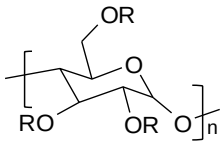
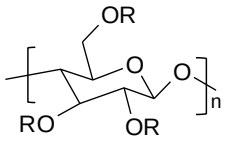
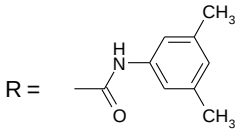
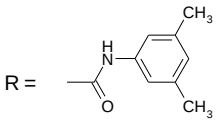
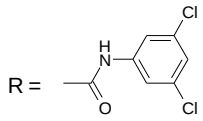
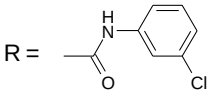
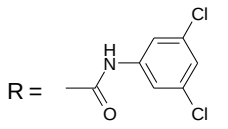
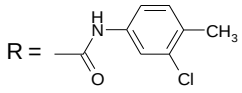
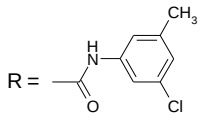
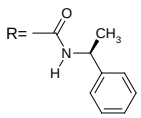
INSTRUCTION MANUAL FOR CHIRALPAK® IA-3, IB-(N)3, IC-3, ID-3, IE-3, IF-3, IG-3, and IH-3

<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.

Column Description

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

Shipping solvent: **n-Hexane / alcohol solvent mixture**

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 Instructions

	250 x 2.1 mm i.d. **150 x 2.1 mm i.d.** Analytical columns	**50 x 4.6 mm i.d.** Analytical columns	**100 x 4.6 mm i.d.** Analytical columns	**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 to 0.5 ml/min	0.5 to 5 ml/min	0.5 to 4 ml/min	0.5 to 2.5 ml/min
Temperature	0 to 40°C			

☞ NOTES: The column is stable to HPLC pressures. 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-(N)3, IC-3, ID-3, IE-3, IF-3, IG-3 and IH-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 (CH_3CN)) 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 optimisation 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	CH_3CN❸/Alcohol❹
Typical starting conditions	50:50	100:0
Advised optimisation 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 CH_3CN 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-3, IB-(N)3, IC-3, ID-3, IE-3, IF-3, IG-3 and IH-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 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 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 optimise 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 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 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.

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

Following extensive use of the column in multiple solvents there may be a change in column 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…).

- Flush with ethanol at 0.5 ml/min for 30 min, followed by 100% THF at 0.5 ml/min for 2 hours.
- Flush with ethanol at 0.05 ml/min(*) for 300 min.

(*) This low flow rate would be critical for the column performance.

If this is not successful, then try with 100% N,N-dimethylformamide (DMF) or N,N-dimethylacetamide (DMAC) at 0.3 ml/min for 3 hours instead of the THF flush.

⇨ *This procedure is also recommended for switching between reversed phase and normal phase.*

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 with the additive-free mobile phases.

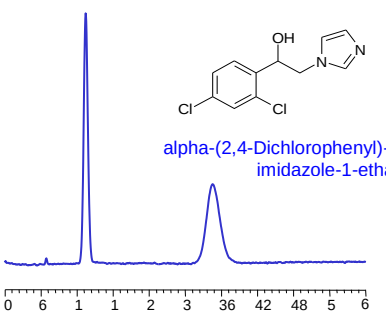
Operating these columns in accordance with the guidelines outlined here will result in a long column life.

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

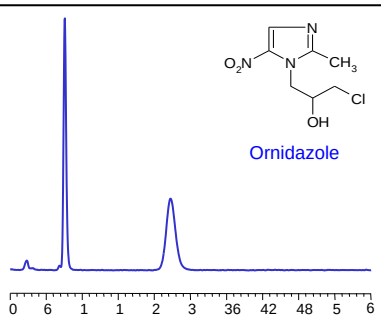
In the USA: questions@chiraltech.com or call 800-6-CHIRAL
In the EU: cte@chiral.fr or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91-40-2338-3700

CHIRALPAK® IA-3 Analytical HPLC applications

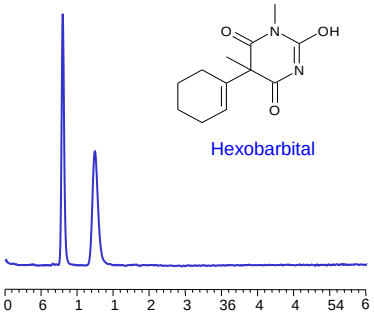
CHIRALPAK® IA-3 (4.6x50mm)



