INSTRUCTION MANUAL FOR CHIRALPAK® IA-U, CHIRALPAK® IB-U, CHIRALPAK® IC-U, and CHIRALPAK® IG-U

<Reverse Phase>

Please read this instruction sheet completely before using these

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

General recommendations

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 use a UHPLC system to preserve the best separation performance of the column.
- 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.
- to adjust the flow rate to uphold the column pressure < 700 bar.

Method Development / Reversed Phase

A - Mobile phases / For both UV and Mass detections

		ACIDIC (AMPHOTERIC) Compounds ❹	**NEUTRAL** Compounds ❹	**BASIC** Compounds ❹
CHIRALPAK® IA-U **CHIRALPAK® IB-U** **CHIRALPAK® IC-U** **CHIRALPAK® IG-U**	Aqueous solution ❶	HCOOH aq. pH 2.0	Water	20mM 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-U **CHIRALPAK® IB-U** **CHIRALPAK® IC-U** **CHIRALPAK® IG-U**	Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF_6 (or $NaPF_6$) aq.

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

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 the column life, it is essential to inject filtered clean sample solutions.**
- ❑ 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: 50mM 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: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, 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 9 Ammonium bicarbonate solution:*

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml 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: 20mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water
Solution B: 20mM potassium dihydrogenophosphate
1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml 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: 20mM sodium tetraborate decahydrate
3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500ml with HPLC grade water
Solution B: 20mM boric acid
0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water
Adjust the pH of solution A to a value of 9.0 using solution B.

Column care / Maintenance

For column care/maintenance, refer to Instruction Manual for normal phase

- 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)

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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