

## INSTRUCTION MANUAL FOR CHIRALPAK® IA-3, IB-(N)3, IC-3, ID-3, IE-3, IF-3, IG-3, and IH-3

### <Reversed 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, the column should be carefully flushed with miscible solvent.

It is highly recommended to use the **regeneration procedure** described in the instruction sheet for normal phase mode. Before using 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 UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ④
<b>CHIRALPAK® IA-3</b> <b>CHIRALPAK® ID-3</b> <b>CHIRALPAK® IE-3</b> <b>CHIRALPAK® IF-3</b> <b>CHIRALPAK® IG-3</b> <b>CHIRALPAK® IH-3</b>  <b>CHIRALPAK® IB-(N)3</b> <b>CHIRALPAK® IC-3</b>	Aqueous solution ①	HCOOH aq. pH 2.0	Water	20 mM NH <sub>4</sub> HCO <sub>3</sub> aq. pH 9.0 adjusted with a <u>basic</u> additive ①
	Organic modifier ②	CH <sub>3</sub> CN or MeOH or EtOH or IPA or THF		
	Typical starting conditions ③	Aqueous solutions CH <sub>3</sub> CN	60%	40% ⑤

☞ 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 ④
<b>CHIRALPAK® IA-3</b> <b>CHIRALPAK® ID-3</b> <b>CHIRALPAK® IE-3</b> <b>CHIRALPAK® IF-3</b> <b>CHIRALPAK® IG-3</b> <b>CHIRALPAK® IH-3</b>  <b>CHIRALPAK® IB-(N)3</b> <b>CHIRALPAK® IC-3</b>	Aqueous solution ①	50 mM Phosphate Buffer pH 2.0  OR  H <sub>3</sub> PO <sub>4</sub> aq. pH 2.0  OR  100 mM KPF <sub>6</sub> (or NaPF <sub>6</sub> ) aq. pH 2.0 adjusted with H <sub>3</sub> PO <sub>4</sub>	Water	20 mM Borate Buffer pH 9.0  OR  20 mM Phosphate Buffer pH 8.0 ⑥  OR  100 mM KPF <sub>6</sub> (or NaPF <sub>6</sub> ) 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<sub>3</sub>CN to start the investigation.
  - The elution power of organic modifiers for these columns is in the descending order of CH<sub>3</sub>CN > EtOH > MeOH: 50% CH<sub>3</sub>CN ≈ 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<sub>3</sub>CN due to their high viscosity in mixtures with water.
- ❸  Retention can be adjusted by changing the proportion of CH<sub>3</sub>CN. Retention may be very sensitive to the amount of CH<sub>3</sub>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.
- 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  
3.40 g KH<sub>2</sub>PO<sub>4</sub> / FW 136.09, make up the volume to 500 ml with HPLC grade water
  - Solution B:** phosphoric acid (H<sub>3</sub>PO<sub>4</sub> 85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.
- Preparation of pH 2 KPF<sub>6</sub> (NaPF<sub>6</sub>) solution:
  - Solution A:** 100 m M potassium (sodium) hexafluorophosphate  
9.20 g KPF<sub>6</sub> / FW 184.06 or 8.40g NaPF<sub>6</sub> / FW 167.95, make up the volume to 500 ml with HPLC grade water
  - Solution B:** phosphoric acid (H<sub>3</sub>PO<sub>4</sub> 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<sub>4</sub>HCO<sub>3</sub> / 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<sub>3</sub>) 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<sub>2</sub>HPO<sub>4</sub> / FW 174.18, make up the volume to 500 ml with HPLC grade water
  - Solution B:** 20 mM potassium dihydrogenophosphate  
1.36g KH<sub>2</sub>PO<sub>4</sub> / 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<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O / FW 381.37, make up the volume to 500 ml with HPLC grade water
  - Solution B:** 20 mM boric acid  
0.62g H<sub>3</sub>BO<sub>3</sub> / 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<sub>3</sub>CN 60:40 (v/v) for instance)

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

## Column storage

- For column storage, remove the acidic or basic additives by flushing the column with the same mobile phase without the additive. Columns can be stored with the additive-free mobile phases.

***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@chiraltech.com](mailto:questions@chiraltech.com) or call 800-6-CHIRAL

In the EU: [cte@chiral.fr](mailto:cte@chiral.fr) or call +33 (0)3 88 79 52 00

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