Hexane-EtOH 80:20 (+0.1% AE)
Flow rate: **5.0** ml/min, Temp.: 25°C



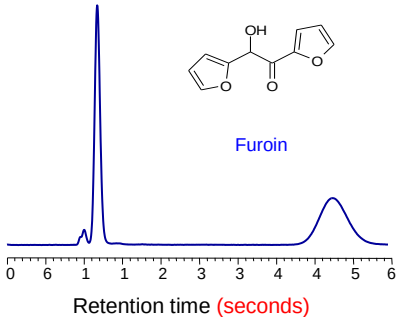
DCM-MeOH 98:2 (+0.1% AE)
Flow rate: **5.0** ml/min, Temp.: 25°C



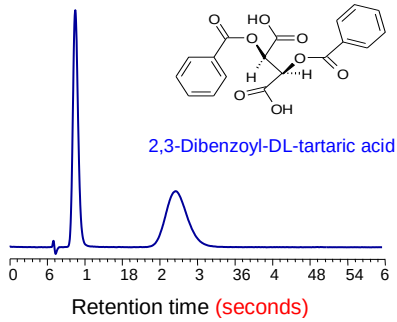
MeOH 100%
Flow rate: **5.0** ml/min, Temp.: 25°C

CHIRALPAK® IC-3 Analytical HPLC applications

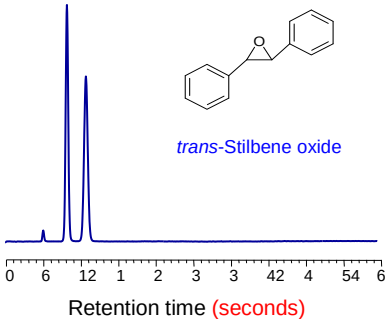
CHIRALPAK® IC-3 (4.6x50mm)



MtBE-EtOH 98:2
Flow rate: **5.0** ml/min, Temp.: 25°C



Hexane-EtOH 80:20 (+0.1% TFA)
Flow rate: **5.0** ml/min, Temp.: 25°C



Hexane-IPA 90:10
Flow rate: **6.0** ml/min, Temp.: 25°C

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

Switching between RP and NP mode

To switch from reversed phase mode to normal phase mode, and vice versa, the column should be carefully flushed with miscible solvent.
It is highly recommended to use the **regeneration procedure** described in the instruction sheet for normal phase mode. Before using this protocol, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts/buffers.

Method Development / Reversed Phase

A - Mobile Phases / For 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-(N)3** **CHIRALPAK® IC-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	CH_3CN or MeOH or EtOH or IPA or THF		
	Typical starting conditions ❸	Aqueous solutions 60% CH_3CN 40% ❺		

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

B – Complementary aqueous and buffer solutions / For UV Detection

		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-(N)3** **CHIRALPAK® IC-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 CH_3CN to start the investigation.
❑ The elution power of organic modifiers for these columns is in the descending order of CH_3CN > EtOH > MeOH: 50% CH_3CN ≈ 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 CH_3CN due to their high viscosity in mixtures with water.

❸ ❑ Retention can be adjusted by changing the proportion of CH_3CN. Retention may be very sensitive to the amount of CH_3CN 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 25ºC for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **<u>may precipitate the buffering salt</u>** 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

➢ *<u>Preparation of pH 2 Phosphate buffer</u>:*
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.

➢ *<u>Preparation of pH 2 KPF_6 ($NaPF_6$) solution</u>:*
Solution A: 100 m 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.

➢ *<u>Preparation of pH 9 Ammonium bicarbonate solution</u>:*
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.

➢ *<u>Preparation of pH 8 Phosphate buffer</u>:*
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.

➢ *<u>Preparation of pH 9 Borate buffer</u>:*
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

- Any traces of salts should be removed before column storage and /or before switching to 100% organic solvent (use Water/CH_3CN 60:40 (v/v) for instance)

Refer to main instruction for normal phase and column care/maintenance.

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 with the additive-free mobile phases.

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

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

In the USA: questions@chiraltech.com or call 800-6-CHIRAL
In the EU: cte@chiral.fr or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91-40-2338-3700