF_6$) aq.

☞ *NOTE 2: The concentration of all the buffering salt should be less than 500 mM.*

❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.

❷ ❑ It is recommended to use ACN to start the investigation.
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50% ACN ≈ 65-70% EtOH ≈ 75-80% MeOH. The use of other organic solvents, **except THF**, has not been investigated and could be harmful to the columns.
- ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their high viscosity in mixtures with water.

❸
- ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present into the mobile phase.
- ❑ Lowering the column temperature may increase the retention time and the selectivity.
- ❑ Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- To maximize column life the use of a guard cartridge is essential when basic conditions are employed.
- **The use of strong basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.**
- When these columns are used at pH > 7**, the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to consequent clogging of the column (refer to the table below).

Water / Organic Modifier	**Buffer solution / Organic Modifier**
90 / 10 to **0 / 100**	**90 / 10** to **15 / 85**

❻ Do not use the phosphate buffer for pH > 8. When pH 9 is necessary, use the ammonium bicarbonate solution or borate buffer for maximum column life.

C – Buffer Preparation – Examples

➢ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50 mM potassium dihydrogenphosphate
3.40 g KH_2PO_4 / FW 136.09, make up the volume to 500 ml with HPLC grade water
Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

Solution A: 100m M potassium (sodium) hexafluorophosphate
9.20 g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500 ml with HPLC grade water
Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *Preparation of pH 9 Ammonium bicarbonate solution:*

Solution A: 20 mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500 ml with HPLC grade water
Solution B Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
** DEA tends to give better peak shape than other bases.*
Adjust the pH of solution A to a value of 9.0 using solution B.

➢ *Preparation of pH 8 Phosphate buffer:*

Solution A: 20 mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500 ml with HPLC grade water
Solution B: 20 mM potassium dihydrogenophosphate
1.36g KH_2PO_4 / FW 136.09, make up the volume to 500 ml with HPLC grade water.
Adjust the pH of solution A to a value of 8.0 using solution B.

➢ *Preparation of pH 9 Borate buffer:*

Solution A: 20 mM sodium tetraborate decahydrate
3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500 ml with HPLC grade water
Solution B: 20 mM boric acid
0.62g H_3BO_3 / FW 61.83, make up the volume to 500 ml with HPLC grade water
Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- 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…).

For detailed Regeneration Procedures, please click here

Column Storage

- For column storage and/or switching to 100% organic solvent, any traces of salts should be removed by flushing the column with a mobile phase which doesn't contain any salts or buffers, for instance Water/ACN = 60/40 (v/v).
- 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 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

INSTRUCTION MANUAL FOR
CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns.

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

Column Description

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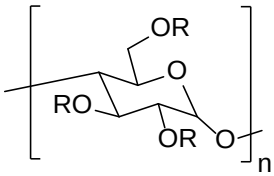
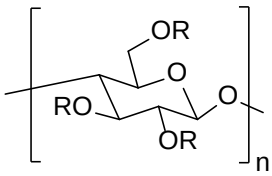
 

Immobilized on 3 µm silica gel</td><td>CELLULOSE-BASED
 

Immobilized on 3 µm silica gel</td></tr>
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CHIRALPAK® IB N-3</td></tr>
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 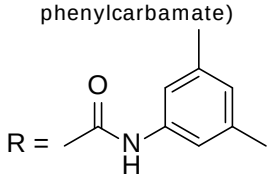
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 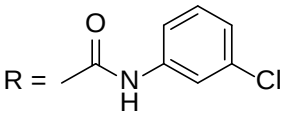
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 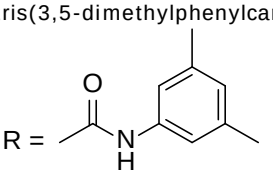
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<tr><td>CHIRALPAK® IE-3</td><td>CHIRALPAK® IF-3</td><td>CHIRALPAK® IC-3</td></tr>
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 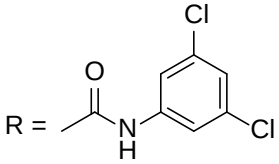
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 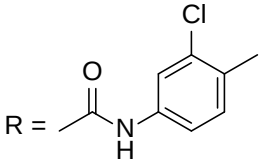
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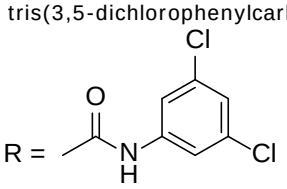
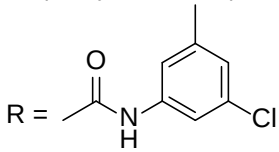
 
</td></tr>
<tr><td>CHIRALPAK® IG-3</td><td>CHIRALPAK® IH-3</td><td>CHIRALPAK® IJ-3</td></tr>
<tr><td>Amylose tris(3-chloro-5-methyl-phenylcarbamate)
 
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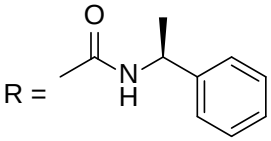
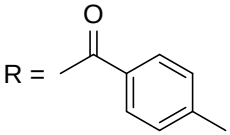
 
</td><td>Cellulose tris(4-methylbenzoate)
 
</td></tr>
<tr><td colspan="3">Shipping Solvent: Hexane/IPA = 90:10 (v/v)

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>

Because different columns are shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in SFC to avoid any damage (see column transfer conditions between LC and SFC on page 4).

THIS INSTRUCTION SHEET IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Columns	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

① The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Method Development / SFC

A - Mobile Phases

CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 can be used *with all ranges of organic miscible solvents as modifiers combined with supercritical carbon dioxide (CO_2),* progressing from the traditional solvents used with other DAICEL columns (mixtures of CO_2 with alcohols or acetonitrile (ACN)) to mobile phases containing CO_2 with 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 trying the Primary Screening with the product dissolved in a stronger solvent (DCM/alcohol...).

3.

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ Alcohols can be added into ACN 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_2 / THF	CO_2 / (DCM+MeOH 90:10)	CO_2 / (EtOAc+MeOH 90:10)	CO_2 / (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

Notes: 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.

All solvent proportions indicated in this manual are by volume.

C – General Comments

- ⇨ Only immobilized CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 are suitable for the Secondary Screening.
- ⇨ Additional modifiers such as $CHCl_3$, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 columns.
- ⇨ 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.
- ⇨ It is also important to ensure 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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ❶	Acidic Samples require Acidic additives ❶
Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid 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*

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_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).

Column Care / Maintenance

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ 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…).

For detailed Regeneration Procedures, please click here

☞ ***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+modifier 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

(*) *This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

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 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

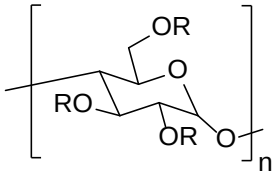
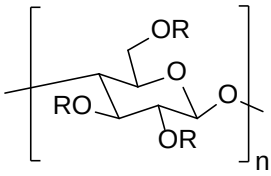
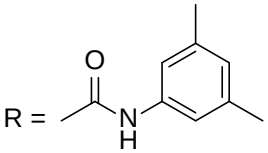
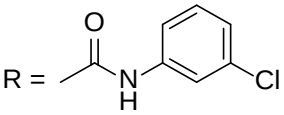
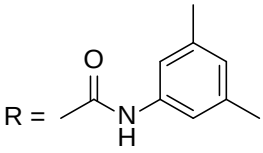
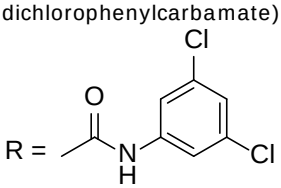
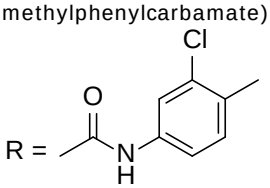
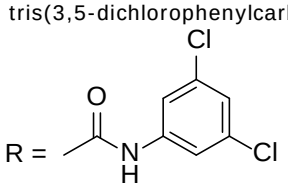
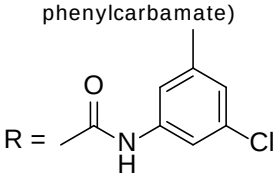
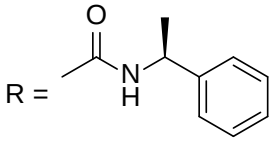
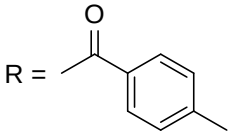
INSTRUCTION MANUAL FOR SFC OPTIMIZED (3.0 mm i.d.)
CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns.

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

Column Description

AMYLOSE-BASED		CELLULOSE-BASED
 **Immobilized** on **3 µm silica gel**		 **Immobilized** on **3 µm silica gel**
CHIRALPAK® IA-3	**CHIRALPAK® ID-3**	**CHIRALPAK® IB-3** **CHIRALPAK® IB N-3**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
CHIRALPAK® IE-3	**CHIRALPAK® IF-3**	**CHIRALPAK® IC-3**
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG-3	**CHIRALPAK® IH-3**	**CHIRALPAK® IJ-3**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 
Shipping Solvent: **Methanol = 100%** 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 different columns, including custom columns, can be shipped in different solvents, 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 5).

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	50 x 3.0 mm i.d. **100 x 3.0 mm i.d.** **150 x 3.0 mm i.d.** Analytical Columns
Flow Rate Direction	As indicated on the column label
Typical Flow Rate **in SFC**	~ 0.5 - 4.0 ml/min
Pressure Limitation①	< 300 bar (4350 psi) for maximum column life ***Typical CO_2 backpressure (BPr) 110-150 bar***
Temperature	0 to 40°C
Column Fitting	Please contact Technical Support for details

① The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Method Development / SFC

A - Mobile Phases

CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 can be used ***with all ranges of organic miscible solvents as co-solvent combined with the carbon dioxide (CO_2)***, progressing from the traditional solvents (mixtures of CO_2 with alcohols or acetonitrile (ACN)), to mobile phases containing CO_2 with tetrahydrofuran (THF), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc), and methyl *tert*-butyl ether (MtBE), 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 Primary Screening.
2. If the compound or compound series are not soluble in any of these mobile phases, we recommend to try the Primary Screening with the product dissolved in a stronger solvent (DCM/alcohol...).

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ Alcohols can be added into ACN to enhance the eluting strength for strongly retained compounds.

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

Table 2. Immobilized Secondary Screening Solvents

Secondary Solvent Mixtures	CO_2 / THF	CO_2 / (DCM+MeOH 90:10)	CO_2 / (EtOAc+MeOH 90:10)	CO_2 / (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

Notes: 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.

All solvent proportions indicated in this manual are by volume.

C – General Comments

⇨ Only immobilized CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 are suitable for the Secondary Screening.

⇨ Additional modifiers such as $CHCl_3$, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, and IJ-3 columns.

⇨ 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.

⇨ It is also important to ensure 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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ❶	Acidic Samples require Acidic additives ❶
Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid 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*

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_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).

Column Care / Maintenance

- ❑ The use of a guard cartridge is highly recommended for maximum column life.
- ❑ 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…).

For detailed Regeneration Procedures, please click here

☞ ***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

(*) *Recommended flow rate for analytical columns (3.0mm i.d.).*

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 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

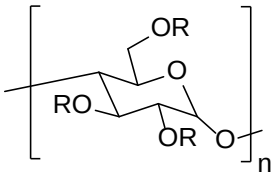
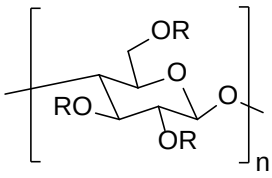
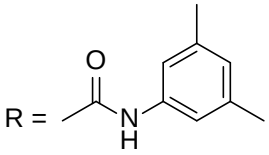
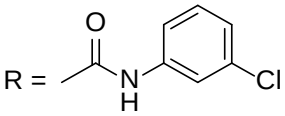
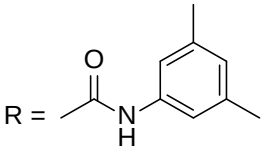
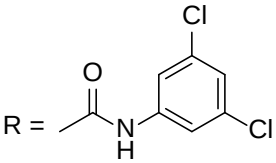
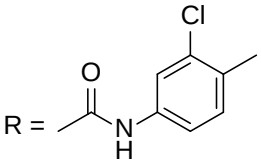
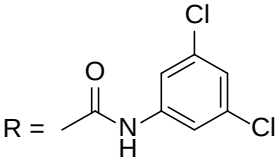
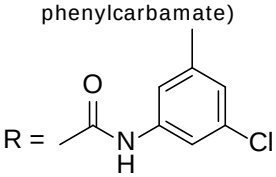
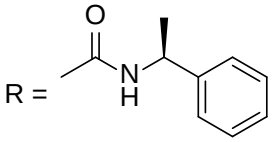
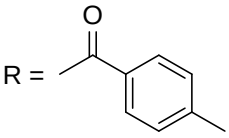
India
Daicel Chiral Technologies (India) Pvt Ltd
Survey No. 542/2 IKP Knowledge Park, Turkapally,
Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
chiral@chiral.daicel.com
www.chiraltech.com

INSTRUCTION MANUAL FOR CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ

<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

AMYLOSE-BASED		CELLULOSE-BASED
 **Immobilized** on **5 µm silica gel**		 **Immobilized** on **5 µm silica gel**
CHIRALPAK® IA	**CHIRALPAK® ID**	**CHIRALPAK® IB** **CHIRALPAK® IB N-5**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
CHIRALPAK® IE	**CHIRALPAK® IF**	**CHIRALPAK® IC**
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG	**CHIRALPAK® IH**	**CHIRALPAK® IJ**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

Shipping Solvent:

1. **Hexane/IPA = 90:10 (v/v) for analytical columns (2.1 mm i.d x 150 mm, 4.6 mm i.d. x 150 and 250 mm), guard, and semi-prep columns**
2. **100% Methanol for analytical (4.6 mm i.d. x 50 and 100 mm) columns**

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 different columns, including custom columns, can be shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 20 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**20 x 10 mm i.d.** **50 x 21 mm i.d.** **50 x 30 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min	5 ml/min (10 mm i.d.) 20 ml/min (20 mm i.d.) 42 ml/min (30 mm i.d.) 118 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② 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.

Method Development / Normal Phase

A - Mobile Phases

CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ 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 (CH_3CN)) 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

- Only immobilized CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ are suitable for the Secondary Screening
- Additional solvent combinations such as $CHCl_3$/Alkane, 1,4-Dioxane/Alkane, Toluene/Alkane, or Acetone/Alkane can also be investigated with CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ 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 adding 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 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 behavior for certain basic compounds than the most commonly used DEA.
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 Samples require Basic additives❶❷	Acidic Samples require Acidic additives
Diethylamine (DEA) 2-Aminoethanol (AE) Ethylenediamine (EDA) Butyl amine (BA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid
< 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*

☞ *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.*

Column Care / Maintenance

- ❑ The use of a guard cartridge or guard column is highly recommended for maximum column life.
- ❑ 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…).

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 the same mobile phase, but without the additive.
- ❑ 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 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

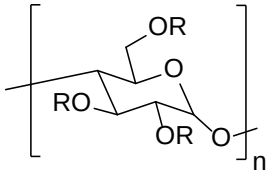
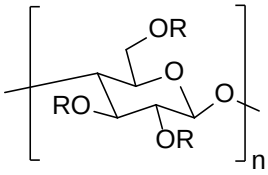
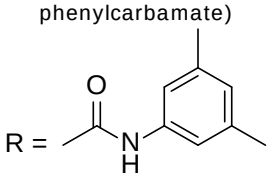
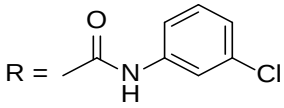
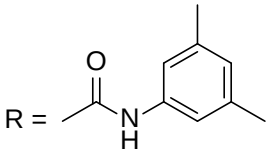
INSTRUCTION MANUAL FOR CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ

<Reversed-Phase>

Please read this instruction manual completely before using these columns.

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

Column Description

AMYLOSE-BASED		CELLULOSE-BASED
		
Immobilized on **5 µm silica gel**		**Immobilized** on **5 µm silica gel**
CHIRALPAK® IA	**CHIRALPAK® ID**	**CHIRALPAK® IB** **CHIRALPAK® IB N-5**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
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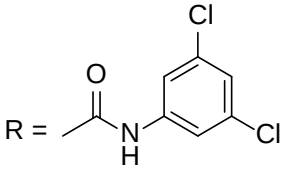
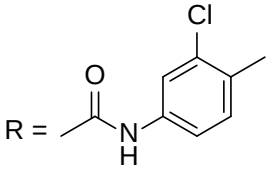
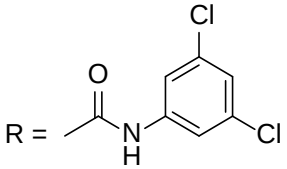
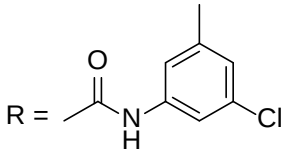
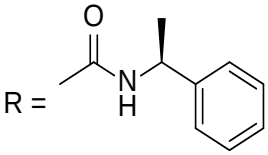
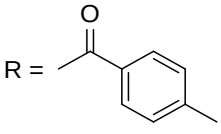
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG	**CHIRALPAK® IH**	**CHIRALPAK® IJ**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

Shipping Solvent:

1. **Hexane/IPA = 90:10 (v/v) for analytical columns (2.1 mm i.d x 150 mm, 4.6 mm i.d. x 150 and 250 mm), guard, and semi-prep columns**
2. **100% Methanol for analytical (4.6 mm i.d. x 50 and 100 mm) columns**

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 different columns, including custom columns, can be shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 20 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**20 x 10 mm i.d.** **50 x 21 mm i.d.** **50 x 30 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min	5 ml/min (10 mm i.d.) 20 ml/min (20 mm i.d.) 42 ml/min (30 mm i.d.) 118 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② 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.

