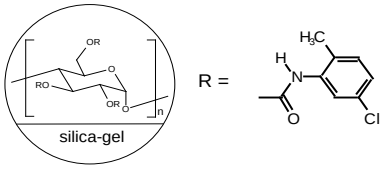
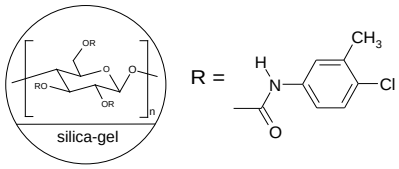
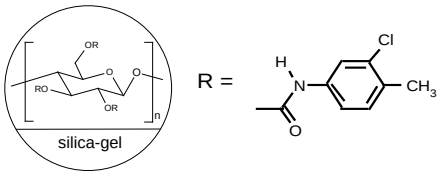
INSTRUCTION MANUAL FOR CHIRALPAK® AY-3R, CHIRALCEL® OX-3R and CHIRALCEL® OZ-3R

Please read this instruction sheet completely before using these columns

Column Description

CHIRALPAK® AY-3R	**CHIRALCEL® OX-3R**
Amylose tris(5-chloro-2-methylphenylcarbamate) **coated** on **3 µm** silica-gel	Cellulose tris(4-chloro-3-methylphenylcarbamate) **coated** on **3 µm** silica-gel
	

CHIRALCEL® OZ-3R
Cellulose tris(3-chloro-4-methylphenylcarbamate) **coated** on **3 µm** silica-gel


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. Microbore columns	**50 x 4.6 mm i.d.** Analytical columns	**100 x 4.6 mm i.d.** Analytical columns	**150 x 4.6 mm i.d.** Analytical columns
Flow rate direction	As indicated on the column label			
Typical Flow rate	0.1 to 0.5 ml/min	0.5 to 5 ml/min	0.5 to 4 ml/min	0.5 to 2.5 ml/min
Temperature	0 to 40°C			

☞ *NOTES:* *The column is stable to HPLC pressures.*
At a given temperature, the column back pressure is linearly proportional to the flow rate.

Operating Procedure

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	**BASIC** Compounds ❹
CHIRALPAK® AY-3R **CHIRALCEL® OX-3R** **CHIRALCEL® OZ-3R**	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		
	Typical starting conditions ❸	Aqueous solutions 60% CH_3CN 40% ❺		

☞ *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	**BASIC** Compounds ❹
CHIRALPAK® AY-3R **CHIRALCEL® OX-3R** **CHIRALCEL® OZ-3R**	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.
	Organic modifier ❷	CH_3CN or MeOH or EtOH or IPA		
	Typical starting conditions ❸	Aqueous solutions 60% CH_3CN 40% ❺		

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

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 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 25ºC 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

- 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/CH_3CN 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% CH_3CN for 2 hours at 0.5ml/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 from the HPLC system and column before changing to 100% CH_3CN or 100% methanol.
- Use Water/CH_3CN 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:

In the USA: questions@chiraltech.com or call 800-6-CHIRAL
In the EU: cte@chiral.fr or call +33 (0)3 88 79 52 00
In India: chiral@chiral.daicel.com or call +91-40-2338-3700

Locations:

North/Latin America
Chiral Technologies. Inc.
800 North Five Points Road
West Chester, PA 19380
800 6 CHIRAL
Tel: 610-594-2100
Fax: 610-594-2325
chiral@chiraltech.com
www.chiraltech.com

Europe
Chiral Technologies Europe
Parc d'Innovation
Bd Gonthier d'Andernach
67400 Illkirch Cedex, France
Tel: +33-388-795-200
Fax: +33-388-667-166
cte@chiral.fr
www.chiral.fr

India
Daicel Chiral Technologies (India) Pvt. Ltd.
Lab No. 4A, Phase III
IKP Knowledge Park
Genome Valley, Turkapally,
Shameerpet, Ranga Reddy Dist.
Hyderabad-500 078, Telangana
Tel: +91-40-2338-3700
Fax: +91-40-2348-0104
chiral@chiral.daicel.com