Prevail™ Carbohydrate ES 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.

Introduction

Prevail™ Carbohydrate ES Columns are packed with a rugged, hydrophilic polymeric gel giving high efficiency, excellent stability without column bleed, good reproducibility, and long column lifetime. The columns are versatile. They are predominantly used to analyze mono- and oligosaccharides by normal-phase liquid chromatography, but can also be used to analyze negatively charged compounds by ion-exchange chromatography.

Under the chromatographic conditions generally used for sugar analysis, Prevail™ Carbohydrate ES Columns provide equal resolution to and greater reproducibility than competitive silica-based columns. Because of its superior gel stability, the Prevail™ Carbohydrate ES Column permits the selection of both organic and aqueous mobile phases. The pH of the aqueous mobile phase may range from pH 2 to pH 13, allowing a variety of buffers for mobile phase optimization and chromatographic efficiency.

Prevail™ Carbohydrate ES Columns reach their full potential when used with the Evaporative Light Scattering Detector (ELSD). This combination of column/detector yields excellent sensitivity, total gradient compatibility, and stable baselines free from noise and drift.

Column Installation

Direction of Flow

Install and use the Carbohydrate ES 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.

Specifications	
Dimensions:	As shown in **Table 1** below
Particle Size:	5µm
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

Table 1: Prevail™ Carbohydrate ES Columns

Column Size (Length x i.d., mm)	Part No.	Particle Size	Flow Rate (mL/min)		Max. (psig)	Pressure Range
			Normal	Max.		
150 x 4.6	**35102**	5	0.5—1.0	1.5	2200	2–13
250 x 4.6	**35101**	5	0.5—1.0	1.5	2200	2–13
53 x 7.0	**35104**	5	1.0—2.5	3.0	2200	2–13
100 x 7.0	**35103**	5	1.0—2.5	3.0	2200	2–13

Mobile Phase Pretreatment

(1) Filtering

Always filter the mobile phase and sample solutions through a 0.5µm 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 (Alltech Part No. **590102**) 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 Prevail™ Carbohydrate ES 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 (75:25) mobile phase.

(2) Set the flow rate at 0.5 mL/min for 4.6mm i.d. columns (1.0 mL/min for 7.0mm 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.

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Mobile Phase (continued)

Saccharide Analysis

(1) Water, acetonitrile, and ethanol, either alone or in mixtures of any ratio, may be used as mobile phase. When using ELS detection, other organic solvents (e.g. acetone) may be chosen as the detector response is unaffected by the spectral properties of the mobile phase. Such solvents can offer alternative selectivity and reduced run times. Take care to ensure that the column backpressure does not exceed the recommended value of 2200psig. As different solvent combinations yield varying viscosities and backpressures, the flow rate may need adjustment.

(2) Various types of buffers soluble in acetonitrile or ethanol (such as tetrapropyl ammonium acetate or sodium acetate) may be used together with the above components so long as no observable precipitation occurs in the mobile phase. When using ELS detection, avoid phosphate or inorganic salt-based buffers; only volatile buffers (such as ammonium carbonate, ammonium acetate, and ammonium formate) may be used.

Ion-Exchange Chromatography

(1) Buffer solutions such as phosphate, acetate, and tris, with or without NaCl, KCl, or Na_2SO_4, may be used.

Mobile Phase Modes

Prevail™ Carbohydrate ES 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 – 50ºC. 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.

Column Cleaning

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.

Pass aqueous 0.01N nitric acid totaling 10 to 20 times the column volume through the column at the normal flow rate or lower. Purge all nitric acid from the column with distilled water and then pass aqueous 0.01N sodium hydroxide totaling 10 to 20 times the column volume at the normal flow rate or lower. Purge all sodium hydroxide from the column with distilled water.

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 30–80% aqueous acetonitrile, 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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