

Operating Instructions for CHIROBIOTIC™ LC Stationary Phases

Column Installation

CHIROBIOTIC columns are shipped in methanol. Before starting to use a new column, wash with 20 mL HPLC grade methanol at 1 mL/min. The column test standard, 5-methyl-5-phenylhydantoin, can be injected at this stage to establish performance.

New columns can take longer to equilibrate, but once baseline stability is achieved it is consistent. New threaded hardware improves column performance. Do not overtighten. This threaded hardware also allows for direct connection to a guard column.

General Operating Conditions

Flow Rate Range:	0.5 to 3.0 mL/min.	Typically-1.0 mL/min.
Pressure Range:	Up to 3500 psi (240 bar)	See details below
pH Range:	CHIROBIOTIC V	3.5 to 7.0
	CHIROBIOTIC V2	3.5 to 7.0
	CHIROBIOTIC T	3.8 to 6.8
	CHIROBIOTIC T2	3.8 to 6.8
	CHIROBIOTIC R	4.0 to 6.8
	CHIROBIOTIC TAG	3.8 to 6.8
Injection Volume:	1 to 5 μ L of a 1 mg/mL solution	See details below

Mobile Phases

All known HPLC solvents and buffers are compatible with CHIROBIOTIC phases. The only critical operating parameter detrimental to these columns is extremes of pH. For best performance we highly recommend the Astec Eluent Degasser (Cat. No. 89703). Proven very beneficial, especially for low viscosity mobile phases, it can handle flow rates from 0.05 to 20.0 mL/min.

Injection Volumes and Concentrations

Injection volumes of 1-5 μ L at concentrations of 1 mg/mL are desirable for good selectivity. The load volume and concentration may be 5 to 10 times higher without affecting resolution. Therefore, begin the separation study at the lowest volumes and concentrations until a proper determination can be made of its effect.

Astec columns may be operated from either direction without loss of performance due to the unique packing system that produces uniform packing density. This is true for all Astec columns.

Temperature

Several temperature effects have been noted with the CHIROBIOTIC phases. At lower temperatures, resolution has been observed to increase with certain compound structures. It is not a generally expected phenomena, however, no deleterious effects on column performance or stability has been observed when operating at column temperatures down to 0 °C. It is possible, however, to prevent racemization from occurring when operating below room temperature as has been observed with diazepines.

At elevated temperatures two effects have been observed. First, an increase in efficiency can be obtained as with standard chromatography. Second, and most importantly, a reversal of elution order can be observed at elevated temperatures. This phenomena is again, compound dependent and not predictable.

The maximum temperature that can be safely employed is 45 °C in all solvent modes. Temperature changes should be made no faster than 2 °C per minute

Pressure

Operating pressure for CHIROBIOTIC columns is generally in the range of 1200 to 1500 psi (80-110 bar) (150 x 4.6 mm) and 2000 to 2500 psi (135-170 bar) (250 x 4.6 mm) at 1.0 mL/minute for 20/80:MeOH/buffer. As with standard reversed phase columns, the higher the water content, the higher the back pressure, with a maximum at 50/50:MeOH/H₂O. Care should always be exercised in prefiltering and degassing the water and solvent used with these columns. In general, pressures should not exceed 3500 psi (240 bar). Columns are packed in excess of 10,000 psi, (680 bar), so column beds are extremely stable to pressure.

Regeneration

Columns showing decreased resolution can sometimes be regenerated by passing several column volumes of 50/50: ACN/50 mM NH₄OAc followed by pure HPLC water and then acetonitrile or methanol through the column at 0.5 mL/min. Acetonitrile or methanol may be used for final displacement and storage. Long term storage (>24 hours) is best done in isopropanol or methanol.

Storage

The column after conditioning with methanol, is tested with 5-methyl-5-phenylhydantoin before storage. When analysis is complete, the column should be returned to this solvent to ensure long life.

Never Store These Phases In Any Buffer Even For Short Periods Of Time. Wash With Water, Then With Either Methanol, Ethanol Or Acetonitrile.

