# Care and Use Note of Fresh Bioscience columns

# Exsil<sup>™</sup> Chiral JM-R HPLC Columns

**IMPORTANT Safety Considerations** 

Columns are intended for use by technically qualified personnel only. Columns operate at high pressures. To avoid leaks or pressure-related failures, follow all manufacturer's directions to ensure all fittings and connections are tight and secure before operating the column. Refer to the QC chromatogram for maximum operating pressures and adjust operating conditions and limits accordingly. Flow rates and pressure should never exceed those listed in Table 1.

Users must be aware of the hazards associated with the mobile phase used and need to use appropriate personal protective equipment and engineering controls based on the MSDS for the mobile phase in use.

#### Specifications

Dimensions:	As shown in Table 1 below
Particle Size:	3/5/10µm
Connecting Fittings:	10-32 Fitting for 1/16" tubing
Column Material:	Type 316 Stainless Steel
Shipping Solvent:	As described in column QC
	chromatogram enclosed with column

Column Size		Particle	FlowRate (mL/min)		Max	Pressure
i.d.,mm)	Part No.	Size	Normal	Max.	(psig)	Range
100x2.0	6136333. s1002	3	0.2—0.5	1.0	5000	2–9
150x3.0	6136333. s1503	3	0.4—0.8	1.2	5000	2–9
150x4.6	6136353. s1546	5	0.5—1.0	1.5	5000	2–9
250x4.6	6136303. s2546	10	1.0-2.5	3.0	5000	2–9

### Table 1: Exsil<sup>™</sup> Chiral JM-R common Columns

#### **Column Installation**

#### Direction of Flow

Install and use the Exsil<sup>™</sup> Chiral Column with the flow through the column matching the flow direction arrow on the column label.

#### **Connecting Tubing**

It is important to use connecting tubing that provides the smallest possible dead volume. For analytical columns, use 0.005–0.010" i.d. tubing from injector to column inlet and from column outlet to the detector. Connecting tubing should be as short as possible. Avoid using a connecting union wherever possible, as it may cause peak broadening.

#### Pump Selection

Use a pump that provides minimum output flow pulsation. Strong pulsation will result in reduced resolution and can degrade the column. Pulsation may be effectively reduced by using a pulse dampener at the pump outlet.

#### Mobile Phase Pretreatment

#### (1) Filtering

Always filter the mobile phase and sample solutions through a  $0.5\mu$ m or smaller mesh filter before use as they may contain undissolved matters and debris that can cause column degradation or chromatographic noise.

#### (2) Degassing

Always thoroughly degas the mobile phase before use or install an On-Line Degassing System on your HPLC system. Alternatively, degassing by helium sparging under reduced pressure may be used, but it must be noted that the organic solvent content in aqueous solutions may be significantly affected by the degassing time.

#### **Column Connection**

The Exsil™ Chiral Column should always be installed in the following manner for proper operation.

- (1) Purge all air from the LC system tubing with an acetonitrile: water (80:20) mobile phase.
- (2) Set the flow rate at 0.3 mL/min for 2.0mm i.d. columns (1.0 mL/min for 4.6mm i.d. columns) and start the pump. As the solution begins to emerge from the column input line, remove the column inlet and outlet stoppers and immediately connect the solvent line to the column inlet. After several drops of the solution emerge from the column outlet, connect the column outlet to the detector.
- (3) Set the flow rate at the rate given in the QC chromatogram and purge the column with a solution flow totaling 10 to 20 times the volume of the column.

#### **Mobile Phase Modifiers**

For basic samples or acidic chiral compounds, it may be necessary to use an appropriate mobile phase modifier in order to achieve chiral resolution and to insure proper peak shapes. Diethylamine, ethanolamine and butyl amine in the concentration range 0.1-0.5 % can be used with basic analytes, while trifluoroacetic or acetic acid (0.1-0.5 %; typically 0.1-0.2 %) with acidic analytes. Mixtures of basic and acidic mobile phase additives are acceptable (e.g. diethylamine acetate or trifluoroacetate). Exsil<sup>™</sup> columns will deliver consistent results when operated with mobile phases containing additives at the concentration levels specified above. However, limited decrease in column efficiency may occur when a column is used in combination with these additives. Therefore, we advise to dedicate columns to mobile phases containing basic additives.

#### **Mobile Phase Restrictions**

Exsil<sup>™</sup> chiral stationary phases are prepared by coating silica with various polysaccharide derivatives. Therefore, any solvent dissolving the polysaccharide derivative (such as tetrahydrofurane, acetone, chlorinated hydrocarbons, ethylacetate, dimethylsulfoxide, dimethylformamide, N-methylformamide, toluene, methylethyl ketone and methyl tert-butyl ether etc...) must be avoided even in trace amounts (e.g. even as sample solvent).

#### **Mobile Phase Modes**

Exsil<sup>™</sup> Chiral Columns may be used with compatible isocratic, continuous gradient, or step gradient mobile phases.

#### Flow Rate

The flow rate should never exceed the maximum flow rate given in **Table 1**. For frequent column usage the normal flow rate is recommended.

#### **Operating Temperatures**

Operating temperatures should generally be within the range  $4 - 50^{\circ}$ C. High-temperature operation may result in bubble generation, necessitating degassing or temperature reduction. Low-temperature operation may require reduced flow rates because of increased eluent viscosity.

#### **Extending Lifetime and Reconditioning**

Long-term, repeated use of the column may cause considerable change in the elution characteristics of saccharides due to accumulation of microadsorbents from the sample solution. In these and other cases the column may be cleaned in the following manner.

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.

Fresh Bioscience highly recommends the use of guard cartridges to extend the lifetime of your column, especially with samples extracted from complex matrixes.

#### **Column Handling and Storage**

When not in use, the column may be left in the LC system without flushing for up to several days, so long as no corrosive agent or propagating bacteria are present. It is essential to ensure that no part of the flow path in the LC system or column becomes dry at any time while not in use. If any possibility of contamination or drying is present, thoroughly purge the column and LC system with water / acetonitrile 80:20 (v/v), disconnect, and stopper the column.

Disconnected columns should be stored in an area free from large temperature changes (preferably in a constant temperature room) with both ends tightly stoppered to prevent internal drying.

Storage in an area exposed to direct sunlight or large temperature changes may cause column degradation.

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