Switching Between RP and NP or SFC

To switch from reversed-phase to normal phase or SFC, and vice versa, the column should be carefully flushed with miscible solvent, such as ethanol or isopropanol. The column should be flushed in a similar manner with ethanol or isopropanol when initially received after purchase, before first used in reversed-phase, as it could contain a hexane/alcohol mixture.

It is highly recommended that the **regeneration procedure** (link below in Column Care section) be used when switching from reversed-phase to normal phase or SFC. Before applying this procedure, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, for example Water/ACN = 60/40, and then flushing with ethanol or isopropanol.

A - Mobile Phases / For Both UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
CHIRALPAK® IA CHIRALPAK® ID CHIRALPAK® IE CHIRALPAK® IF CHIRALPAK® IG CHIRALPAK® IH CHIRALPAK® IB CHIRALPAK® IB N-5 CHIRALPAK® IC CHIRALPAK® IJ	Aqueous Solution ❶	HCOOH aq. pH 2.0	Water	20 mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
	Organic Modifier ❷	ACN or MeOH or EtOH or IPA or THF		
	Typical Starting Conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary aqueous solutions*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
CHIRALPAK® IA CHIRALPAK® ID CHIRALPAK® IE CHIRALPAK® IF CHIRALPAK® IG CHIRALPAK® IH CHIRALPAK® IB CHIRALPAK® IB N-5 CHIRALPAK® IC CHIRALPAK® IJ	Aqueous Solution ❶	50 mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100 mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20 mM Borate Buffer pH 9.0 OR 20 mM Phosphate Buffer pH 8.0 ❻ OR 100 mM KPF_6 (or $NaPF_6$) aq.

☞ *NOTE 2: The concentration of all the buffering salt should be less than 500 mM.*

❶ Refer to **section C** for the preparation of an aqueous solution and choice of basic additives.

❷
- It is recommended to use ACN to start the investigation
- The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50%ACN ≈ 65-70%EtOH ≈ 75-80%MeOH.
- The use of other organic solvents, **except THF**, has not been investigated and could be harmful to the columns.
- The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.

❸
- Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
- Lowering the column temperature may increase the retention time and the selectivity.
- Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- To maximize column life, the use of a guard cartridge or guard column is essential when basic conditions are employed.
- The use of strong basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
- When these columns are used at pH > 7**, the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to subsequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

❻ Do not use a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

C – Buffer Preparation – Examples

- *Preparation of pH 2 Phosphate buffer:*

  Solution A: 50 mM potassium dihydrogenphosphate
  3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water
  Solution B: phosphoric acid (H_3PO_4 85% by weight)
  Adjust the pH of solution A to a value of 2.0 using solution B.

- *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

  Solution A: 100 mM potassium (sodium) hexafluorophosphate
  9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500 ml with HPLC grade water
  Solution B: phosphoric acid (H_3PO_4 85% by weight)
  Adjust the pH of solution A to a value of 2.0 using solution B.

- *Preparation of pH 9 Ammonium bicarbonate solution:*

  Solution A: 20 mM ammonium bicarbonate
  0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500 ml with HPLC grade water
  Solution B Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
  ** DEA tends to give better peak shape than other bases.*
  Adjust the pH of solution A to a value of 9.0 using solution B.

- *Preparation of pH 8 Phosphate buffer:*

  Solution A: 20 mM potassium hydrogenophosphate
  1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500 ml with HPLC grade water
  Solution B: 20 mM potassium dihydrogenophosphate
  1.36g KH_2PO_4 / FW 136.09, make up the volume to 500 ml with HPLC grade water.
  Adjust the pH of solution A to a value of 8.0 using solution B.

- *Preparation of pH 9 Borate buffer:*

  Solution A: 20 mM sodium tetraborate decahydrate
  3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500 ml with HPLC grade water
  Solution B: 20 mM boric acid
  0.62g H_3BO_3 / FW 61.83, make up the volume to 500 ml with HPLC grade water
  Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- The use of a guard cartridge or guard column is highly recommended for maximum column life.
- 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…).

For detailed Regeneration Procedures, please click here

Column Storage

- For column storage and/or switching to 100% organic solvent, any traces of salts should be removed by flushing the column with a mobile phase which doesn't contain any salts or buffers, for instance Water/ACN = 60/40 (v/v).
- 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 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

INSTRUCTION MANUAL FOR CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ

<Supercritical Fluid Chromatography (SFC)>

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

Column Description

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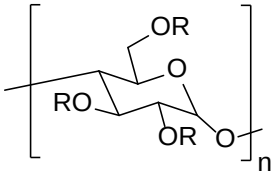
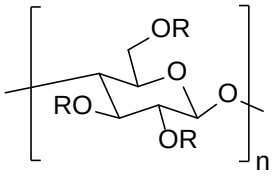
 

Immobilized on 5 µm silica gel</td><td>CELLULOSE-BASED
 

Immobilized on 5 µm silica gel</td></tr>
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CHIRALPAK® IB N-5</td></tr>
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 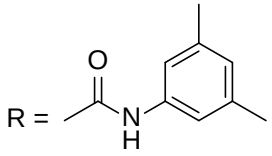
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 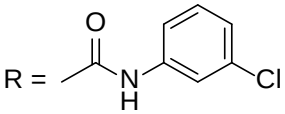
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 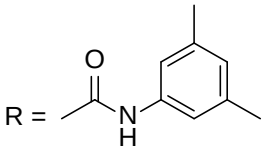
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<tr><td>CHIRALPAK® IE</td><td>CHIRALPAK® IF</td><td>CHIRALPAK® IC</td></tr>
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 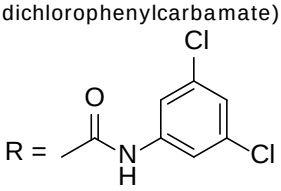
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 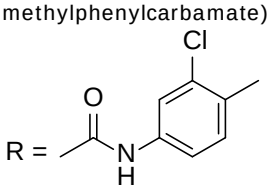
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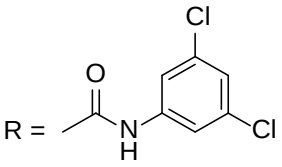
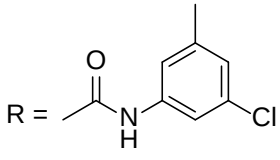
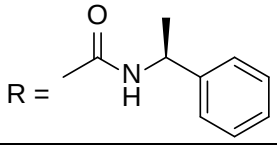
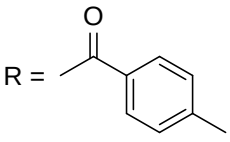
 
</td></tr>
<tr><td>CHIRALPAK® IG</td><td>CHIRALPAK® IH</td><td>CHIRALPAK® IJ</td></tr>
<tr><td>Amylose tris(3-chloro-5-methyl-phenylcarbamate)
 
</td><td>Amylose tris[(S)- α-methylbenzylcarbamate]
 
</td><td>Cellulose tris(4-methylbenzoate)
 
</td></tr>
<tr><td colspan="3">Shipping Solvent:
1. Hexane/IPA = 90:10 (v/v) for analytical columns (2.1 mm i.d x 150 mm, 4.6 mm i.d. x 150 and 250 mm), guard, and semi-prep columns
2. 100% Methanol for analytical (4.6 mm i.d. x 50 and 100 mm) columns

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>

Because different columns, including custom columns, can be shipped in different solvents, 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 5).

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 20 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**10 x 4.0 mm i.d.** Guard Cartridge	**20 x 10 mm i.d.** **50 x 21 mm i.d.** **50 x 30 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate **in SFC**	0.5-1.0 ml/min	1.0-5.0 ml/min	15 ml/min (10 mm i.d.) 60 ml/min (20 mm i.d.) 120 ml/min (30 mm i.d.) 350 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Method Development / SFC

A - Mobile Phases

CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ can be used *with all ranges of organic miscible solvents as modifiers combined with supercritical carbon dioxide (CO_2),* progressing from the traditional solvents used with other DAICEL columns (mixtures of CO_2 with alcohols or acetonitrile (CH_3CN)) to mobile phases containing CO_2 with 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 trying the Primary Screening with the product dissolved in a stronger solvent (DCM/alcohol...).

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ Alcohols can be added into ACN 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_2 / THF	CO_2 / (DCM+MeOH 90:10)	CO_2 / (EtOAc+MeOH 90:10)	CO_2 / (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

Notes: 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.

All solvent proportions indicated in this manual are by volume.

C – General Comments

⇨ Only immobilized CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ are suitable for the Secondary Screening.

⇨ Additional modifiers such as $CHCl_3$, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ.

⇨ 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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

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

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_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.

Column Care / Maintenance

- ❑ The use of a guard cartridge or guard column is highly recommended for maximum column life.
- ❑ 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…).

For detailed Regeneration Procedures, please click here

☞ ***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+modifier 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

(*) *This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

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 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

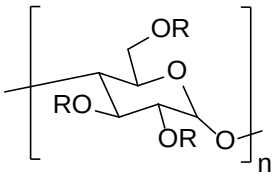
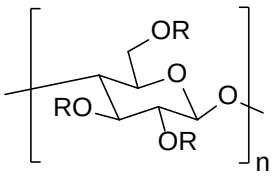
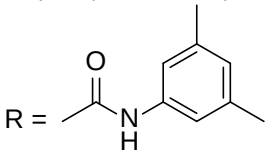
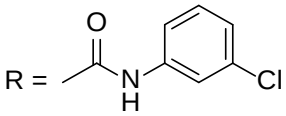
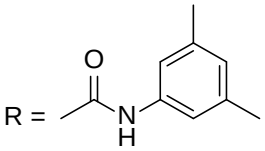
INSTRUCTION MANUAL FOR CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10

<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

AMYLOSE-BASED		CELLULOSE-BASED
 **Immobilized** on **10 µm silica gel**		 **Immobilized** on **10 µm silica gel**
CHIRALPAK® IA-10	**CHIRALPAK® ID-10**	**CHIRALPAK® IB N-10**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
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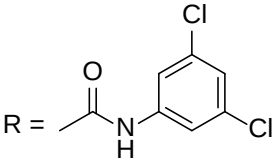
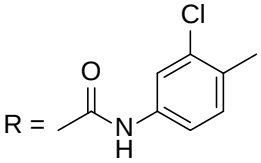
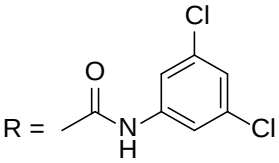
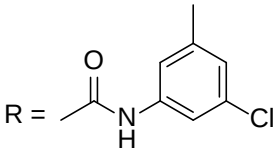
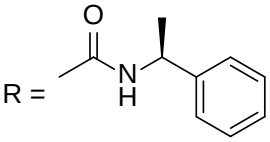
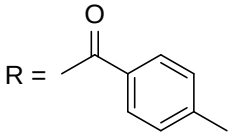
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG-10	**CHIRALPAK® IH-10**	**CHIRALPAK® IJ-10**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

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.

***Because different columns, including custom columns, can be shipped in different solvents, we recommend**

flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.*

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**50 x 4.6 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

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.

Method Development / Normal Phase

A - Mobile Phases

CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10 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 (CH_3CN)) 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

- Only immobilized CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10 are suitable for the Secondary Screening
- Additional solvent combinations such as $CHCl_3$/Alkane, 1,4-Dioxane/Alkane, Toluene/Alkane, or Acetone/Alkane can also be investigated with CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10 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 adding 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 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 behavior for certain basic compounds than the most commonly used DEA.
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 Samples require Basic additives❶❷	Acidic Samples require Acidic additives
Diethylamine (DEA) 2-Aminoethanol (AE) Ethylenediamine (EDA) Butyl amine (BA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid
< 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*

☞ *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.*

Column Care / Maintenance

- ❑ The use of a guard column is highly recommended for maximum column life.
- ❑ 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…).

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 the same mobile phase, but without the additive.
- ❑ 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 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

INSTRUCTION MANUAL FOR CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10

<Reversed-Phase>

Please read this instruction manual completely before using these columns.

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

Column Description

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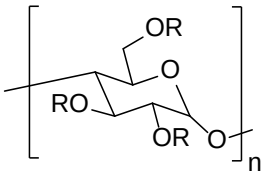
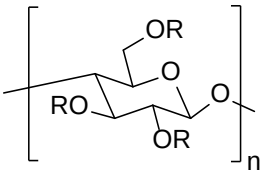
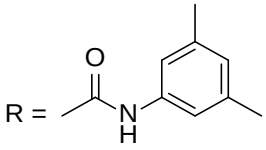
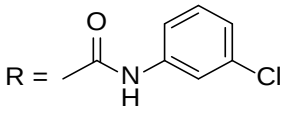
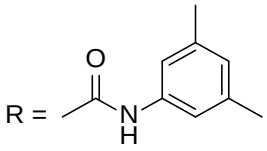
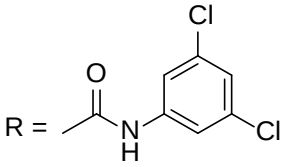
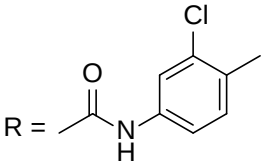
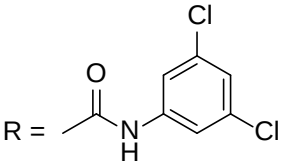
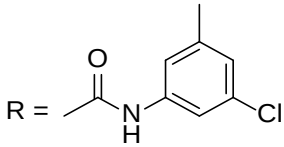
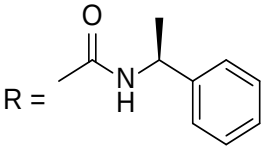
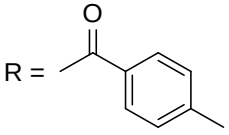
		
Immobilized on **10 µm silica gel**		**Immobilized** on **10 µm silica gel**
CHIRALPAK® IA-10	**CHIRALPAK® ID-10**	**CHIRALPAK® IB N-10**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
CHIRALPAK® IE-10	**CHIRALPAK® IF-10**	**CHIRALPAK® IC-10**
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG-10	**CHIRALPAK® IH-10**	**CHIRALPAK® IJ-10**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

Shipping Solvent:

Water/ACN = 60:40 (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.

Because different columns, including custom columns, can be shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**/ /**	**50 x 4.6 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

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.

Switching Between RP and NP or SFC

To switch from reversed-phase to normal phase or SFC, and vice versa, the column should be carefully flushed with miscible solvent, such as ethanol or isopropanol. The column should be flushed in a similar manner with ethanol or isopropanol when initially received after purchase, before first used in reversed-phase, as it could contain a hexane/alcohol mixture.

It is highly recommended that the **regeneration procedure** (link below in Column Care section) be used when switching from reversed-phase to normal phase or SFC. Before applying this procedure, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, for example Water/ACN = 60/40, and then flushing with ethanol or isopropanol.

A - Mobile Phases / For Both UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
CHIRALPAK® IA-10 CHIRALPAK® ID-10 CHIRALPAK® IE-10 CHIRALPAK® IF-10 CHIRALPAK® IG-10 CHIRALPAK® IH-10 CHIRALPAK® IB N-10 CHIRALPAK® IC-10 CHIRALPAK® IJ-10	Aqueous Solution ❶	HCOOH aq. pH 2.0	Water	20 mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
	Organic Modifier ❷	ACN or MeOH or EtOH or IPA or THF		
	Typical Starting Conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary aqueous solutions*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❹
CHIRALPAK® IA-10 CHIRALPAK® ID-10 CHIRALPAK® IE-10 CHIRALPAK® IF-10 CHIRALPAK® IG-10 CHIRALPAK® IH-10 CHIRALPAK® IB N-10 CHIRALPAK® IC-10 CHIRALPAK® IJ-10	Aqueous Solution ❶	50 mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100 mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20 mM Borate Buffer pH 9.0 OR 20 mM Phosphate Buffer pH 8.0 ❻ OR 100 mM KPF_6 (or $NaPF_6$) aq.

☞ *NOTE 2: The concentration of all the buffering salt should be less than 500 mM.*

❶ Refer to **section C** for the preparation of an aqueous solution and choice of basic additives.

❷
- ❑ It is recommended to use ACN to start the investigation
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50%ACN ≈ 65-70%EtOH ≈ 75-80%MeOH.
- ❑ The use of other organic solvents, **except THF**, has not been investigated and could be harmful to the columns.
- ❑ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.

❸
- ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
- ❑ Lowering the column temperature may increase the retention time and the selectivity.
- ❑ Increasing the column temperature and decreasing the flow rate may increase the resolution.

