INSTRUCTION MANUAL FOR
CHIRALPAK® IA, CHIRALPAK® IB, CHIRALPAK® IC,
CHIRALPAK® ID, CHIRALPAK® IE, CHIRALPAK® IF and CHIRALPAK IG

<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></th>
<th>ACIDIC (AMPHOTERIC) Compounds</th>
<th>NEUTRAL Compounds</th>
<th>BASIC Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIRALPAK® IA</td>
<td>Aqueous solution</td>
<td>HCOOH aq. pH 2.0</td>
<td>20 mM NH4HCO3 aq. pH 9.0 adjusted with a basic additive</td>
</tr>
<tr>
<td>CHIRALPAK® ID</td>
<td></td>
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<td></td>
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<tr>
<td>CHIRALPAK® IE</td>
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<tr>
<td>CHIRALPAK® IF</td>
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<tr>
<td>CHIRALPAK® IG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHIRALPAK® IB</td>
<td>Organic modifier</td>
<td>CH3CN or MeOH or EtOH or IPA or THF</td>
<td></td>
</tr>
<tr>
<td>CHIRALPAK® IC</td>
<td>Typical starting conditions</td>
<td>Aqueous solutions</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH3CN</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

<table>
<thead>
<tr>
<th></th>
<th>ACIDIC (AMPHOTERIC) Compounds</th>
<th>NEUTRAL Compounds</th>
<th>BASIC Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIRALPAK® IA</td>
<td>Aqueous solution</td>
<td></td>
<td>20 mM Borate Buffer pH 9.0</td>
</tr>
<tr>
<td>CHIRALPAK® ID</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CHIRALPAK® IE</td>
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<tr>
<td>CHIRALPAK® IF</td>
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<tr>
<td>CHIRALPAK® IG</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CHIRALPAK® IB</td>
<td>50 mM Phosphate Buffer pH 2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHIRALPAK® IC</td>
<td>OR H3PO4 aq. pH 2.0 OR</td>
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</tr>
<tr>
<td></td>
<td>100 mM KPF6 (or NaPF6) aq.</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>pH 2.0 adjusted with H3PO4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
<td>20 mM Phosphate Buffer pH 8.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 CH$_3$CN > EtOH > MeOH: 50%CH$_3$CN $\approx$ 65-70%EtOH $\approx$ 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$_3$CN due to their high viscosity in mixtures with water.

Retention can be adjusted by changing the proportion of CH$_3$CN. Retention may be very sensitive to the amount of CH$_3$CN 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 KH$_2$PO$_4$ / FW 136.09, make up the volume to 500ml with HPLC grade water
  Solution B: phosphoric acid (H$_3$PO$_4$ 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) hexafluorophosphat
  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$_3$PO$_4$ 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$_4$HCO$_3$ / 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$_2$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.

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

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