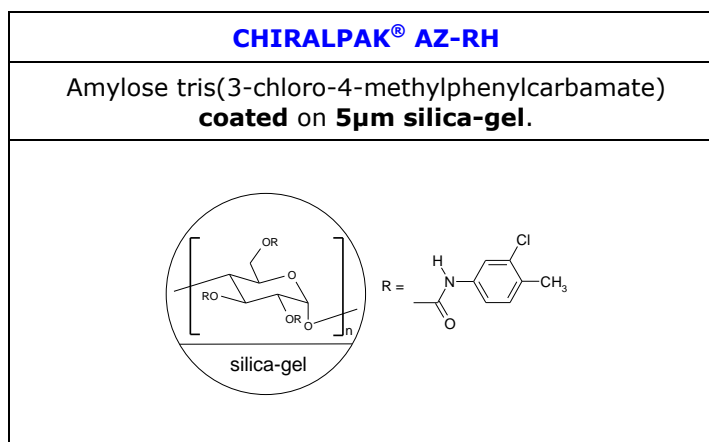


INSTRUCTION MANUAL FOR CHIRALPAK® AZ-RH

Please read this instruction sheet completely before using this column

Column Description



CAUTION

The entire HPLC system including the injector and the injection loop must be flushed with a solvent compatible with the column and its storage solvent prior to connecting the column. Many of the solvents commonly used in HPLC eluents such as acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride and THF may DESTROY the chiral stationary phase if they are present, even in residual quantities, in the system.

If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed and the relevant solvent lines flushed.

Operating Conditions

	150 x 2.1 mm i.d. Analytical column	150 x 4.6 mm i.d. Analytical column
Flow rate direction	As indicated on the column label	
Typical Flow rate	~ 0.1 to 0.3ml/min	~ 0.5 to 1.0ml/min
Pressure limitation	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.	
Temperature	5°C to 40°C	

Operating Procedure

CAUTION

Basic conditions SHOULD BE AVOIDED, either in the sample solution or in the mobile phase.

**Please contact Chiral Technologies for further assistance
before trying any solvents not mentioned below.**

A - Mobile Phases / For UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds
CHIRALPAK® AZ-RH	Aqueous solution ❶	HCOOH aq. pH 2.0	Water
	Organic modifier ❷	CH ₃ CN or MeOH or EtOH or IPA	
	Typical starting conditions ❸	Aqueous solutions CH ₃ CN	60% 40% ❹

☞ NOTE 1: If you cannot achieve sufficient resolution, try the complementary mobile phases:

B – Complementary Mobile Phases / For UV Detection

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds
CHIRALPAK® AZ-RH	Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H ₃ PO ₄ aq. pH 2.0 OR 100mM KPF ₆ (or NaPF ₆) aq. pH 2.0 adjusted with H ₃ PO ₄	Water
	Organic modifier ❷	CH ₃ CN or MeOH or EtOH or IPA	
	Typical starting conditions ❸	Aqueous solutions CH ₃ CN	60% 40% ❹

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

- ❶ Refer to **section C** for preparation of aqueous solution.
- ❷
 - ❑ 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 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.

- ⑥ 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.
- ④ 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

C – Buffer preparation – Examples

➤ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50mM 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: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF₆ / FW 184.06 or 8.40g NaPF₆ / FW 167.95, 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.

Column Care / Maintenance

- The use of a guard cartridge is highly recommended for maximum column life.
- Samples should preferably be dissolved in the mobile phase. The mobile phase and the sample solution should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.
- Before disconnecting the column from the HPLC, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, e.g. Water/CH₃CN 60:40 (v/v).
- If the column is contaminated with non eluted components, wash it with a mobile phase that does not contain any salts / buffers then with 100% CH₃CN for 2 hours at 0.3ml/min. If the non-eluting components are more soluble in methanol, this solvent may be used for the washing step.
- All salts must be flushed out from the HPLC system and the column before changing to 100% CH₃CN or 100% methanol.
- Use Water/CH₃CN 60:40 (v/v) to store the column.

Important Notice

⇒ STRONGLY BASIC solvent additives or sample solutions MUST BE AVOIDED, because they are likely to damage the silica gel used in these columns.

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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