❹
- ❑ To maximize column life, the use of a guard column is essential when basic conditions are employed.
- ❑ The use of strong basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
- ❑ When these columns are used at pH > 7**, the temperature should be maintained between 5°C and 25°C for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to subsequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

❻ Do not use a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

C – Buffer Preparation – Examples

- *Preparation of pH 2 Phosphate buffer:*

  Solution A: 50 mM potassium dihydrogenphosphate
  3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water
  Solution B: phosphoric acid (H_3PO_4 85% by weight)
  Adjust the pH of solution A to a value of 2.0 using solution B.

- *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

  Solution A: 100 mM potassium (sodium) hexafluorophosphate
  9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500 ml with HPLC grade water
  Solution B: phosphoric acid (H_3PO_4 85% by weight)
  Adjust the pH of solution A to a value of 2.0 using solution B.

- *Preparation of pH 9 Ammonium bicarbonate solution:*

  Solution A: 20 mM ammonium bicarbonate
  0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500 ml with HPLC grade water
  Solution B Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH_3) and so on.
  ** DEA tends to give better peak shape than other bases.*
  Adjust the pH of solution A to a value of 9.0 using solution B.

- *Preparation of pH 8 Phosphate buffer:*

  Solution A: 20 mM potassium hydrogenophosphate
  1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500 ml with HPLC grade water
  Solution B: 20 mM potassium dihydrogenophosphate
  1.36g KH_2PO_4 / FW 136.09, make up the volume to 500 ml with HPLC grade water.
  Adjust the pH of solution A to a value of 8.0 using solution B.

- *Preparation of pH 9 Borate buffer:*

  Solution A: 20 mM sodium tetraborate decahydrate
  3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500 ml with HPLC grade water
  Solution B: 20 mM boric acid
  0.62g H_3BO_3 / FW 61.83, make up the volume to 500 ml with HPLC grade water
  Adjust the pH of solution A to a value of 9.0 using solution B.

Column Care / Maintenance

- The use of a guard column is highly recommended for maximum column life.
- 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…).

For detailed Regeneration Procedures, please click here

Column Storage

- For column storage and/or switching to 100% organic solvent, any traces of salts should be removed by flushing the column with a mobile phase which doesn't contain any salts or buffers, for instance Water/ACN = 60/40 (v/v).
- 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 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

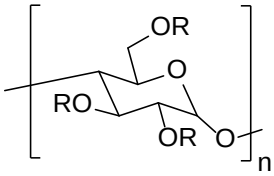
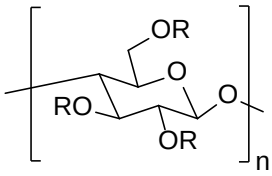
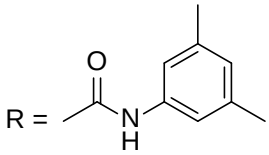
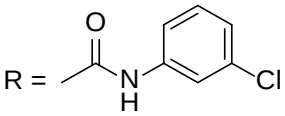
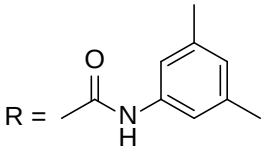
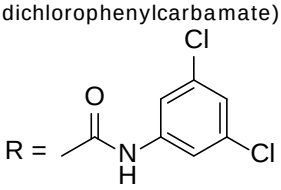
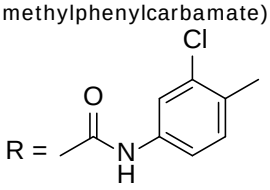
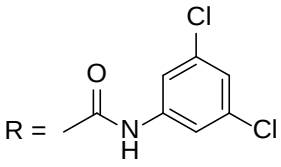
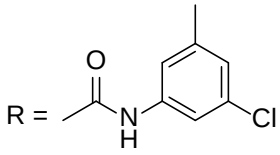
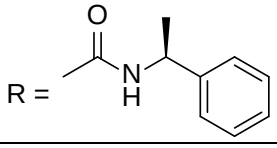
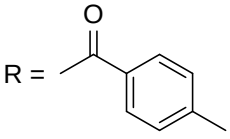
INSTRUCTION MANUAL FOR CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction manual completely before using these columns.

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

Column Description

AMYLOSE-BASED		CELLULOSE-BASED
		
Immobilized on **10 µm silica gel**		**Immobilized** on **10 µm silica gel**
CHIRALPAK® IA-10	**CHIRALPAK® ID-10**	**CHIRALPAK® IB N-10**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethylphenylcarbamate) 
CHIRALPAK® IE-10	**CHIRALPAK® IF-10**	**CHIRALPAK® IC-10**
Amylose tris(3,5-dichlorophenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(3,5-dichlorophenylcarbamate) 
CHIRALPAK® IG-10	**CHIRALPAK® IH-10**	**CHIRALPAK® IJ-10**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(4-methylbenzoate) 

Shipping Solvent:

1. **Methanol = 100%**

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 different columns, including custom columns, can be shipped in different solvents, 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 5).

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	**150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns	**250 x 10 mm i.d.**① **250 x 21 mm i.d.**① **250 x 30 mm i.d.**① **250 x 50 mm i.d.**① Semi-Prep Columns
Guard	**/ /**	**50 x 4.6 mm i.d.** Guard Column	**/ /**
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate **in SFC**	0.5-1.0 ml/min	1.0-5.0 ml/min	15 ml/min (10 mm i.d.) 60 ml/min (20 mm i.d.) 120 ml/min (30 mm i.d.) 350 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 21 mm i.d) of eluent at the beginning of each preparative work.

② The relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) 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 withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Method Development / SFC

A - Mobile Phases

CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10 can be used *with all ranges of organic miscible solvents as modifiers combined with supercritical carbon dioxide (CO_2),* progressing from the traditional solvents used with other DAICEL columns (mixtures of CO_2 with alcohols or acetonitrile (CH_3CN)) to mobile phases containing CO_2 with 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 trying the Primary Screening with the product dissolved in a stronger solvent (DCM/alcohol...).

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

❶ Alcohols can be added into ACN 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_2 / THF	CO_2 / (DCM+MeOH 90:10)	CO_2 / (EtOAc+MeOH 90:10)	CO_2 / (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

Notes: 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.

All solvent proportions indicated in this manual are by volume.

C – General Comments

⇨ Only immobilized CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10 are suitable for the Secondary Screening.

⇨ Additional modifiers such as $CHCl_3$, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA-10, IB N-10, IC-10, ID-10, IE-10, IF-10, IG-10, IH-10, and IJ-10.

⇨ 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

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

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO_2) are sometimes enough to elute the product properly.

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

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_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.

Column Care / Maintenance

- The use of a guard column is highly recommended for maximum column life.
- 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…).

For detailed Regeneration Procedures, please click here

☞ ***Column transfer between modes:***

From LC to SFC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with 100% CO_2 or CO_2+modifier at 0.25 ml/min(*) for 45 min

From SFC to LC

- Flush with 100% EtOH at 0.25 ml/min(*) for 45 min
- Flush with the mobile phase at 0.25 ml/min(*) for 45 min

(*) *This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

Column Storage

- For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% EtOH 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 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

INSTRUCTION MANUAL FOR 2L-ChiralTLC (IA, IC, ID, IE, IF)

Please read this instruction sheet completely before using these plates

TLC Description

Plate Specification :

Immobilized-type CSPs (Chiral stationary phases) and silica-gel with a fluorescence indicator (254nm) in the form of a bilayer on an aluminum plate.

Base Material : Aluminum

Chiral selectors of CSP layer :

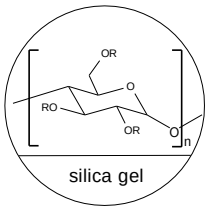
IA : Amylose tris(3,5-dimethylphenylcarbamate) IC

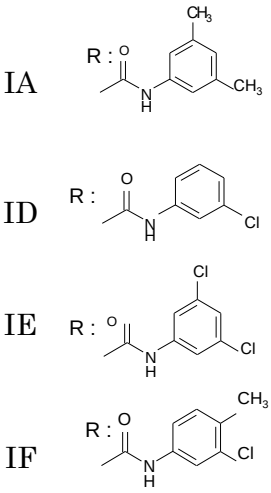
: Cellulose tris(3,5-dichlorophenylcarbamate)

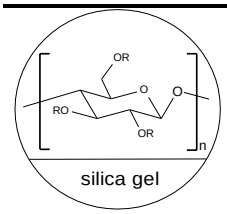
ID : Amylose tris(3-chlorophenylcarbamate)

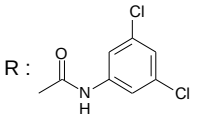
IE : Amylose tris(3,5-dichlorophenylcarbamate)

IF : Amylose tris(3-chloro-4-methylphenylcarbamate)









Operating Instructions

Size	200 mm width X 100 mm length
Layer Thickness	approximately 270 μm (CSP layer + silica-gel layer)
Particle size	CSP : 20μm
Recommended sample amount	1 ～ 5 μL
Detection	Silica-gel layer includes a fluorescence indicator (UV254nm). Samples which have ultraviolet absorption can be observed as spots (shadow). Staining reagent can also be used.

Important reminder

- 2L-ChiralTLC are intended only for research and experimental proposes.
- Intended for use with organic solvents only. Use with aqueous solvents can cause coating to wash off.
- An impact shock or stress should be avoided as, the base material is aluminium. TLC plates that are cut may have sharp edges. To prevent from injuring fingers, please use proper protective equipment.
- Do not press and rub the surface of TLC plates. Silica-gel layer may lift off. When you cut a TLC plate, protection of the surface of the TLC plate is recommended.
- UV lamps should be handled in accordance with their instruction manuals.
- Once the vacuum-sealed package is opened, unused plates should be stored in a desiccator with a desiccant.

How to use 2L-ChiralTLC

1) Preparation of TLC plates

Open a vacuum-sealed package and remove a 2L-ChiralTLC plate. Confirm the position of "sample application zone" and the direction of developing by reference to Fig. 1. "Sample application zone" has only CSP layer (no silica-gel layer). The type of CSP is printed on the top of TLC plate.

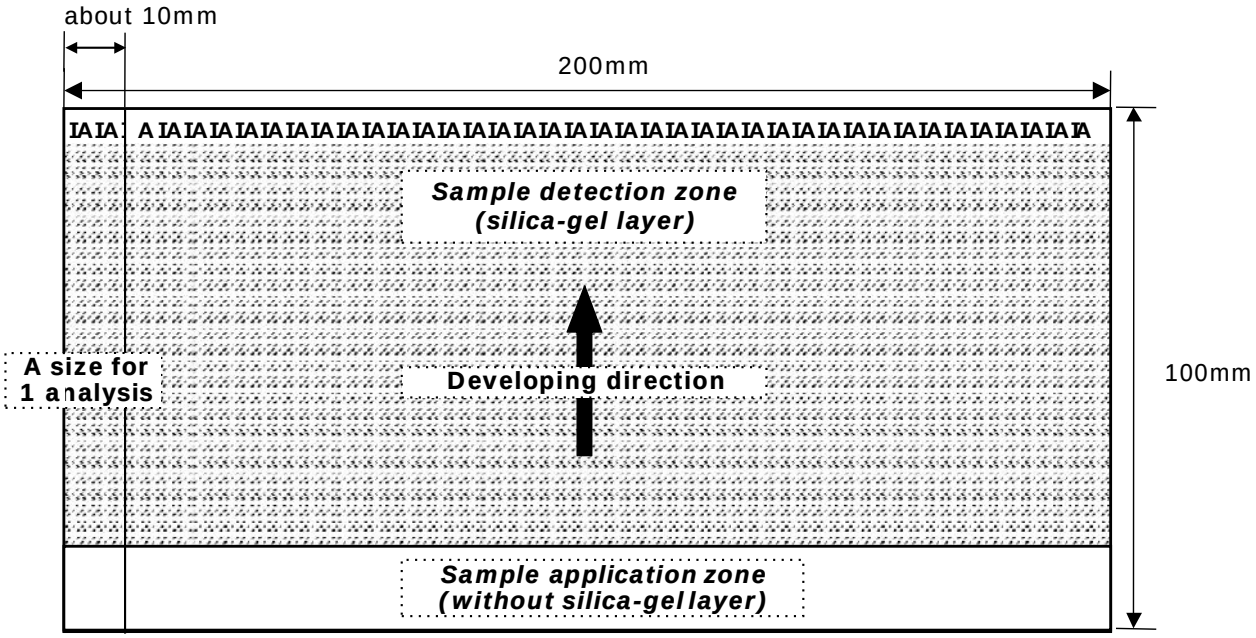


Fig. 1. An example of 2L-ChiralTLC and the size for 1 analysis (20 analyses per 1 plate)

Cut 2L-ChiralTLC into a proper size with scissors. 10mm in width per one analysis is recommended. Do not press the surface of the TLC plate with fingers to prevent from peeling the Silica-gel coat. When you cut a TLC plate, it is recommended to protect the surface to avoid contamination with your fingers. In addition, to prevent from injuring fingers, please use proper protective equipment.

2) Sample spotting

Spot the sample on "sample application zone". ("Sample application zone" is the bottom end of the TLC plate where only the CSP is coated) The recommended spotting position is within approximately 10 mm of the bottom end of the TLC. (Fig. 2). Best spotting results are obtained by dipping a microcapillary pipet into a sample solution, removing the pipet, and gently touching the end of the pipet to the surface of the TLC plate until a spot of no more than 2 mm in diameter is obtained. Allow spot to dry, and repeat spotting procedure if necessary, to increase the amount of sample on the plate. All spots should be dry before proceeding to developing step.

Before spotting, it is recommended to verify the UV detection of the sample by spotting on the top of the silica-gel layer zone. (Fig. 3)

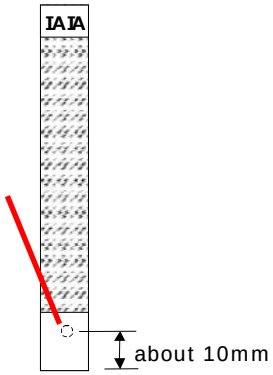


Fig. 2. The position of Sample spotting

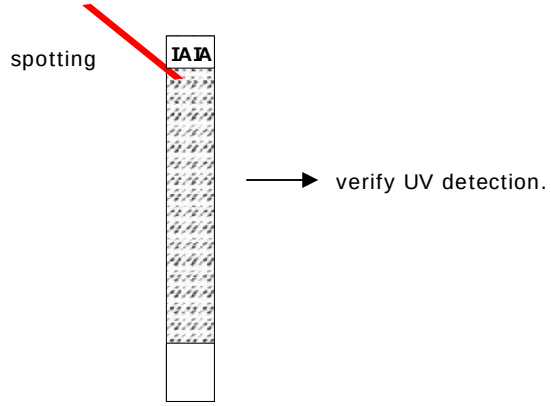


Fig. 3. The verification of UV detection of the Sample

3) Developing (Sample separation)

Put a filter paper into the developing chamber and fill with a saturated vapor of the solvent. Then, put the TLC plate in a developing chamber. Confirm that the solvent level is lower than the position of sample spot (Fig. 4). After the TLC has been developed, dry the TLC plate.

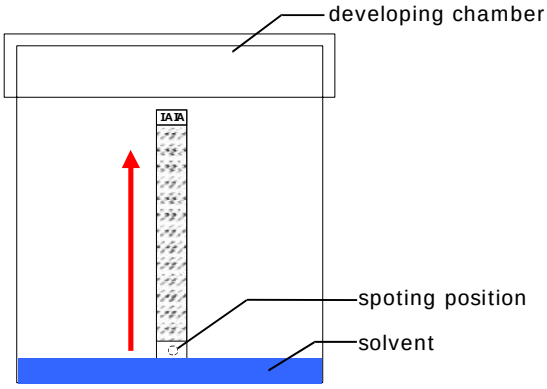


Fig. 4. Developing

4) UV detection

Irradiate UV light to the TLC plate and observe the sample spot (shadow) in the sample detection zone. Measure the distance of the sample spots from the sample spotted position (A and B in Fig. 5) and the solvent front from the sample spotted position (C in Fig. 5). Calculate Rf (Relative to Front) value. (Fig. 5)

If sample spots can't be observed, spraying and drying an organic solvent (e.g. Ethanol) on the surface of TLC plate may help the detection. An accompanying atomizer is for this purpose.

In the case of detection of samples which don't have enough UV absorption, use staining reagent to detect the spot. (staining reagent : phosphomolybdic acid-ethanol, iodine stain, p-anisaldehyde, or ninhydrin) If you need to use staining reagent, it is recommended to verify the coloring and the detection of the sample by spotting the sample on a TLC plate before using 2L-ChiralTLC® (an achiral standard silica-gel TLC plate is recommended).

