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

<Reverse 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, column should be carefully flushed with miscible solvent.
It is highly recommended to apply the regeneration procedure described in the instruction sheet for normal phase mode. Before applying 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 both UV and Mass detections

<table>
<thead>
<tr>
<th>CHIRALPAK® IA</th>
<th>CHIRALPAK® ID</th>
<th>CHIRALPAK® IE</th>
<th>CHIRALPAK® IF</th>
<th>CHIRALPAK® IG</th>
<th>CHIRALPAK® IH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIRALPAK® IB(N-5)</td>
<td>CHIRALPAK® IC</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compounds</th>
<th>ACIDIC (AMPHOTERIC)</th>
<th>NEUTRAL Compounds</th>
<th>BASIC Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous solution</td>
<td>HCOOH aq. pH 2.0</td>
<td>Water</td>
<td>20 mM NH₄HCO₃ aq. pH 9.0 adjusted with a basic additive</td>
</tr>
<tr>
<td>Organic modifier</td>
<td>CH₃CN or MeOH or EtOH or IPA or THF</td>
<td>Aqueous solutions</td>
<td>60%</td>
</tr>
<tr>
<td>Typical starting conditions</td>
<td>CH₄CN</td>
<td>60%</td>
<td></td>
</tr>
</tbody>
</table>

NOTE 1: If you cannot achieve sufficient resolution, try the complementary aqueous solutions
B – Complementary aqueous and buffer solutions / For UV detection

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<tr>
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<tr>
<td>CHIRALPAK® IH</td>
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</tr>
</tbody>
</table>

### ACIDIC (AMPHOTERIC) Compounds
- 50 mM Phosphate Buffer pH 2.0
  - OR H₃PO₄ aq. pH 2.0
  - OR 100 mM KPF₆ (or NaPF₆) aq. pH 2.0 adjusted with H₃PO₄

### NEUTRAL Compounds
- Water

### BASIC Compounds
- 20 mM Borate Buffer pH 9.0
  - OR 20 mM Phosphate Buffer pH 8.0
  - OR 100 mM KPF₆ (or NaPF₆) aq.

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

- Refer to section C for preparation of aqueous solution and choice of basic additives.
- It is recommended to use CH₃CN to start the investigation
  - The elution power of organic modifiers for these columns is in the descending order of CH₃CN > EtOH > MeOH: 50%CH₃CN ≈ 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 CH₃CN due to their high viscosity in mixtures with water.
- Retention can be adjusted by changing the proportion of CH₃CN. Retention may be very sensitive to the amount of CH₃CN 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.
- 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.
- 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).

<table>
<thead>
<tr>
<th>Water / Organic Modifier</th>
<th>Buffer solution / Organic Modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 / 10 to 0 / 100</td>
<td>90 / 10 to 15 / 85</td>
</tr>
</tbody>
</table>

- 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 3.40g KH₂PO₄ / FW 136.09, make up the volume to 500ml with HPLC grade water
- **Solution B**: phosphoric acid (H₃PO₄ 85% by weight)
  - Adjust the pH of solution A to a value of 2.0 using solution B.

#### Preparation of pH 2 KPF₆ (NaPF₆) solution:
- **Solution A**: 100 mM potassium (sodium) hexafluorophosphate 9.20g KPF₆ / FW 184.06 or 8.40g NaPF₆ / FW 167.95, make up the volume to 500 ml with HPLC grade water
- **Solution B**: phosphoric acid (H₃PO₄ 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
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$_2$HPO$_4$ / FW 174.18, make up the volume to 500 ml with HPLC grade water
  - **Solution B:** 20 mM potassium dihydrogenophosphate
  - 1.36g KH$_2$PO$_4$ / 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$_2$B$_4$O$_7$.10H$_2$O / FW 381.37, make up the volume to 500 ml with HPLC grade water
  - **Solution B:** 20 mM boric acid
  - 0.62g H$_3$BO$_3$ / 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.

- Any traces of salts should be removed before column storage and/or before switching to 100% organic solvent (use Water/CH$_3$CN 60:40 (v/v) for instance)

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