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

		ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
CHIRALPAK® IA **CHIRALPAK® ID** **CHIRALPAK® IE** **CHIRALPAK® IF** **CHIRALPAK® IG** **CHIRALPAK® IH** **CHIRALPAK® IB(N-5)** **CHIRALPAK® IC**	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 **CHIRALPAK® ID** **CHIRALPAK® IE** **CHIRALPAK® IF** **CHIRALPAK® IG** **CHIRALPAK® IH** **CHIRALPAK® IB(N-5)** **CHIRALPAK® IC**	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 25°C for maximum column life.**

❺ 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).

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

➢ *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 $Na_2B_4O_7.10H_2O$ / 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 main instruction for normal phase and column care/maintenance.