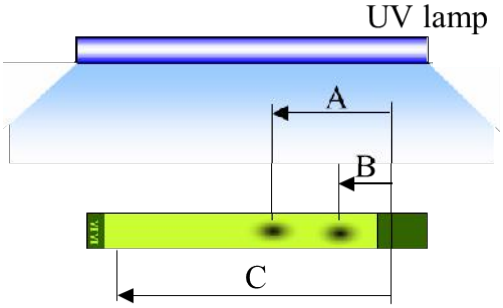


Fig. 5. UV detection

$$Rf_1 = A / C$$
$$Rf_2 = B / C$$

Recommended Method

A - Solvents

2L-ChiralTLC can be used with a wide range of organic miscible solvents, such as mixtures of alkanes/alcohol, pure alcohol or acetonitrile (CH_3CN)) to developing solvents containing methyl *tert*-butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform ($CHCl_3$), ethyl acetate (EtOAc) among others.

We recommend that the conditions shown in Table 1 are used as the basis for initial method development for 2L-ChiralTLC. After the initial evaluation the most promising methods can be optimised using the suggested ranges below. MtBE and chlorinated solvents may also be used in their pure form as developing solvents. Moreover, in the case of solvents with strong elution intensity, such as THF and ethyl acetate, it is advised to mix them with a hydrocarbon solvent (e.g. hexane or heptane) to modulate retention and selectivity.

Table 1. Recommended organic miscible solvents

	Alkane❶/ Alcohol❷	Alkane❶/ EtOAc	Alkane❶/ $CHCl_3$	Alkane❶/ THF	MTBE / EtOH
Typical starting conditions	90:10	90:10	70:30	90:10	100:0
Advised optimization range	95:5 ~ 0:100❸	95:5 ~ 0:100	95:5 ~ 0:100	95:5 ~ 0:100	100:0 ~ 40:60

❶ Alkane = n-Hexane, iso-Hexane or n-Heptane. Some small selectivity differences may sometimes be found.
❷ Methanol, ethanol, and 2-propanol are raised as typical alcohol.
Moreover, as alcohol other than the above, 1-propanol, 1-butanol, 2-butanol, etc. can be used.
Depending on a sample, separation may change greatly with kinds of alcohol.
❸ As for the mixed solvent of alcohol, viscosity may become high with composition. Assuming to use CHIRALFLASH, please adjust the composition of solvent mixture not to exceed the maximum working pressure range of CHIRALFLASH. Usually, higher composition of alcohol gives shorter retention time and higher Rf value.
Due to limited miscibility of MeOH in Alkane, it is necessary to add an appropriate volume of EtOH or the other alcohol listed above together with MeOH in order to obtain homogenous solvent mixtures. A maximum of 5% MeOH in n-Hexane only may be used without adding other alcohol.

For initial and routine method development, it is recommended to use pure alcohol, EtOAc, THF, MTBE, $CHCl_3$ and mixtures of alkanes. For higher productivity on CHIRALFLASH, it is effective to use a solvent where the solubility of the sample is comparatively high as an initial examination solvent.

B – Additives

For basic samples or acidic samples, it is necessary to add an additive into the developing solvent in order to get appropriate spots, otherwise broad and/or tailing spot form may be observed.

1. Add 0.1 volume percent of acid (for acidic samples) or base (for basic samples) to developing solvent.
2. Before developing, soak TLC plate for about 1 minute in above solvent and dry it. This will ensure that the entire plate has been conditioned with the chosen additive.
3. Once the above steps are taken, the TLC plate is ready to be used.

Depending on samples, EDA or AE is often more effective than DEA. If you use these additives, 2 volume percent of alcohol can be necessary to be completely mixed, because of their low miscibility to low polar solvents.

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

⇨ ***STRONGLY BASIC solvent additives or sample solutions <u>MUST BE AVOIDED</u>, as they are likely to damage the silica gel used in this TLC.***

Method Development for CHIRALFLASH

The CSP used for 2L-ChiralTLC and CHIRALFLASH is the same (i.e. 20μm CSP). Therefore, the eluent used on 2L-ChiralTLC is directly applicable to CHIRALFLASH, if the sample is separated on 2L-ChiralTLC. The protocol of method development for CHIRALFLASH using 2L-ChiralTLC is described below.

1. Spot sample solutions and develop with each 2L-ChiralTLC (IA, IC, ID, IE and IF) and each solvent (Refer to the Table 1). [Screening]

2. If good separation is achieved on TLC, the mobile phase can be directly transfered to the separation on the corresponding CHIRALFLASH. If a good separation on CHIRALFLASH is confirmed, move to the optimization of separation conditions (injection amount or mobile phase etc.). If the method is being transferred to CHIRALFLASH, it may be desirable to optimize the separation to obtain higher Rf values, since this will speed up the separation and use less solvent

- The example of separation of Flavanone(FLV)

1. Sample solutions are spotted on 2L-ChiralTLC IA, IC and developed with several solvents. (Fig. 6) (In case of no separation, try other combinations of CSP types and mobile phases.)

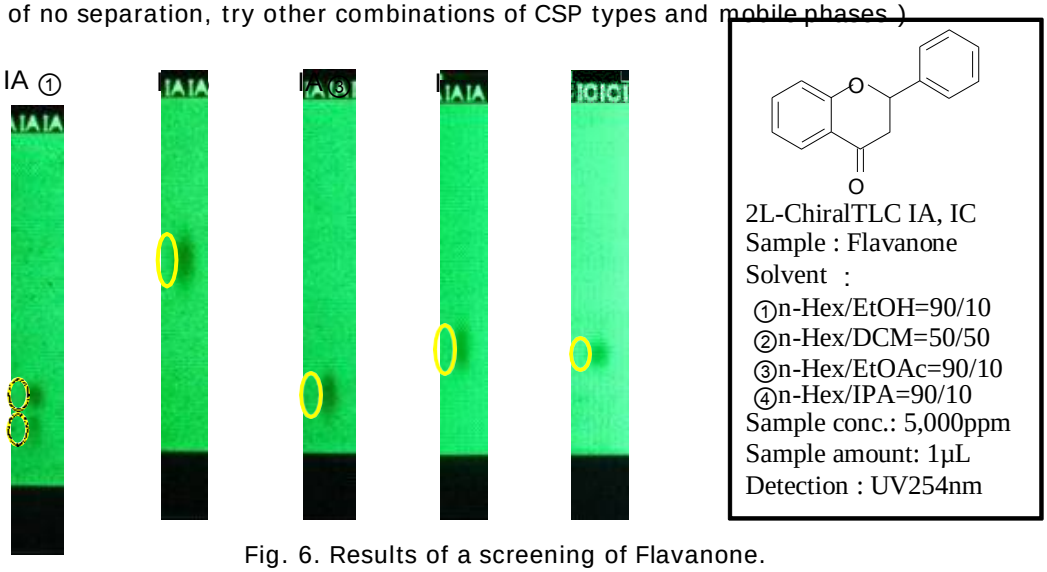


Fig. 6. Results of a screening of Flavanone.
(n-Hex=n-Hexane, EtOH=Ethanol, DCM=Dichloromethane, EtOAc=Ethyl acetate, IPA=2-Propanol)

2. Inject a certain amount of sample on CHIRALFLASH with the same mobile phase with TLC. If a good separation on CHIRALFLASH is confirmed, increase the sample loading amount and fix the separation conditions. (Fig. 7)

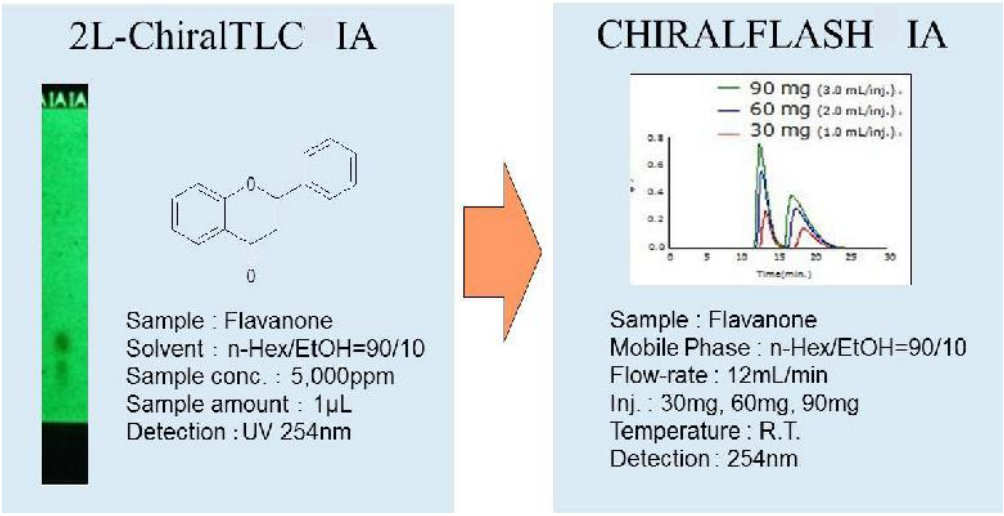
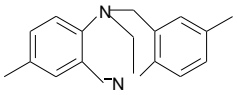


Fig 7. The optimization of separation conditions on CHIRALFLASH IA

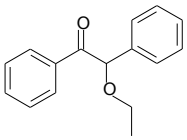
Applications of 2L-ChiralTLC

Applications of 2L-ChiralTLC IA

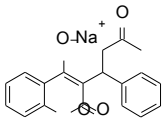
1. Tröger's base (TB)
Solvent : n-Hex/EtOH=90/10 (v/v)
Sample conc. : 20mg/mL
Sample amount : 1μL
Detection : : UV254nm



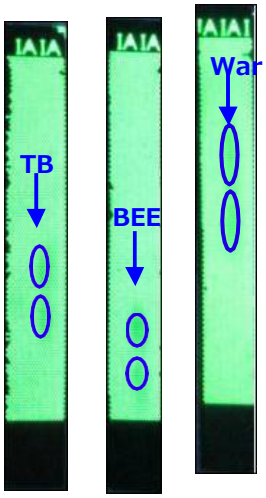
2. Benzoin Ethyl Ether (BEE)
Solvent : n-Hex/EtOAc=90/10 (v/v)
Sample conc. : 10mg/mL
Sample amount : 1μL
Detection : : UV254nm



3. Warfarin Sodium (War)
Solvent : n-Hex/IPA/TFA=50/50/0.1 (v/v/v)
Sample conc. : 25mg/mL
Sample amount : 1μL
Detection : : UV254nm

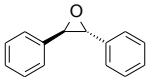


1. TB : Rf_1=0.49, Rf_2=0.35
2. BEE : Rf_1=0.33, Rf_2=0.19
3. War : Rf_1=0.78, Rf_2=0.60

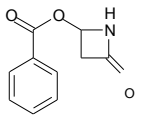


Applications of 2L-ChiralTLC IC

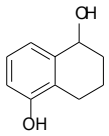
1. trans-Stilbene oxide (TSO)
Solvent : n-Hex/IPA=90/10 (v/v)
Sample conc. : 50mg/mL
Sample amount : 1μL Detection
: : UV254nm



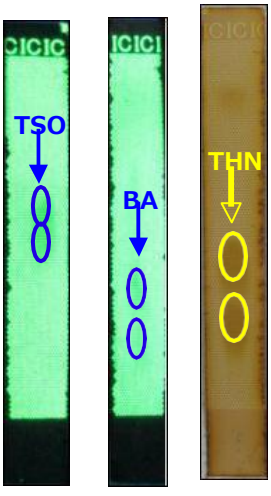
2. 4-Benzoyloxy-2-azetidinone (BA)
Solvent : n-Hex/IPA=50/50 (v/v)
Sample conc. : 100mg/mL
Sample amount : 1μL
Detection : : UV254nm



3. 1,5-dihydroxy-1,2,3,4-tetrahydronaphthalene(THN)
Solvent : DCM/MeOH/AcOH=100/0.1/0.1(v/v/v)
(twice developing)
Sample conc. : 50mg/mL
Sample amount : 1μL
Detection : : Iodine stain



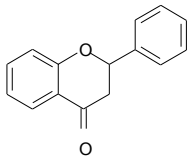
1. TSO : Rf_1=0.61, Rf_2=0.54
2. BA : Rf_1=0.41, Rf_2=0.28
3. THN : Rf_1=0.46, Rf_2=0.26



Applications of 2L-ChiralTLC ID

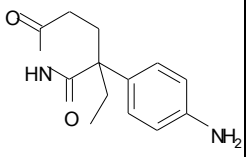
1. Flavanone (FLV)
Solvent : MeOH=100
Sample conc. : 5mg/mL
Sample amount : 1μL

Detection : : UV 254nm



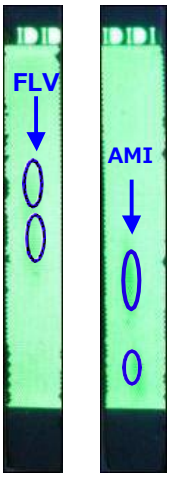
2. Aminoglutethimide (AMI)
Solvent : EtOAc/DEA=100/0.1 (v/v)
Sample conc. : 20mg/mL
Sample amount : 1μL

Detection : : UV254nm



1. FLV : Rf_1=0.64, Rf_2=0.49

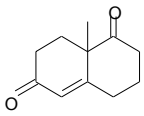
2. AMI : Rf_1=0.44, Rf_2=0.16



Applications of 2L-ChiralTLC IE

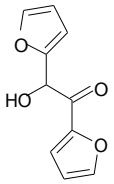
1. Wieland Miescher ketone (WMK)
Solvent : n-Hex/EtOH=60/40 (v/v)
(twice developing)
Sample conc. : 10mg/mL
Sample amount : 1μL

Detection : : UV 254nm



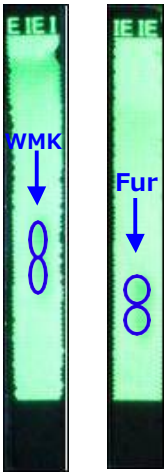
2. Furoin (Fur)
Solvent : n-Hex/EtOH=70/30 (v/v)
Sample conc. : 20mg/mL
Sample amount : 1μL

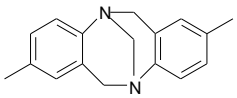
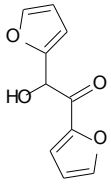
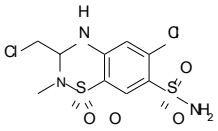
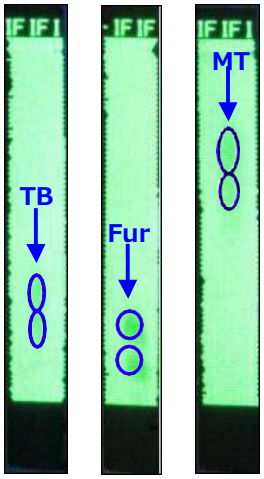
Detection : : UV254nm



1. WMK : Rf_1=0.51, Rf_2=0.41

2. Fur : Rf_1=0.37, Rf_2=0.28



Applications of 2L-ChiralTLC IF	
1. Tröger's base (TB) Solvent : MeOH=100 Sample conc. : 20mg/mL Sample amount : 1μL Detection : : UV 254nm  2. Furoin (Fur) Solvent : n-Hex/EtOH/MeOH=75/15/15 (v/v/v) Sample conc. : 50mg/mL Sample amount : 1μL Detection : : UV254nm  3. Methyclothiazide (MT) Solvent : n-Hex/EtOH/DEA=50/50/0.1 (v/v/v) (twice developing) Sample conc. : 10mg/mL Sample amount : 2μL Detection : : UV254nm 	1. TB : Rf_1=0.38, Rf_2=0.28 2. Fur : Rf_1=0.28, Rf_2=0.16 3. MT : Rf_1=0.76, Rf_2=0.65 

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

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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

INSTRUCTION MANUAL FOR CHIRALPAK® AGP

Please read this instruction sheet completely before using this column

Column Description

CHIRALPAK® AGP : α_1-acid glycoprotein immobilized on 5 µm silica-gel.

Shipping solvent: **Water / 2-Propanol (2-PrOH) solvent mixture (85/15 v/v)**

All columns have been pre-tested before packaging. The test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

Application Scope

CHIRALPAK® AGP has very broad applicability and is suitable for enantiomer resolution of all types of compounds, including:

- amines (primary, secondary, tertiary and quaternary ammonium compounds)
- strong and weak acids
- non-ionisable compounds (amides, esters, alcohols, sulfoxides, etc)

Operating Conditions

	50 x 2.1 mm i.d.*1 100 x 2.1 mm i.d.*1 150 x 2.1 mm i.d.*1 Analytical column	**50 x 3 mm i.d.*1 100 x 3 mm i.d. 150 x 3 mm i.d.** Analytical column	**50 x 4 mm i.d.*1 100 x 4 mm i.d. 150 x 4 mm i.d.** Analytical column	**100 x 10 mm i.d. 150 x 10 mm i.d.** Semi-prep. column
Flow direction	As indicated on the column label			
Typical Flow rate	0.2 mL/min	0.5 mL/min	0.9 mL/min	4.0 mL/min
pH range	4.0 - 7.0			
Recommended temperature*2	20 - 30°C			
Buffer concentration	up to 100 mM, typically 10-20 mM			
Organic modifier ratio	0-15% by volume			
Charged additive concentration	up to 10 mM			

*1 It is very important that the HPLC system is optimized in terms of void volume for work with columns of small dimensions.

