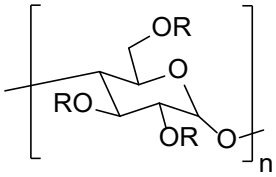
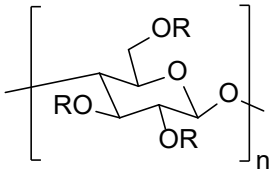
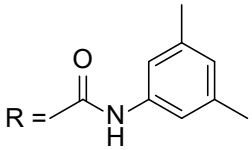
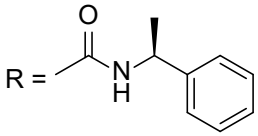
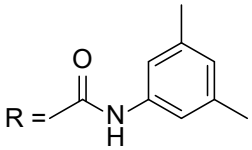
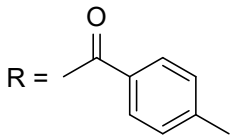
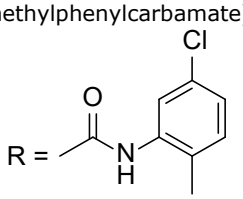
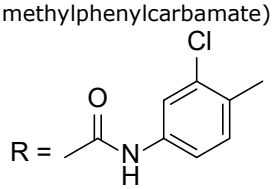
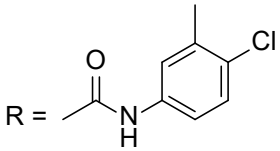
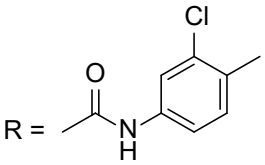
INSTRUCTION MANUAL FOR Analytical Columns of CHIRALPAK® AD-3R, AS-3R, AY-3R, AZ-3R CHIRALCEL® OD-3R, OJ-3R, OX-3R, and OZ-3R

<Reversed-Phase>

Please read this instruction sheet completely before using these columns

Column Description

AMYLOSE-BASED		CELLULOSE-BASED	
			
Coated on **3 µm silica gel**		**Coated** on **3 µm silica gel**	
CHIRALPAK® AD-3R	**CHIRALPAK® AS-3R**	**CHIRALCEL® OD-3R**	**CHIRALCEL® OJ-3R**
Amylose tris(3,5-dimethylphenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dimethylphenylcarbamate) 	Cellulose tris (4-methylbenzoate) 
CHIRALPAK® AY-3R	**CHIRALPAK® AZ-3R**	**CHIRALCEL® OX-3R**	**CHIRALCEL® OZ-3R**
Amylose tris(5-chloro-2-methylphenylcarbamate) 	Amylose tris(3-chloro-4-methylphenylcarbamate) 	Cellulose tris(4-chloro-3-methylphenylcarbamate) 	Cellulose tris(3-chloro-4-methylphenylcarbamate) 

Shipping Solvent: **Water/Acetonitrile = 60/40 (v/v)**

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, were included with the column when purchased.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

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 as HPLC eluents including acetone, chloroform, DMF, dimethylsulfoxide, ethyl acetate, methylene chloride, and THF, may DESTROY the chiral stationary phase if they are present, even in residual quantities, within the system.
If an auto-sampler is used, then the solvent employed to flush this unit between injections should also be changed to something compatible and the relevant solvent lines flushed.

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 x 2.1 mm i.d.** Analytical Column	**50 x 4.6 mm i.d.** **100 x 4.6 mm i.d.** **150 x 4.6 mm i.d.** **250 x 4.6 mm i.d.** Analytical Columns
Guard	**//**	**10 x 4.0 mm i.d.** Guard Column
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min
Pressure Limitation①	Please contact Technical Support for details	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

① The column pressure is the total pressure minus the system pressure. 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 Both UV and Mass Detections

CAUTION

Basic conditions SHOULD BE AVOIDED, both in the sample solution and the mobile phase, for CHIRALPAK® AZ-3R.

	CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALPAK® AZ-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R		CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
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	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *NOTE 1: If you cannot achieve sufficient resolution, try the complementary mobile phases (Section B)*

B – Complementary Aqueous and Buffer Solutions / For UV Detection Only

	CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALPAK® AZ-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R		CHIRALPAK® AD-3R CHIRALPAK® AS-3R CHIRALPAK® AY-3R CHIRALCEL® OD-3R CHIRALCEL® OJ-3R CHIRALCEL® OX-3R CHIRALCEL® OZ-3R
	ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO4 aq. pH 2.0 OR 100mM KPF_6 (or NaPF6) aq. pH 2.0 adjusted with H_3PO4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF6 (or $NaPF_6$) aq.
Organic modifier ❷	ACN or MeOH or EtOH or 2-PrOH		
Typical starting conditions ❸	Aqueous solutions 60% ACN 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 ACN to start the investigation
- ❑ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50% ACN ≈ 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 ACN due to their higher viscosity in mixtures with water.

❸
- ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the

amount of ACN present in 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 strongly 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 a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended 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/ACN 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% ACN 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% ACN or 100% methanol.
- Use Water/ACN 60:40 (v/v) to store the column, at room temperature

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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