Column Assessment Parameters

Column assessment parameters provide useful data as to the repeatability of column performance and can be used to evaluate your column for signs of deterioration. Each column is individually tested and assigned a serial number for traceability of all column components. Since virtually every molecule, organic or inorganic, can be "included" no useful marker has been found to measure void volume. The retention volume of the solutes is used as a relative measure of consistent packing.

Stability Data

After testing for selectivity, a 250 x 4.6 mm column was run at 1.0 mL/min. with 10/90: CH₃CN/1% TEAA buffer at pH 7.0. A second column was similarly tested at pH 4.0. After 100 hours of continuous operation there was no observable change in alpha. The k', after showing an initial drop of approximately 10%, was stable throughout the experiment. Publications on clinical applications have verified operating performance. As many as 3000 plasma extracts have been reported injected before any deterioration was observed.

A precolumn (before injector) of 40 µm silica should always be used when operating silica based columns with aqueous mobile phases.

LC-MS

CHIROBIOTIC columns are ideally compatible for LC-MS application using either the polar ionic mode or reversed phase with volatile salts and buffers. See CHIROBIOTIC Handbook for details.

Register Your Column Purchase using the enclosed card. Comprehensive details of method development and optimization techniques are contained in the CHIROBIOTIC handbook. If you do not have a copy, please complete and return the enclosed card or e-mail techservice@sial.com.

Preparative Columns and Media

CHIROBIOTIC CSPs are available in 2" and 4" prepacked columns as well as in bulk 10 μ m and 16 μ m media for preparative separations. Please contact our Technical Service Department for specific information (Phone: 800-359-3041 or 814-359-3041, email: techservice@sial.com).

Guard Column Systems

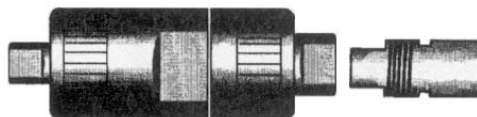
Astec guard columns are available in a variety of configurations.

1. Integral Guard System

The holder threads directly onto the analytical column. There is no dead volume from connectors. They are available in two different internal diameters: (A) for 2.0 mm I.D. columns; and, (B) for 3.0, 4.0 and 4.6 mm I.D. columns. This configuration results in maximum NTP, minimal symmetry distortion leading to the lowest possible detection limits.

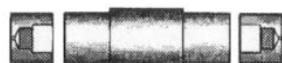
Cartridges are made of stainless steel with Delrin® end caps. They are available in 2 cm x 2.0 mm for 2.0 mm I.D. columns and 2 cm x 4.0 mm for 3.0, 4.0 and 4.6 mm I.D. columns which fit both the Integral and Universal Guard Systems.

Integral Guard Column Holder



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Guard Cartridge Assembly fits both Universal and Integral Holders



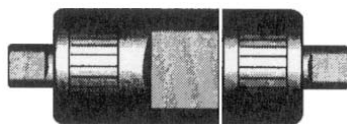
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2. Universal Guard System

This is an independent guard column holder that requires a column coupler and this guard column system fits all internal diameters. Precision manufactured from 316 stainless steel, the inlet and outlet of holder connections are made using standard capillary tubing and fittings. Cartridges are packed with 3.5 or 5 μ m materials.

Cartridges are made of stainless steel with Delrin end caps (see above). They are available in 2 cm x 2.0 mm for 2.0 mm I.D. columns and 2 cm x 4.0 mm for 3.0, 4.0 and 4.6 mm I.D. columns which fit both the Integral and Universal Guard System

Universal Guard Cartridge Holder



G003950

3. Microbore Guard System

This is an independent guard column system requiring a column coupler. Column dimensions are 2 cm x 1.0 mm. Used only for 2.0 mm I.D. and smaller columns. These are prepacked and ready to use.

4. Prep Guard System

A 5 cm x 10 mm stainless steel column is packed with the same 5 μ m media as the analytical column. This guard column can be used for 10 mm and 21.2 mm I.D. columns.

Trademarks

CHIROBIOTIC — Sigma-Aldrich Co.

Delrin — E.I. duPont de Nemours & Co., Inc.