*2 The column lifetime might be reduced if used at higher temperature.

A - Mobile Phase Starting Conditions

	ACIDIC Compounds	**NEUTRAL** Compounds	**BASIC** Compounds
Typical starting conditions	**10 mM Ammonium acetate buffer (pH 5.8)❶ / 2-PrOH = 95 / 5 (v/v)**		

❶ Refer to section B for preparation of the buffer.

B – Buffer Preparation - Example

➢ *Preparation of 10 mM Ammonium acetate buffer (1Liter):*

1. Weigh 770.8 mg of ammonium acetate (CH_3COONH_4, purity > 99%) into a beaker.
2. Dissolve the salt with about 800 mL water (HPLC grade), equilibrated at room temperature (20-25℃).
3. Adjust pH to the target value by using either diluted acetic acid or a diluted ammonium hydroxide solution.
4. Filter the solution through a membrane of 0.22 µm into a measuring flask.
5. Add water until the limit line of the measuring flask. Place the stopper in the neck and homogenize the solution by agitation.

When buffer should be mixed with an organic modifier, the measurements are normally by volumes, using preferably volumetric flasks or measuring pipettes.
After mixing, de-gas the mobile phase in an ultrasonic bath.

Note that in the case where a charged additive is needed in the mobile phase, the charged additive should be added into the aqueous solution before the pH adjustment.

C – Mobile Phases

Bacteria grow fast in eluents containing no or low alcoholic organic modifier. Such mobiles phases must be freshly prepared.

❖ ***Buffer***
The salt concentration of ammonium acetate buffer is typically 10-20 mM but can be varied up to 100 mM. The other kinds of buffers, such as sodium or potassium phosphate buffers, sodium acetate buffers, formate or citrate buffers, can also be used. However, the LC-MS compatibility of the method may be sometimes compromised.

❖ ***Organic modifiers***
2-PrOH is the most frequently used. However, methanol, ethanol and acetonitrile can also be investigated. The relative eluting strength can be ranked as follows: 2-PrOH > EtOH ≥ ACN > MeOH

❖ ***Charged additives***
Cationic and anionic additives, such as *N,N*-dimethyloctyl amine (DMOA), trifluoroacetic acid (TFA), octanoic acid (OA), heptafluorobutyric acid (HFBA), can be usedin low concentration (≤ 10 mM) to regulate retention and enantioselectivity. However, some of these additives may be difficult to be removed totally from the column, due to very high affinity to the matrix. Thus, the properties of the column may be affected.

CAUTION: The miscibility of OA and DMOA to water is very limited. Only 2 mM OA or 5 mM DMOA can be homogeneously incorporated into the aqueous solution at ambient temperature. A phase separation may occur beyond these concentrations.

Once a charged additive is used in the mobile phase, the column should be dedicated for the purpose.

D – Samples

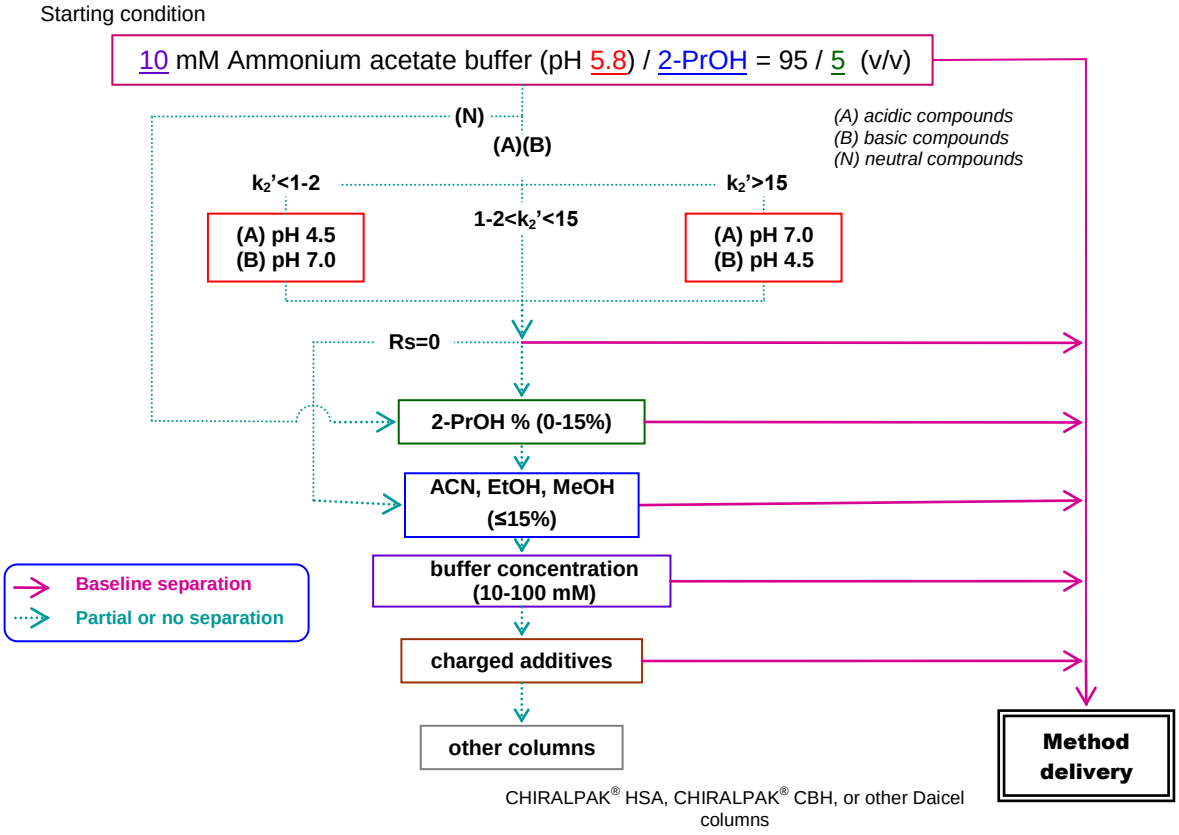
The sample amount injected onto the column should be kept low. The recommended sample concentration is 0.20 mg/mL or lower with an injection volume of 5-10 µL, preferably.

Dissolve the sample in the mobile phase when it is possible. If the sample is insoluble in the mobile phase, add a higher concentration of the organic modifier. The sample solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.

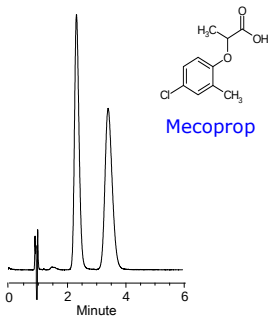
CAUTION: Dissolution of the sample in pure or high percentage of organic solvents may cause on-line sample precipitation.
Do not inject unclear sample solutions or solutions containing undissolved compounds.

Method Development

The following scheme offers a guide for method development and method optimization.

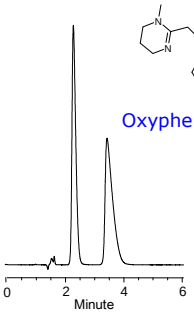


CHIRALPAK® AGP
100 x 4 mm i.d.



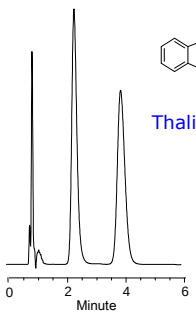
Mecoprop

Buffer (pH 5.8) / 2-PrOH = 95 / 5
Buffer : 10 mM Ammonium acetate



Oxyphencyclimine

Buffer (pH 4.5) / 2-PrOH = 90 / 10
Buffer : 10 mM Ammonium acetate



Thalidomide

Buffer (pH 7.0) / 2-PrOH = 98 / 2
Buffer : 10 mM Ammonium acetate
+ 5mM DMOA

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- If the column has been contaminated with very hydrophobic material, wash the column backwards (no detector connected) over night with Water/2-PrOH 75/25(v/v) at a reduced flow-rate (e.g. 0.3 mL/min for 4 mm ID columns).
- Before disconnecting the column from the HPLC system, flush the column with a mobile phase that does not contain any salts / buffers, e.g. Water/2-PrOH 90/10(v/v).
- For the storage of the column, it is recommended to fill it with Water/2-PrOH 85/15(v/v).
  For short storage period, the column can be placed at ambient temperature (<30°C).
  For longer storage periods, however, it is recommended to place it in a refrigerator.

Important Notice

We recommend the use of a CHIRALPAK® AGP guard cartridge in order to protect the analytical column from any particulates and impurities with high affinity to the stationary phase. Change the guard cartridge regularly, especially in bioanalysis.

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

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

North/Latin America
Chiral Technologies. Inc.
1475 Dunwoody Dr. Ste 310
West Chester, PA 19380
800 6 CHIRAL
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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
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INSTRUCTION MANUAL FOR CHIRALPAK® BSA, CHIRALPAK® DSA, CHIRALPAK® MSA, CHIRALPAK® RSA, and CHIRALPAK® PSA

Please read this instruction sheet completely before using this column

Operating Conditions

Column Size	Column Flushing-1 (mL/min)	Colum Flushing-2 (mL/min)	Typical Flow Rate (mL/min)
2.1 mm i.d. x 50, 100, and 150 mm L	0.15	0.25	0.25
3.0 mm i.d. x 50, 100, and 150 mm L	0.3	0.5	0.5
4.0 mm i.d. x 50, 100, and 150 mm L	0.5	0.9	0.9

- The column is filled with 10% 2-PrOH in water.
- Start at a low flow rate (column flushing-1) and maintain this flow for 2 min.
- Increase the flow rate (column flushing-2) and continue the washing for 10 min.
- Equilibrate the column with the mobile phase to be used.
- Recommended flow rate is 0.25 mL/min with a 2.0 mm I.D. column; 0.5 mL/min with a 3.0 mm I.D. column; 0.9 mL/min with a 4.0 mm I.D. column

Temperature

The column should be used at room temperature or below.

Mobile Phase Composition

- Buffer: Phosphate or acetate buffers should be used (0.01 to 0.1 M)
- pH: The column can be used in the pH-range 5 to 7.
- Organic modifier: 2-Propanol and Acetonitrile are the most commonly used (less than 10%)

Samples

The recommended sample concentration is less than 0.1 mg/mL with an injection volume of 5-20 µL. If possible, dissolve the sample in the mobile phase. If the sample is insoluble in the mobile phase, add a higher concentration of the organic modifier. However, be aware of that too high organic modifier concentration might precipitate the buffer salts.

Avoid dissolving the sample in pure organic solvent.

Do not inject unclear sample solutions or samples containing undissolved compounds.

Column Care /Maintenance

1. The column can be stored at room temperature.
2. When in use, the column can be left in the chromatographic system. However, be careful when buffers without organic modifiers, or buffers containing low concentration of acetonitrile are used, since in such mobile phases bacteria grow fast. Such mobile phases must be freshly prepared.
3. When column is stored for a longer period of time, it is recommended to fill it with 10% 2-PrOH in distilled water.
4. It is recommended to store the column in the refrigerator. Before use, repeat the Operating Conditions.

If the column has been contaminated, wash the column overnight with 10% 2-PrOH in distilled water at a flow rate indicated under (column flushing-1).

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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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
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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

INSTRUCTION MANUAL FOR CHIRALPAK® CBH COLUMN

Please read this instruction sheet completely before using this column

Column Description

CHIRALPAK® CBH : Cellobiohydrolase immobilized on 5 µm silica-gel.

Shipping solvent : **Water / 2-Propanol (2-PrOH) solvent mixture (85/15 v/v)**

All columns have been pre-tested before packaging. The test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

Application Scope

CHIRALPAK® CBH can offer high enantioselectivity for basic compounds, including:

- primary and secondary amines
- non-ionisable compounds (amides, esters, alcohols, sulfoxides, etc)

For compounds of acidic category, however, it is preferred to use CHIRALPAK® AGP and CHIRALPAK® HSA columns.

Operating Conditions

	50 x 2.1 mm i.d.*1 100 x 2.1 mm i.d.*1 150 x 2.1 mm i.d.*1 Analytical column	**50 x 3 mm i.d.*1 100 x 3 mm i.d. 150 x 3 mm i.d.** Analytical column	**50 x 4 mm i.d.*1 100 x 4 mm i.d. 150 x 4 mm i.d.** Analytical column	**100 x 10 mm i.d. 150 x 10 mm i.d.** Semi-prep. column
Flow direction	As indicated on the column label			
Typical Flow rate	0.2 mL/min	0.5 mL/min	0.9 mL/min	4.0 mL/min
pH range	4.0 - 7.0			
Recommended temperature*2	20 - 30°C			
Buffer concentration	up to 100 mM, typically 10-20 mM			
Organic modifier ratio	0-15% by volume			
Charged additive concentration	up to 10 mM			

*1 It is very important that the HPLC system is optimized in terms of void volume for work with columns of small dimensions.

*2 The column lifetime might be reduced if used at higher temperature.

A - Mobile Phase Starting Conditions

	NEUTRAL Compounds	BASIC Compounds
Typical starting conditions	**10 mM Ammonium acetate buffer (pH 5.8)❶ / 2-PrOH = 95 / 5 (v/v)**	

❶ Refer to section B for preparation of the buffer.

B – Buffer Preparation - Example

➢ *Preparation of 10mM Ammonium acetate buffer (1Liter):*

1. Weigh 770.8 mg of ammonium acetate (CH_3COONH_4, purity > 99%) into a beaker.
2. Dissolve the salt with about 800 mL water (HPLC grade), equilibrated at room temperature (20-25℃).
3. Adjust pH to the target value by using either diluted acetic acid or a diluted ammonium hydroxide solution.
4. Filter the solution through a membrane of 0.22 µm into a measuring flask.
5. Add water until the limit line of the measuring flask. Place the stopper in the neck and homogenize the solution by agitation.

When buffer should be mixed with an organic modifier, the measurements are normally by volumes, using preferably volumetric flasks or measuring pipettes.
After mixing, degas the mobile phase in an ultrasonic bath.

C – Mobile Phases

Bacteria grow fast in eluents containing no or low alcoholic organic modifier. Such mobiles phases must be freshly prepared.

❖ ***Buffer***
The salt concentration of ammonium acetate buffer is typically 10-20 mM but can be varied up to 100 mM. The other kinds of buffers, such as sodium or potassium phosphate buffers, sodium acetate buffers, formate or citrate buffers, can also be used. However, the LC-MS compatibility of the method may be sometimes compromised.

❖ ***Organic Modifiers***
2-PrOH is the most frequently used. However, methanol, ethanol and acetonitrile can also be investigated. The relative eluting strength can be ranked as follows: 2-PrOH > EtOH ≥ ACN > MeOH

❖ ***Charged additives***
No charged additives are needed.

D – Samples

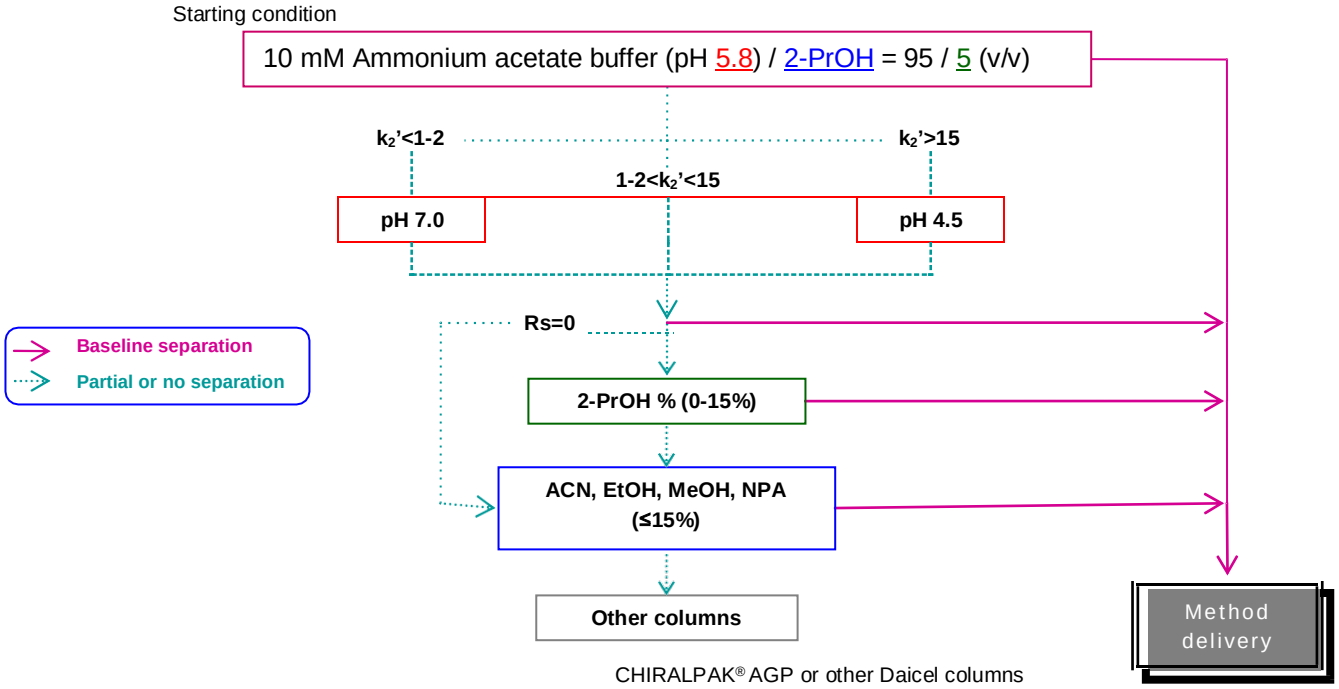
The sample amount injected onto the column should be kept low enough. The recommended sample concentration is 0.20 mg/mL or lower with an injection volume of 5-10 µL, preferably.

