# *INSTRUCTION MANUAL FOR CHIRALPAK® IA-3, CHIRALPAK IB-3, CHIRALPAK IC-3, CHIRALPAK ID-3, CHIRALPAK IE-3, CHIRALPAK IF-3 and CHIRALPAK IG-3*

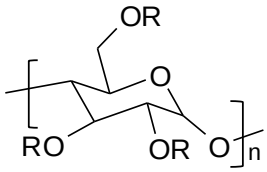
## *<Normal Phase>*

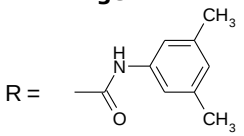
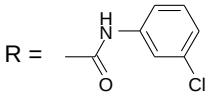
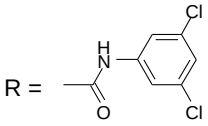
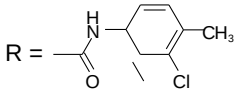
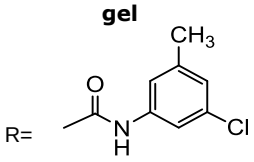
***Please read this instruction sheet completely before using these columns***

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

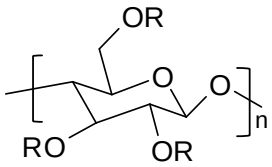
## Column Description

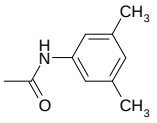
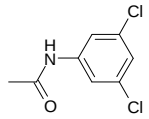
**AMYLOSE-BASED**




| CHIRALPAK® IA-3 | CHIRALPAK® ID-3 | CHIRALPAK® IE-3 | CHIRALPAK® IF-3 |
|---|---|---|---|
| Amylose tris(3,5-dimethylphenylcarbamate) **immobilized** on **3µm silica-gel**  | Amylose tris (3-chlorophenylcarbamate) **immobilized** on **3µm silica gel**  | Amylose tris(3,5-dichlorophenylcarbamate) **immobilized** on **3µm silica gel**  | Amylose tris(3-chloro-4-methylphenylcarbamate) **immobilized** on **3µm silica gel**  |
| **CHIRALPAK® IG-3** | | | |
| Amylose tris(3-chloro-5-methylphenylcarbamate) **immobilized** on **3µm silica gel**  | | | |

## CELLULOSE-BASED




| CHIRALPAK® IB-3 | CHIRALPAK® IC-3 |
|---|---|
| Cellulose tris(3,5-dimethylphenylcarbamate) **immobilized** on **3µm silica-gel** | Cellulose tris(3,5-dichlorophenylcarbamate) **immobilized** on **3µm silica-gel** |
| R =  | R =  |

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.**<br>**150 x 2.1 mm i.d.**<br>Analytical columns | **50 x 4.6 mm i.d.**<br>Analytical columns | **100 x 4.6 mm i.d.**<br>Analytical columns | **150 x 4.6 mm i.d.**<br>**250 x 4.6 mm i.d.**<br>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-3, IC-3, ID-3 and IE-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 | $CH_3CN$❸/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-3, IC-3, ID-3, IE-3 and IF-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.

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

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.*

⇨ *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***

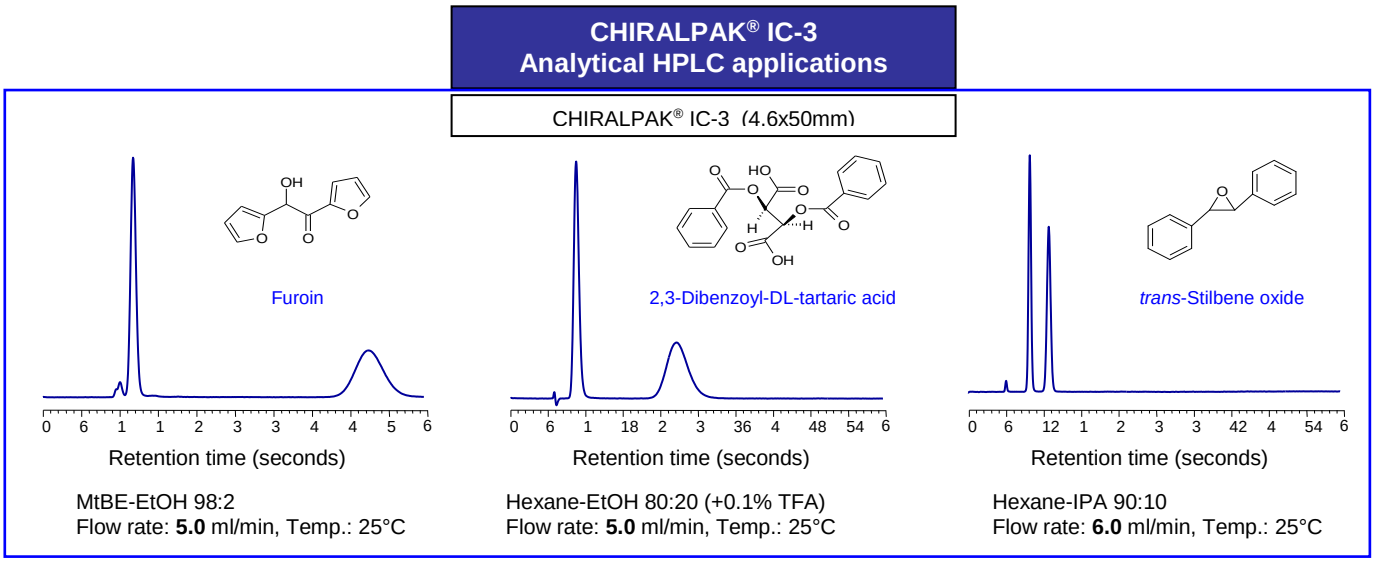
Following extensive use of the column in multiple solvents there may be a change in column reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in chiral recognition due to the history of the column (mobile phases, additives…).

- 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: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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