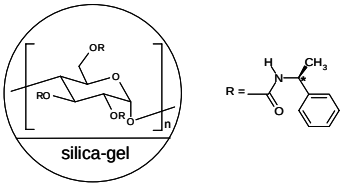
INSTRUCTION MANUAL FOR CHIRALPAK® AS-RH COLUMNS

150 x 4.6 mm ID analytical column

Please read this instruction sheet completely before using this column

Column description:

Packing composition: Amylose tris [(S)-α-methylbenzylcarbamate] coated on **5µm silica-gel**.



Shipping solvent: **H_2O / CH_3CN (50:50 v/v)**

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

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. 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 restrictions

	150 x 4.6 mm ID Analytical column
Flow rate direction	As indicated on the column label
Typical Flow rate ①	~ 0.5 to 1.0ml/min Do not exceed 1.5ml/min
Pressure limitation ②	Should be maintained < 50 Bar (~700 psi)③ for maximum column life Do not exceed 100 Bar (~1400 psi)
pH ④	Between pH 2.0 and pH 9.0
Temperature ⑤	5°C to 40°C

① The maximum flow rate depends on the mobile phase viscosity (mobile phase composition), and should be adjusted in accordance with the pressure upper's limit (i.e. 100 Bar).

② The back pressure value that should be taken into account is the one generated by the column itself. This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

③ Ideal value for maximum column life, but stable up to 100 Bar.
④ A pH less than 8.0 is recommended for maximum column life.
⑤ Keep the temperature between 5°C to 25°C when used with a pH higher than 7.0.

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Operating procedure

Please contact CHIRAL TECHNOLOGIES EUROPE for further assistance before trying any solvents not mentioned below.

A - Mobile phases

		BASIC Compounds	**ACIDIC** Compounds	**NEUTRAL** Compounds
CHIRALPAK® AS-RH 150 x 4.6 mm ID	**Aqueous solution ❶**	20mM Borate Buffer ($H_3BO_3/Na_2B_4O_7$) pH 9.0 ❷+❸ 20mM Phosphate Buffer (KH_2PO_4/K_2HPO_4) pH 8.0 ❸+❹	50mM Phosphate Buffer pH 2.0 H_3PO_4 aqueous solution pH 2.0	Water
	Organic modifier ❺	CH_3CN or MeOH or EtOH or IPA		
	Typical starting conditions ❻	Aqueous solution: 50% Organic modifier: 50% ❼		

❶ Concentration of the buffering salt should be less than 500mM.

❷
- **To secure column life the use of a guard cartridge is necessary when basic conditions are required.**
- The use of strong basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel support used to make this stationary phase.

❸ When this column is used at a pH > 7, the temperature has to be maintained between **5°C and 25°C** for maximum column life.

❹ Do not use phosphate buffer at a pH > 8. When pH 9 is necessary, use borate buffer for maximum column life.

❺
- Acetonitrile is recommended first to start the analyses
- The elution power of organic modifiers for this column is in the descending order of Acetonitrile > Ethanol > Methanol: 50%CH_3CN # 65-70%EtOH # 75-80%MeOH
- The use of other organic solvents has not been investigated and could be harmful to the column.
- The use of alcohol causes the back pressure to be significantly higher than when using acetonitrile as organic modifier due to their high viscosity in mixtures with water.

❻
- Suggested starting mobile phase composition is water (buffer) / acetonitrile 50:50 (v/v). If the sample elutes too early or too late, the percentage of acetonitrile can be decreased or increased accordingly.
- Small changes in acetonitrile concentration usually make large differences in retention times.
- The mobile phase should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before using.
- Lowering the column temperature may increase the selectivity.
- Increasing the column temperature and decreasing the flow rate may increase the resolution.

❼ 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

B – Buffer preparation – example

➢ *Preparation of pH 2 buffer:*

Solution A: 50mM potassium dihydrogenophosphate (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 8 buffer:*

Solution A: 20mM potassium dihydrogenophosphate (1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml with HPLC grade water).
Solution B: 20mM potassium hydrogenophosphate (1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water).
Adjust the pH of solution B to a value of 8.0 using solution A.

➢ *Preparation of pH 9 buffer:*
Solution A: 20mM boric acid (0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water).
Solution B: 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).
Adjust the pH of solution B to a value of 9.0 using solution A.

Column care / Maintenance

- 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.
- If the column has become contaminated with non eluted components, wash it with 100% acetonitrile for two hours at 0.3ml/min. Alternatively, 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 of the HPLC system and column before changing to 100% acetonitrile or 100% methanol.
- Use water / acetonitrile 50:50 (v/v) to store the column.

Important Notice

⇨ **This instruction sheet is not applicable to any other DAICEL columns.**

⇨ If you have any questions about the use of this column, or encounter a problem, please contact CHIRAL TECHNOLOGIES EUROPE for assistance (cte@chiral.fr).

Operation of this column in accordance with the guidelines outlined here will result in a long column life.

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TABLE OF DAICEL CHIRAL COLUMNS

Type of Adsorbent	Column Trade Name	Phase Type		Particle Size	
		Normal Phase	Reversed phase	5 µm	10µm
Amylose Carbamate	CHIRALPAK® AD	◆			◆
	CHIRALPAK® AD-H	◆		◆	
	CHIRALPAK® AD-RH		◆	◆	
	CHIRALPAK® AS	◆			◆
	CHIRALPAK® AS-H	◆		◆	
	CHIRALPAK® AS-RH		◆	◆	
Cellulose Carbamate	CHIRALCEL® OD	◆			◆
	CHIRALCEL® OD-H	◆		◆	
	CHIRALCEL® OD-R		◆		◆
	CHIRALCEL® OD-RH		◆	◆	
	CHIRALCEL® OC	◆			◆
	CHIRALCEL® OF	◆			◆
	CHIRALCEL® OG	◆			◆
Cellulose Ester	CHIRALCEL® OJ	◆			◆
	CHIRALCEL® OJ-H	◆		◆	
	CHIRALCEL® OJ-RH		◆	◆	
	CHIRALCEL® OA	◆			◆
	CHIRALCEL® OB	◆			◆
	CHIRALCEL® OB-H	◆		◆	
	CHIRALCEL® OK	◆			◆
	CHIRALCEL® CA	◆		NA	NA
Crown Ether	CROWNPAK® CR(+)		◆	◆	
	CROWNPAK® CR(-)		◆	◆	
Ligand Exchange	CHIRALPAK® MA(+)		◆	3 µm	
	CHIRALPAK® WH		◆		◆
Polymethacrylate	CHIRALPAK® OP(+)	◆			◆
	CHIRALPAK® OT(+)	◆			◆

Columns packed with 20µm material dedicated to preparative scale applications (50 & 100mm I.D.) are also available from Chiral Technologies Europe.
For more detailed information, refer to our catalogue also available on our website: http://www.chiral.fr or contact Chiral Technologies Europe.