Dissolve the sample in the mobile phase when it is possible. If the sample is insoluble in the mobile phase, add a higher concentration of the organic modifier. The sample solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.

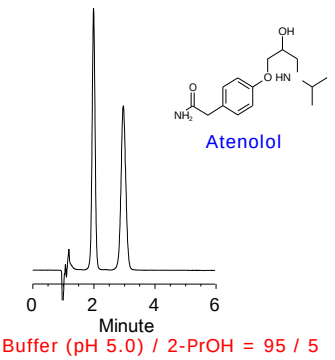
CAUTION: Dissolution of the sample in pure or high percentage of organic solvents may cause on-line sample precipitation.
Do not inject unclear sample solutions or solutions containing undissolved compounds.

Method Development

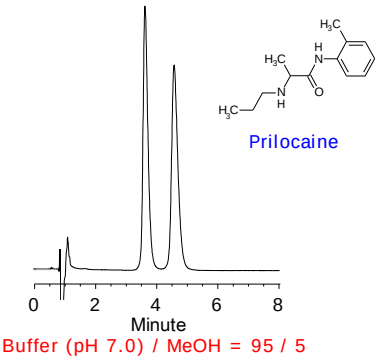
The following scheme offers a guide for method development and method optimization.



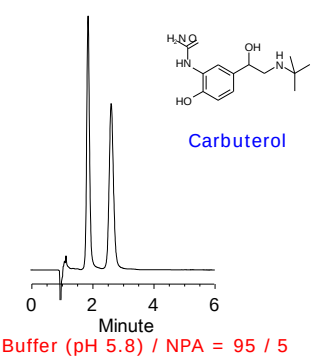
CHIRALPAK® CBH
100 x 4 mm i.d.



Buffer (pH 5.0) / 2-PrOH = 95 / 5
Buffer : 10 mM Ammonium acetate



Buffer (pH 7.0) / MeOH = 95 / 5
Buffer : 10 mM Ammonium acetate



Buffer (pH 5.8) / NPA = 95 / 5
Buffer : 10 mM Ammonium acetate

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- If the column has been contaminated, wash the column backwards (no detector connected) with a mobile phase containing 2.5 mM disodium EDTA (ethylenediamine tetraacetic acid). In case the column still shows bad chromatographic performance, backflush with 10 mM phosphate buffer pH 3.5 containing 15% 2-PrOH at a reduced flow rate (e.g. 0.5 mL/min for 4 mm ID columns) for about 1 hour.
- Before disconnecting the column from the HPLC system, flush the column with a mobile phase that does not contain any salts / buffers, e.g. Water/2-PrOH 90/10(v/v).
- For the storage of the column, it is recommended to fill it with Water/2-PrOH 85/15(v/v).
  For short storage period, the column can be placed at ambient temperature (<30°C).
  For longer storage periods, however, it is recommended to place it in a refrigerator.

Important Notice

We recommend the use of a CHIRALPAK® CBH guard cartridge in order to protect the analytical column from any particulates and impurities with high affinity to the stationary phase. Change the guard cartridge regularly, especially in bioanalysis.

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

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

USE OF GUARD CARTRIDGES
CHIRALPAK® AGP, CHIRALPAK® CBH, CHIRALPAK® HSA

We recommend the use of a guard cartridge, in order to protect the analytical column from impurities with high affinity and particulate impurities.
The guard cartridge should be replaced regularly; otherwise the column performance can be affected.
This is of special importance in bioanalysis.

For each type of chiral analytical columns, **CHIRALPAK® AGP**, **CHIRALPAK CBH** and **CHIRALPAK HSA**, **there are specific guard cartridges available.**

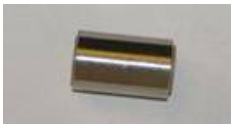
Choose The Right Guard Cartridge

In order to optimize the performance of the chromatographic system, the guard cartridge should have a dimension that matches the dimension of the analytical column:

- Guard cartridges matching each diameter of analytical columns are available *(refer to the table below)*
- A unique cartridge holder, Ref. 00081, can be used for all guard dimensions (2, 3 and 4 mm i.d.).

Guard column holder (Ref. 00081)

Guard cartridges



CHIRALPAK®-AGP / Guard 5 µm, 2-pack	*CHIRALPAK®-CBH / Guard* 5 µm, 2-pack	*CHIRALPAK®-HSA / Guard* 5 µm, 2-pack	*Guard accessories*
Ref. 30791 10x2 mm i.d..	*Ref. 33791 10x2 mm i.d.*	*Ref. 34791 10x2 mm i.d.*	*Ref. 000D2 micro column coupler*
Ref. 30781 10x3 mm i.d.	*Ref. 33781 10x3 mm i.d.*	*Ref. 34781 10x3 mm i.d.*	*Ref. 000D1 column coupler*
Ref. 30711 10x4 mm i.d.	*Ref. 33711 10x4 mm i.d.*	*Ref. 34711 10x4 mm i.d.*	

Important Notice

⇨ 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

INSTRUCTION MANUAL FOR CHIRALPAK® HSA

Please read this instruction sheet completely before using this column

Column Description

CHIRALPAK® HSA : Human serum albumin immobilized on 5 µm silica-gel.

Shipping solvent : **Water / 2-Propanol (2-PrOH) solvent mixture (90/10 v/v)**

All columns have been pre-tested before packaging. The test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

Application Scope

CHIRALPAK® HSA can offer high enantioselectivity for compounds bearing carboxylic groups, including:

- strong and weak acids
- zwitter-ionic molecules
- non-ionisable compounds (amides, esters, alcohols, sulfoxides, etc)

For compounds of basic category, however, it is preferred to use CHIRALPAK® AGP and CHIRALPAK® CBH columns.

Operating Conditions

	50 x 2.1 mm i.d.[*1] **100 x 2.1 mm i.d.**[*1] **150 x 2.1 mm i.d.**[*1] Analytical column	**50 x 3 mm i.d.**[*1] **100 x 3 mm i.d. 150 x 3 mm i.d.** Analytical column	**50 x 4 mm i.d.**[*1] **100 x 4 mm i.d. 150 x 4 mm i.d.** Analytical column	**100 x 10 mm i.d. 150 x 10 mm i.d.** Semi-prep. column
Flow direction	As indicated on the column label			
Typical Flow rate	0.2 mL/min	0.5 mL/min	0.9 mL/min	4.0 mL/min
pH range	5.0 - 7.0			
Recommended temperature[*2]	20 - 30°C			
Buffer concentration	up to 100 mM, typically 10-20 mM			
Organic modifier ratio	0-15% by volume			
Charged additive concentration	up to 10 mM			

*1 It is very important that the HPLC system is optimized in terms of void volume for work with columns of small dimensions.

*2 The column lifetime might be reduced if used at higher temperature.

A - Mobile Phase Starting Conditions

	ACIDIC Compounds	**NEUTRAL** Compounds
Typical starting conditions	**10 mM Ammonium acetate buffer (pH 7.0)❶ / 2-PrOH = 85 / 15 (v/v)**	

❶ Refer to section B for preparation of the buffer.

B – Buffer Preparation - Example

➢ *Preparation of 10 mM Ammonium acetate buffer (1Liter):*

1. Weigh 770.8 mg of ammonium acetate (CH_3COONH_4, purity > 99%) into a beaker.
2. Dissolve the salt with about 800 mL water (HPLC grade), equilibrated at room temperature (20-25°C).
3. Adjust pH to the target value by using either diluted acetic acid or a diluted ammonium hydroxide solution.
4. Filter the solution through a membrane of 0.22 µm into a measuring flask.
5. Add water until the limit line of the measuring flask. Place the stopper in the neck and homogenize the solution by agitation.

When buffer should be mixed with an organic modifier, the measurements are normally by volumes, using preferably volumetric flasks or measuring pipettes.
After mixing, degas the mobile phase in an ultrasonic bath.

Note that in the case where a charged additive is needed in the mobile phase, the charged additive should be added into the aqueous solution before the pH adjustment.

C – Mobile Phases

Bacteria grow fast in eluents containing no or low alcoholic organic modifier. Such mobiles phases must be freshly prepared.

❖ ***Buffer***
The salt concentration of ammonium acetate buffer is typically 10-20 mM but can be varied up to 100 mM. The other kinds of buffers, such as sodium or potassium phosphate buffers, sodium acetate buffers, formate or citrate buffers, can also be used. However, the LC-MS compatibility of the method may be sometimes compromised.

❖ ***Organic Modifiers***
2-PrOH is the most frequently used. However, methanol, ethanol and acetonitrile can also be investigated. The relative eluting strength can be ranked as follows: 2-PrOH > EtOH ≥ ACN > MeOH

❖ ***Charged additives***
Cationic and anionic additives, such as *N,N*-dimethyloctyl amine (DMOA), trifluoroacetic acid (TFA), octanoic acid (OA), heptafluorobutyric acid (HFBA), can also be used to regulate retention and enantioselectivity. However, some of these additives may be difficult to remove totally, due to very high affinity to the matrix. Thus, the properties of the column may be affected.

CAUTION: The miscibility of OA and DMOA to water is very limited. Only 2 mM OA or 5 mM DMOA can be homogeneously incorporated into the aqueous solution at ambient temperature. A phase separation may occur beyond these concentrations.

Once a charged additive is used in the mobile phase, the column should be dedicated for the purpose.

D – Samples

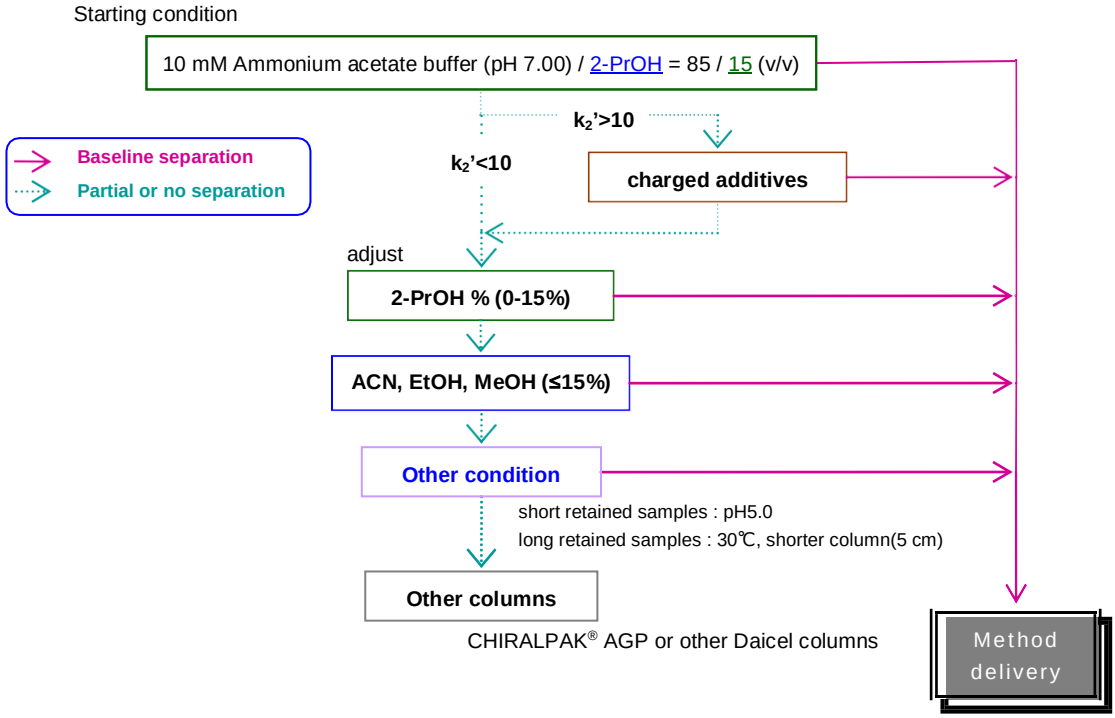
The sample amount injected onto the column should be kept low enough. The recommended sample concentration is 0.20 mg/mL or lower with an injection volume of 5-10 µL, preferably.

Dissolve the sample in the mobile phase when it is possible. If the sample is insoluble in the mobile phase, add a higher concentration of the organic modifier. The sample solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.

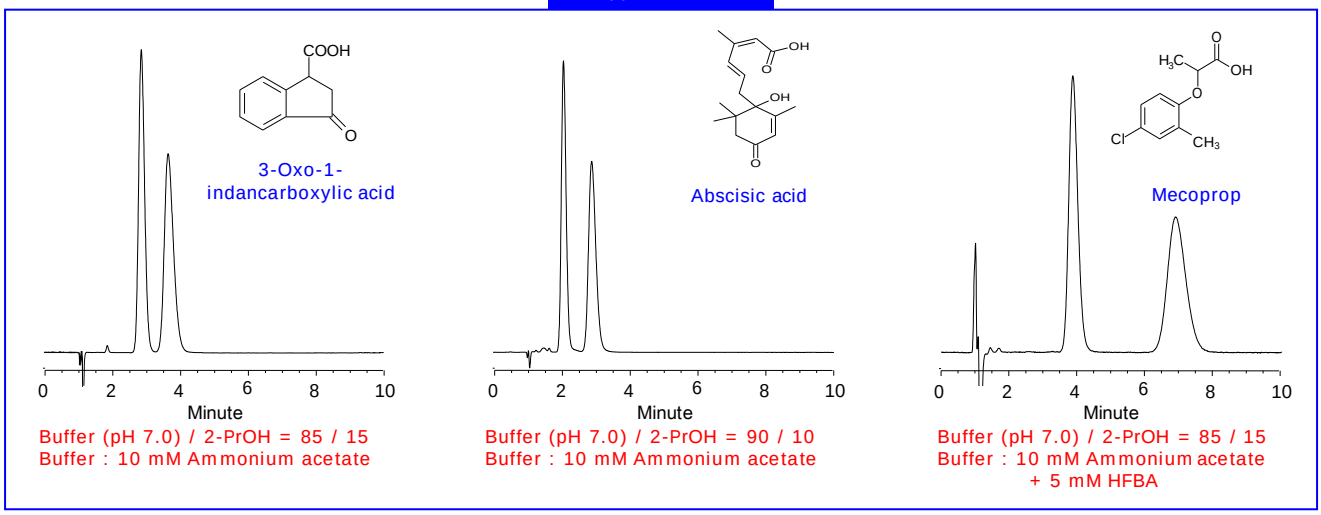
CAUTION: Dissolution of the sample in pure or high percentage of organic solvents may cause on-line sample precipitation.
Do not inject unclear sample solutions or solutions containing undissolved compounds.

Method Development

The following scheme offers a guide for method development and method optimization



CHIRALPAK® HSA
100 x 4 mm ID



Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- If the column has been contaminated with very hydrophobic material, wash the column backwards (no detector connected) over night with Water/2-PrOH 75/25(v/v) at a reduced flow-rate (e.g. 0.3 mL/min for 4 mm ID columns).
- Before disconnecting the column from the HPLC system, flush the column with a mobile phase that does not contain any salts / buffers, e.g. Water/2-PrOH 90/10(v/v).
- For the storage of the column, it is recommended to fill it with Water/2-PrOH 90/10(v/v).
  For short storage period, the column can be placed at ambient temperature (<30°C).
  For longer storage periods, however, it is recommended to place it in a refrigerator.

Important Notice

We recommend the use of a CHIRALPAK® HSA guard cartridge in order to protect the analytical column from any particulates and impurities with high affinity to the stationary phase. Change the guard cartridge regularly, especially in bioanalysis.

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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Parc d'Innovation
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REGENERATION PROCEDURES FOR CHIRALPAK® IA, IB, IB N, IC, ID, IE, IF, IG, IH, and IJ

The following regeneration procedures are ONLY intended for CHIRALPAK® immobilized-type columns. These procedures should never be used for any CHIRALCEL® or CHIRALPAK® coated-type columns.

Regeneration Overview

Following the extensive use of a 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…).

Because of the unique characteristics of each phase, different regeneration procedures have been developed to achieve optimal results. The following procedures can be used on all inner diameter columns and column lengths. Please see the specific section for the particle size column you are attempting to regenerate for important adjustments related to flow rate and time.

These procedures are also highly recommended when switching from reversed-phase (RP) to normal phase (NP) or SFC.

If there are any questions or concerns about performing these procedures, please contact Technical Support before proceeding.

