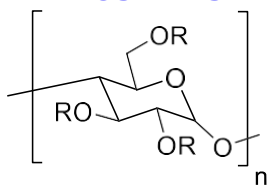
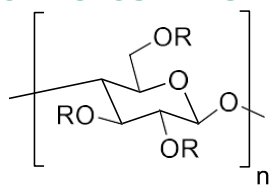
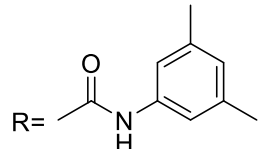
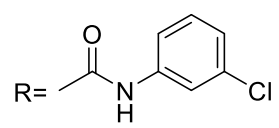
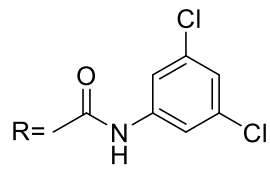
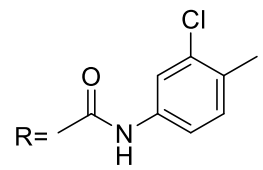
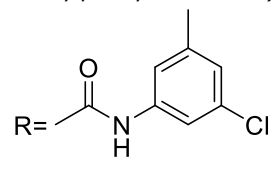
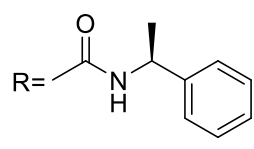
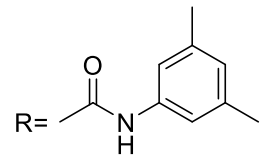
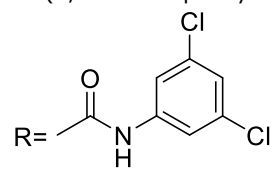
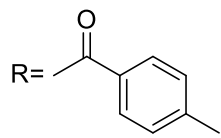
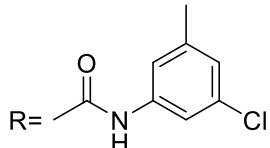
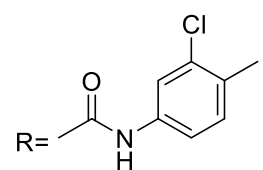


INSTRUCTION MANUAL FOR CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, IJ, IK and IM

<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.

| AMYLOSE-BASED | | CELLULOSE-BASED | |
|---|---|--|--|
|  <p style="text-align: center; color: blue;">Immobilized on 5 µm silica gel</p> | |  <p style="text-align: center; color: green;">Immobilized on 5 µm silica gel</p> | |
| CHIRALPAK® IA | CHIRALPAK® ID | CHIRALPAK® IE | |
| Amylose tris(3,5-dimethylphenylcarbamate) | Amylose tris(3-chlorophenylcarbamate) | Amylose tris(3,5-dichlorophenylcarbamate) | |
|  |  |  | |
| CHIRALPAK® IF | CHIRALPAK® IG | CHIRALPAK® IH | |
| Amylose tris(3-chloro-4-methylphenylcarbamate) | Amylose tris(3-chloro-5-methylphenylcarbamate) | Amylose tris[(S)-α-methylbenzylcarbamate] | |
|  |  |  | |
| CHIRALPAK® IB CHIRALPAK® IB N-5 | | CHIRALPAK® IC | |
| Cellulose tris(3,5-dimethylphenylcarbamate) | Cellulose tris(3,5-dichlorophenylcarbamate) | Cellulose tris(4-methylbenzoate) | |
|  |  |  | |
| CHIRALPAK® IK | | CHIRALPAK® IM | |
| Cellulose tris(3-chloro-5-methylphenylcarbamate) | Cellulose tris(3-chloro-4-methylphenylcarbamate) | | |
|  |  | | |

Shipping Solvent:

1. **Hexane/Isopropanol (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**

Columns should be flushed with 100% Ethanol or IPA before connecting to a reversed phase system to ensure all Hexane is removed before introducing an aqueous mobile phase to the column.

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 20 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** described in the instruction manual for normal phase (located in the column care and maintenance 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 ④ |
|--|-------------------------------|--|-----------------------------|--|
| CHIRALPAK® IA CHIRALPAK® ID CHIRALPAK® IE CHIRALPAK® IF CHIRALPAK® IG CHIRALPAK® IH CHIRALPAK® IB CHIRALPAK® IB N-5 CHIRALPAK® IC CHIRALPAK® IJ CHIRALPAK® IK CHIRALPAK® IM | Aqueous Solution ① | HCOOH aq. pH 2.0 | Water | 20 mM NH ₄ HCO ₃ aq. pH 9.0 adjusted with a basic additive ① |
| | Organic Modifier ② | ACN or MeOH or EtOH or IPA or THF | | |
| | Typical Starting Conditions ③ | Aqueous solutions 60% ACN 40% ⑤ | | |

☞ 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 ④ |
|--|--------------------|---|-----------------------------|--|
| CHIRALPAK® IA CHIRALPAK® ID CHIRALPAK® IE CHIRALPAK® IF CHIRALPAK® IG CHIRALPAK® IH CHIRALPAK® IB CHIRALPAK® IB N-5 CHIRALPAK® IC CHIRALPAK® IJ CHIRALPAK® IK CHIRALPAK® IM | Aqueous Solution ① | 50 mM Phosphate Buffer pH 2.0 OR H ₃ PO ₄ aq. pH 2.0 OR 100 mM KPF ₆ (or NaPF ₆) aq. pH 2.0 adjusted with H ₃ PO ₄ | Water | 20 mM Borate Buffer pH 9.0 OR 20 mM Phosphate Buffer pH 8.0 ⑥ OR 100 mM KPF ₆ (or NaPF ₆) aq. |

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

- ① Refer to **section C** for the preparation of an aqueous solution and choice of basic additives.
- ②
 - ☐ 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.
- ③
 - ☐ 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.
- ④
 - ☐ 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 silicagel matrix.
 - ☐ When these columns are used at pH > 7, **the temperature should be maintained between 5°C and 25°C for maximum column life.**

- 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 |

- 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 $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ / 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 0.5 µm 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 does not 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

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