Sub-2 µm Regeneration

1.) For CHIRALPAK® IA-U and ID-U

- Flush the column with ethanol at 0.21 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 0.17 ml/min for 240 min.
- Flush the column with ethanol at 0.02 mL/min(*) for 600 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

2.) For CHIRALPAK® IB-U and IC-U

- Flush with ethyl acetate (EtOAc) at 0.42 ml/min for 30 min (if a mobile phase containing additives was used, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.
- Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

3.) For CHIRALPAK® IG-U

- Flush with ethyl acetate (EtOAc) at 0.42 ml/min for 60 min (if a mobile phase containing additives was used, flush for 2 hours or more) at room temperature.
- Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase

Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

4.) For CHIRALPAK® IH-U

- Flush with ethanol at 0.21 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 0.13 ml/min for 3 hours.
- Flush with ethanol at 0.13 ml/min(*) for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.21 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

** This low flow rate is critical for column performance*

3 µm Regeneration

Analytical Columns (2.1, 3.0, and 4.6 mm i.d.)

The following procedures are for 4.6 mm inner diameter analytical columns, but can be used for all analytical column dimensions by scaling the flow rate proportional to the cross-sectional area (i.e a 4.6 mm i.d. column flowing at 1 ml/min has an equivalent flow rate of 0.43 ml/min on 3.0 mm i.d. column, and 0.21 ml/min on a 2.1 mm i.d. column):

1.) For CHIRALPAK® IA-3, ID-3, and IF-3

- Flush the column with ethanol at 0.5 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 0.4 ml/min for 240 min.
- Flush the column with ethanol at 0.05 ml/min(*) for 600 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

2.) For CHIRALPAK® IB-3, IB N-3, and IC-3

- Flush with ethyl acetate (EtOAc) at 1 ml/min for 30 min (if a mobile phase containing additives was used, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.
- Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

3.) For CHIRALPAK® IE-3 and IH-3

a. Flush the column with ethanol at 0.5 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 0.3 ml/min for 180 min.

b. Flush the column with ethanol at 0.3 ml/min for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

4.) For CHIRALPAK® IG-3

- Flush with ethyl acetate (EtOAc) at 1 ml/min for 60 min (if a mobile phase containing additives was used, flush for 2 hours or more) at room temperature.
- Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

5.) For CHIRALPAK® IJ-3

- Flush with ethanol at 0.5 ml/min for 30 min, followed by dichloromethane (DCM) at 0.3 ml/min for 3 hours.
- Flush with ethanol at 0.05 ml/min(*) for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

** This low flow rate is critical for column performance*

5 µm Regeneration

Analytical Columns (2.1, 3.0, and 4.6 mm i.d.)

The following procedures are for 4.6 mm inner diameter analytical columns, but can be used for all analytical column dimensions by scaling the flow rate proportional to the cross-sectional area (i.e a 4.6 mm i.d. column flowing at 1 ml/min has an equivalent flow rate of 0.43 ml/min on 3.0 mm i.d. column, and 0.21 ml/min on a 2.1 mm i.d. column):

1.) For CHIRALPAK® IA, ID, IE, IF, and IH

- Flush the column with ethanol at 0.5 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 0.3 ml/min for 180 min.
- Flush the column with ethanol at 0.3 mL/min(*) for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

2.) For CHIRALPAK® IB, IB N-5, and IC

- Flush with ethyl acetate (EtOAc) at 1 ml/min for 30 min (if a mobile phase containing additives was used, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.
- Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

6.) For CHIRALPAK® IG

- Flush with ethyl acetate (EtOAc) at 1 ml/min for 60 min (if a mobile phase containing additives was used, flush for 2 hours or more) at room temperature.
- Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 1 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

7.) For CHIRALPAK® IJ

- Flush with ethanol at 0.5 ml/min for 30 min, followed by dichloromethane (DCM) at 0.3 ml/min for 3 hours.
- Flush with ethanol at 0.3 ml/min(*) for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 0.5 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

** This low flow rate is critical for column performance*

Semi-Prep and Preparative Columns (1, 2, 3 and 5 cm i.d.)

For Semi-Prep and Preparative columns, a similar procedure as above can be used, but the time of each flush needs to be adjusted. The following procedures are for 1 cm semi-prep columns, but can be used for all semi-prep column dimensions by scaling the flow rate proportional to the cross-sectional area (i.e a 1 cm i.d. column flowing at 1 ml/min has an equivalent flow rate of 4.5 ml/min on 2 cm i.d. column, 9 ml/min on a 3 cm i.d. column, and 25 ml/min on a 5 cm i.d. column):

1.) For CHIRALPAK® IA, ID, IE, IF, and IH

a. Flush with ethanol at 2 ml/min for 30 min, followed by N,N-dimethylformamide (DMF) at 1.2 ml/min for 3 hours.

b. Flush with ethanol at 1.2 ml/min(*) for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

2.) For CHIRALPAK® IB, IB N-5, and IC

a. Flush with ethyl acetate (EtOAc) at 4 ml/min for 30 min (after flushing with mobile phase containing additives, flush for 2 hours or more), plug both ends of the column, and let stand at room temperature for 48 hours or more.

b. Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

3.) For CHIRALPAK® IG

a. Flush with ethyl acetate (EtOAc) at 4 ml/min for 60 min (after flushing with mobile phase containing additives, flush for 2 hours or more) at room temperature.

b. Equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

4.) For CHIRALPAK® IJ

- Flush with ethanol at 2 ml/min for 30 min, followed by dichloromethane (DCM) at 1.2 ml/min for 3 hours.
- Flush with ethanol at 1.2 ml/min(*) for 50 min and then equilibrate with shipping solvent (see column description section in the corresponding Normal Phase Instruction Manual) at 2 ml/min for 1 hour or more prior to testing the column under the original test conditions listed on the performance chromatogram.

** This low flow rate is critical for column performance*

**** For regeneration procedures of 10 or 20 µm immobilized columns, please contact us at the corresponding email address or telephone number below. ****

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

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Europe
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India
Daicel Chiral Technologies (India) Pvt Ltd
Survey No. 542/2 IKP Knowledge Park, Turkapally,
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Technical Support

If you are in need of an instruction manual or if you have any questions about the use of our columns, please contact the Technical Support team in your region.

For North and Latin America
Email: questions@cti.daicel.com
Tel: 1 800 6CHIRAL

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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

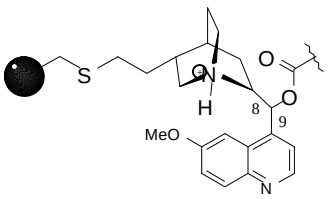
INSTRUCTION MANUAL FOR CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-)

Please read this instruction sheet completely before using these columns

Column Description

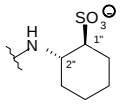
Packing composition:

⇨ Quinine combined with (S,S)-ACHSA(*) for CHIRALPAK® ZWIX(+),
⇨ Quinidine combined with (R,R)-ACHSA(*) for CHIRALPAK® ZWIX(-),
⇨ *Both of the chiral selectors are immobilized on 3µm silica-gel.*



CHIRALPAK® ZWIX(+)

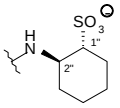
Quinine -derived (8S, 9R)



(1"S, 2"S)

CHIRALPAK® ZWIX(-)

Quinidine -derived (8R, 9S)



(1"R, 2"R)

Covalently bonded on 3µm spherical silica gel

(*) *trans-2-aminocyclohexanesulfonic acid (ACHSA)*

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 Recommendations

	150 x 3 mm i.d. 250 x 3 mm i.d.	150 x 4 mm i.d. 250 x 4 mm i.d.
Flow rate direction	As indicated on the column label	
Flow rate range	~ 0.2 - 0.5 ml/min	~ 0.3 – 1.0 ml/min
Temperature range	5 to 45°C	

- Samples should be filtered through a membrane filter of approximately 0.5 µm porosity.
- Mobile phases should be filtered through an appropriate filtration membrane.

Operating Procedure

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) are zwitterionic chiral stationary phases developed mainly for chiral separations of free amino acids. They exhibit remarkable stereoselectivity for zwitterionic molecules, especially amino acids and peptides, without derivatization.

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) columns are compatible for use in LC-MS detection. The suitability of the mobile phase systems to MS detection/identification makes the chromatographic method from the zwitterionic columns extremely valuable in analyzing numerous amino acids which are deficient of chromophors for UV detection.

Owing to the feature of pseudo-enantiomers of the two chiral selectors, the elution order of enantiomers can be systematically reversed on CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-), although their column performance may not be exactly equal towards each analyte.

They are compatible with all common HPLC solvents (e.g. methanol, acetonitrile, tetrahydrofuran, water).

Practical Method Development Scheme

In zwitterionic mode, the mobile phase should provide efficient solvation to all the ionised species involved in the double ion-exchange equilibria. This requires the consequent proton activities of the mobile phase media.

☞ *Bulk mobile phase:*

- Owing to its pronounced protic properties, MeOH is an essential mobile phase component for chiral separations on CHIRALPAK® ZWIX(+) and CHIRALPAK®ZWIX(-).
- To adjust the eluting strength and separation degree, MeOH can be mixed with acetonitrile (ACN) or THF at various proportions (preferably with MeOH ≥ 20%, v/v) as the bulk stationary phase. Higher MeOH contents lead to decrease in retention time of zwitterionic compounds.
- Addition of a low percentage of water (e.g. 2%) to the mobile phase has no detrimental effect on enantio-selectivity. On the contrary, this gives the benefits of improving MS detection, increasing sample solubility (avoiding on-line precipitation) and reducing peak tailing when working with relatively low amount of MeOH in the mobile phase.

☞ *Additives:*

- Due to the intra-molecular counter-ion effect of the chiral selectors, the combined presence of acidic and basic additives in eluent is necessary. The additive pair of formic acid (FA)-diethyl amine (DEA) at 50mM-25mM is proved to be versatile for operating the zwitterionic CSPs. They contribute to the proton activity of mobile phase as well.
- For fully LC-MS compatible conditions, FA/DEA can be replaced by FA/ammonium formate or a mixture of FA/ammonia. For MS applications, we would recommend the following starting conditions:

25mM FA + 25mM ammonium formate in MeOH/H_2O 98:2 (v/v).

☞ *Approaches for method development:*

STARTING CONDITIONS (standard LC)

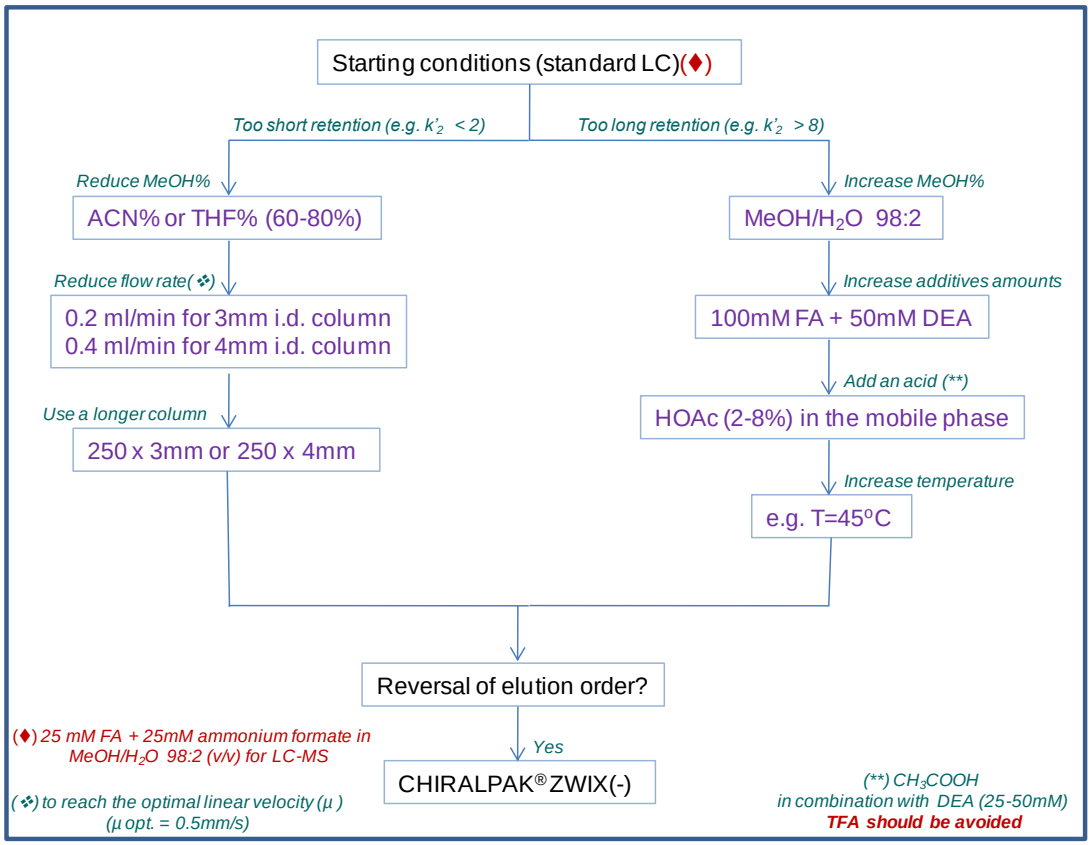
- Mobile Phases: (1). MeOH / ACN / H_2O 49:49:2 (v/v/v) 50mM FA + 25mM DEA(*)

  (2). MeOH / THF / H_2O 49:49:2 (v/v/v) 50mM FA + 25mM DEA(*)

(*) *Add 1.9ml of formic acid and 2.6ml of diethyl amine to 1L of bulk mobile phase.*

- Column and flow rate: CHIRALPAK® ZWIX(+) 150 x 3mm i.d. / 0.4-0.5 ml/min or 150 x 4mm i.d. / 0.8-1.0 ml/min
- Temperature: 25°C

OPTIMIZATION STEPS



Column Care / Maintenance

☞ *Starting:*

Before initial use, the column should be equilibrated with 20 column volumes of the mobile phase (ca. 30 -40ml).

☞ *Cleaning AND Storage:*

100% MeOH and 100% ACN can be used to wash the column. Mixtures of these solvents with H_2O (50:50, v/v) may also be efficient. We recommend flushing the column with 100% MeOH before storage (20 column volumes). The column can be stored at room temperature.

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:

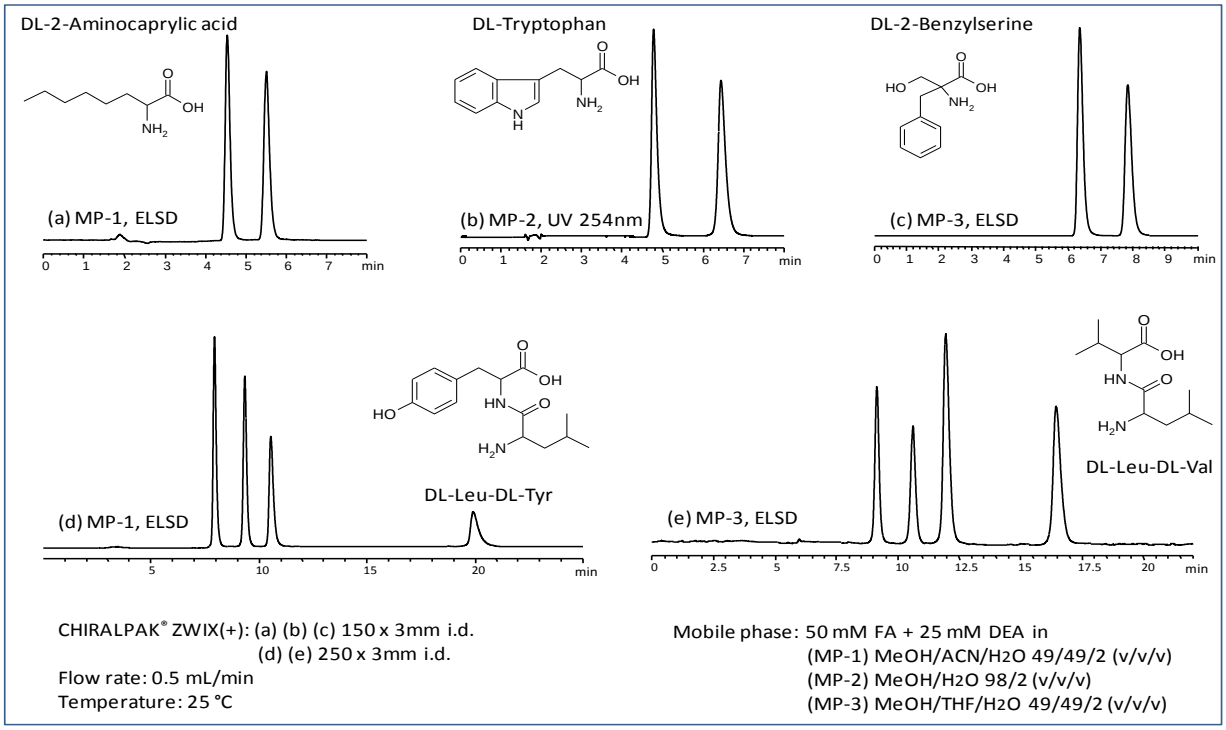
In the USA: questions@cti.daicel.com or call 800-6-CHIRAL
In the EU: cte@cte.daicel.com or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91 84 1866 0700 & 703

Examples of Chiral Separations for Standard Amino Acids

Column: CHIRALPAK® ZWIX(+) / 250 x 3mm i.d.
Mobile phase: MeOH/ACN/H_2O 49:49:2 (50mM FA + 25mM DEA); 0.5ml/min; 25°C

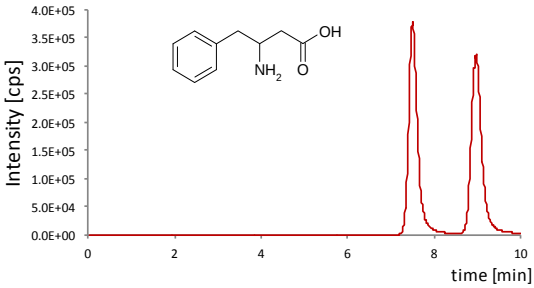
Amino acid	t_1 (min)	t_2 (min)	α	Rs	Elution order	Detection
Leucine	7.3	8.9	1.36	5.1	L/D	ELSD
Methionine	8.9	10.0	1.19	3.6	L/D	ELSD
Phenylalanine	7.9	9.1	1.24	4.1	L/D	ELSD
Proline	6.6	9.8	1.86	12.0	L/D	ELSD
Tyrosine	9.3	11.2	1.29	4.1	L/D	UV 230
Threonine	9.1	10.9	1.29	3.5	L/D	ELSD
Valine	7.3	8.8	1.34	4.8	L/D	ELSD

Examples of Chiral Analyses

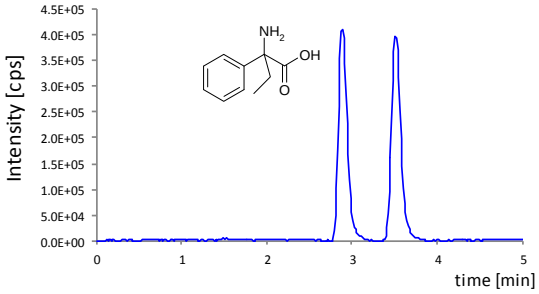


LC-MS applications

β-Homophenylalanine



2-Amino-2-phenylbutyric acid



LC-MS: Q-Trap (MRM Mode)
CHIRALPAK® ZWIX(-) (150 x 3mm i.d.)
Mobile phase: 25mM FA + 12.5 mM ammonium formate in MeOH/ACN/H_2O 49/49/2 (v/v/v)
Flow rate: 0.5 mL/min; Temperature: 40 °C;

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
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Shamirpet Mandal, Medchal-Malkajgiri District,
Hyderabad-500101. Telangana, India
Tel: +91 84 1866 0700 & 703
Fax: +91 84 1866 0730
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INSTRUCTION MANUAL FOR CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-)

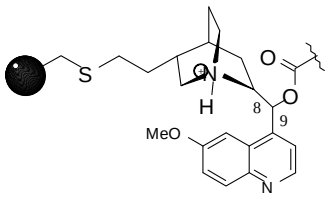
Semi-Preparative Columns

Please read this instruction sheet completely before using these columns

Column Description

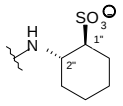
Packing composition:

- ⇨ Quinine combined with (S,S)-ACHSA(*) for CHIRALPAK® ZWIX(+),
- ⇨ Quinidine combined with (R,R)-ACHSA(*) for CHIRALPAK® ZWIX(-),
- ⇨ *Both of the chiral selectors are immobilized on **5µm** silica-gel.*



CHIRALPAK® ZWIX(+)

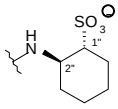
Quinine -derived (8S, 9R)



(1"S, 2"S)

CHIRALPAK® ZWIX(-)

Quinidine -derived (8R, 9S)



(1"R, 2"R)

Covalently bonded on **5µm** spherical silica gel

(*) *trans-2-aminocyclohexanesulfonic acid (ACHSA)*

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 Recommendations

	150 x 10 mm i.d. 250 x 10 mm i.d.	150 x 20 mm i.d. 250 x 20 mm i.d.
Flow rate direction	As indicated on the column label	
Flow rate range	2 to 6 ml/min	8 to 24 ml/min
Temperature range	5 to 45°C	

- Pressure drop over the column should be maintained <150 Bar (2100psi) for maximum column life time.
- Samples should be filtered through a membrane filter of approximately 0.5 µm porosity.
- Mobile phases should be filtered through an appropriate filtration membrane.

Operating Procedure

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) are zwitterionic chiral stationary phases developed mainly for chiral separations of free amino acids. They exhibit remarkable stereoselectivity for zwitterionic molecules, especially amino acids and peptides, without derivatization.

CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-) columns are compatible for use in LC-MS detection. The suitability of the mobile phase systems to MS detection/identification makes the chromatographic method from the zwitterionic columns extremely valuable in analyzing numerous amino acids which are deficient of chromophors for UV detection.

Owing to the feature of pseudo-enantiomers of the two chiral selectors, the elution order of enantiomers can be systematically reversed on CHIRALPAK® ZWIX(+) and CHIRALPAK® ZWIX(-), although their column performance may not be exactly equal towards each analyte.
They are compatible with all common HPLC solvents (e.g. methanol, acetonitrile, tetrahydrofuran, water).

Practical Method Development Scheme / Analytical Column

In zwitterionic mode, the mobile phase should provide efficient solvation to all the ionized species involved in the double ion-exchange equilibria. This requires the consequent proton activities of the mobile phase media.

☞ *Bulk mobile phase:*

- Owing to its pronounced protic properties, MeOH is an essential mobile phase component for chiral separations on CHIRALPAK® ZWIX(+) and CHIRALPAK®ZWIX(-).
- To adjust the eluting strength and separation degree, MeOH can be mixed with acetonitrile (ACN) or THF at various proportions (preferably with MeOH ≥ 20%, v/v) as the bulk stationary phase. Higher MeOH contents lead to decrease in retention time of zwitterionic compounds.
- Addition of a low percentage of water (e.g. 2%) to the mobile phase has no detrimental effect on enantio-selectivity. On the contrary, this gives the benefits of improving MS detection, increasing sample solubility (avoiding on-line precipitation) and reducing peak tailing when working with relatively low amount of MeOH in the mobile phase.

☞ *Additives:*

- Due to the intra-molecular counterion effect of the chiral selectors, the combined presence of acidic and basic additives in eluent is necessary. The additive pair of formic acid (FA)-diethyl amine (DEA) at 50mM-25mM is proved to be versatile for operating the zwitterionic CSPs. They contribute to the proton activity of mobile phase as well.
- For fully LC-MS compatible conditions, FA/DEA can be replaced by FA/ammonium formate or a mixture of FA/ammonia. For MS applications, we would recommend the following starting conditions:

  25mM FA + 25mM ammonium formate in MeOH/H_2O 98:2 (v/v).

☞ *Approaches for method development:*

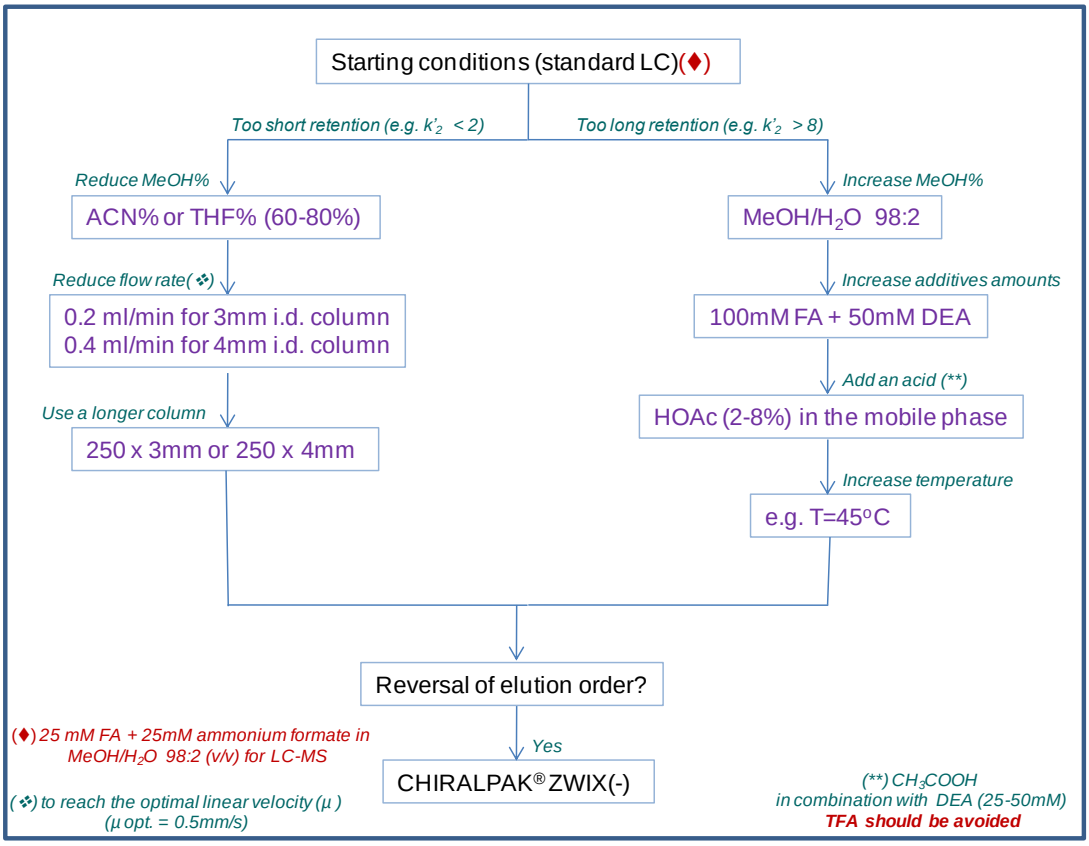
STARTING CONDITIONS (standard LC) / Analytical Column

- ❖ Mobile Phases:
  - (1). MeOH / ACN / H_2O 49:49:2 (v/v/v) + 50mM FA + 25mM DEA(*)
  - (2). MeOH / THF / H_2O 49:49:2 (v/v/v) + 50mM FA + 25mM DEA(*)

(*) *Add 1.9ml of formic acid and 2.6ml of diethyl amine to 1L of bulk mobile phase.*

- ❖ Column and flow rate: CHIRALPAK® ZWIX(+) 150 x 3mm i.d. / 0.4-0.5 ml/min or 150 x 4mm i.d. / 0.8-1.0 ml/min
- ❖ Temperature: 25°C

OPTIMIZATION STEPS / Analytical Column



Column Care / Maintenance

☞ *Starting:*

Before initial use, a 10 x 250 column should be equilibrated with 300mL of the mobile phase; a 20 x 250 column should be equilibrated with 1.2 Liters of the mobile phase.

☞ *Cleaning:*

100% MeOH and 100% ACN can be used to wash the column.
Mixtures of these solvents with H_2O (50:50, v/v) may also be efficient.

☞ *Storage*

We recommend flushing the column with 100% MeOH before storage: (10 x 250 300mL; 20 x 250 1.2 Liters)
The column can be stored at room temperature.

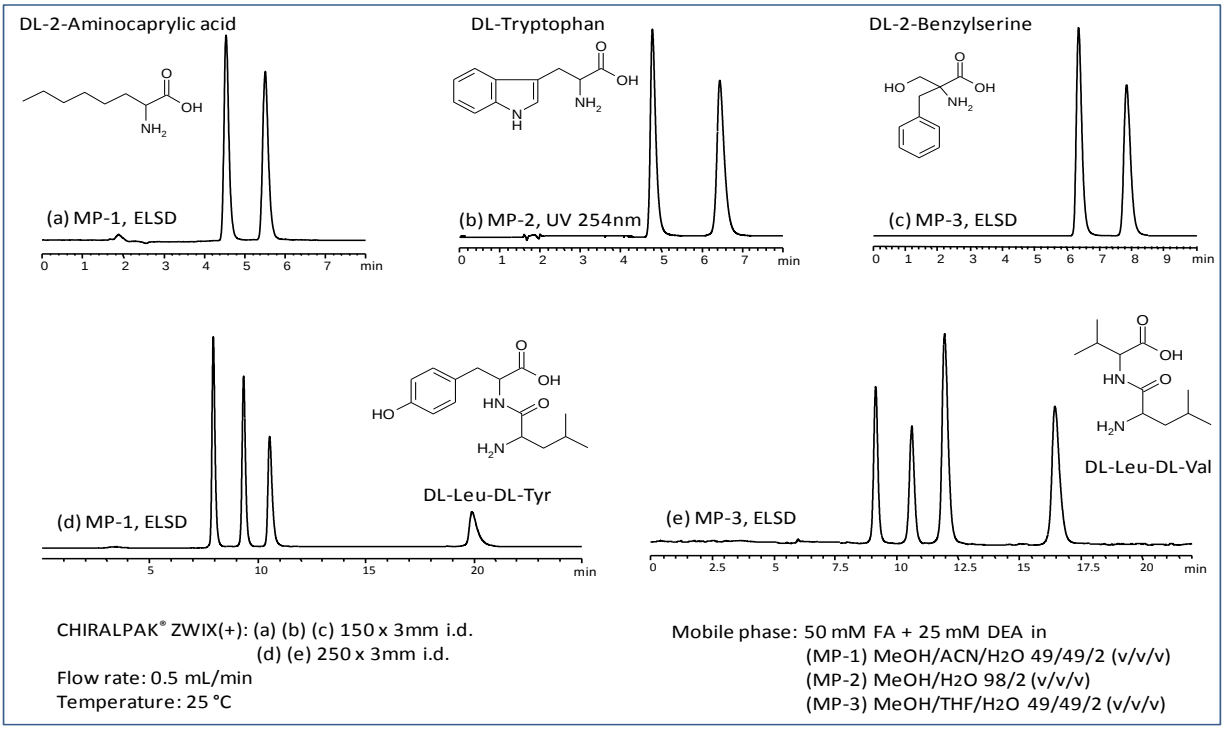
Operating this column in accordance with the guidelines outlined here will result in a long column life.

Examples of Chiral Separations for Standard Amino Acids

Column: CHIRALPAK® ZWIX(+) / 250 x 3mm i.d.
Mobile phase: MeOH/ACN/H_2O 49:49:2 (50mM FA + 25mM DEA); 0.5ml/min; 25°C

Amino acid	t_1 (min)	t_2 (min)	α	Rs	Elution order	Detection
Leucine	7.3	8.9	1.36	5.1	L/D	ELSD
Methionine	8.9	10.0	1.19	3.6	L/D	ELSD
Phenylalanine	7.9	9.1	1.24	4.1	L/D	ELSD
Proline	6.6	9.8	1.86	12.0	L/D	ELSD
Tyrosine	9.3	11.2	1.29	4.1	L/D	UV 230
Threonine	9.1	10.9	1.29	3.5	L/D	ELSD
Valine	7.3	8.8	1.34	4.8	L/D	ELSD

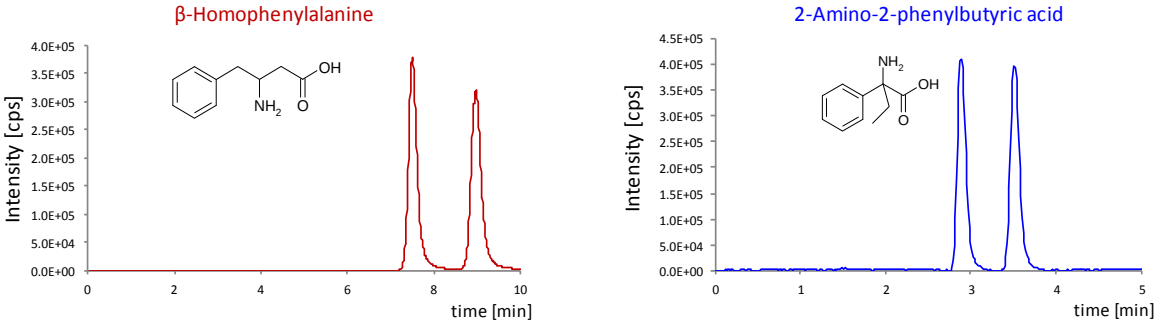
Examples of Chiral Analyses



CHIRALPAK® ZWIX(+): (a) (b) (c) 150 x 3mm i.d.
(d) (e) 250 x 3mm i.d.
Flow rate: 0.5 mL/min
Temperature: 25 °C

Mobile phase: 50 mM FA + 25 mM DEA in
(MP-1) MeOH/ACN/H_2O 49/49/2 (v/v/v)
(MP-2) MeOH/H_2O 98/2 (v/v/v)
(MP-3) MeOH/THF/H_2O 49/49/2 (v/v/v)

LC-MS applications



LC-MS: Q-Trap (MRM Mode)
CHIRALPAK® ZWIX(-) (150 x 3mm i.d.)
Mobile phase: 25mM FA + 12.5 mM ammonium formate in MeOH/ACN/H2O 49/49/2 (v/v/v)
Flow rate: 0.5 mL/min; Temperature: 40 °C;

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

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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
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Fax: +91 84 1866 0730
chiral@chiral.daicel.com
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