Agilent ZORBAX Carbohydrate Analysis Column

Data Sheet

General Description

The ZORBAX Carbohydrate Analysis column is an application-specific column for the separation of mono-and other saccharides. This packing is produced by reacting 3-aminopropylsilane with ZORBAX SIL (silica) particles. The reaction conditions used to produce ZORBAX Carbohydrate were specifically developed to maximize surface coverage with a monolayer bonded phase. The uniform, spherical, ZORBAX particles are 5 µm in diameter, and have a controlled pore size to give optimum column efficiency. Columns are packed to a uniform bed density using a proprietary, high-pressure, slurry-loading technique.

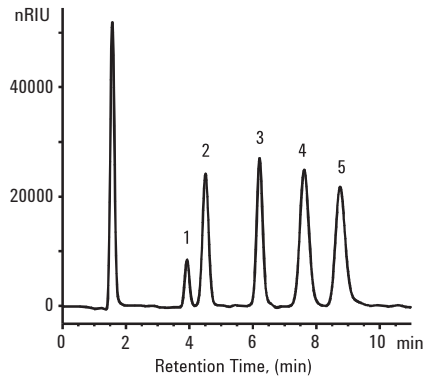
Column Characteristics

Figure 1 shows typical chromatographic performance for a 4.6 x 150 mm column. The actual performance of your column may be slightly different. Each column is individually tested in a normal phase mode to ensure a quality packed-bed structure and reproducible separation. The actual QC test and performance of your column is described on the Column Performance Report enclosed with your column.

Figure 1
Typical Performance of ZORBAX Carbohydrate Analysis Column

OPERATING CONDITIONS
Column: ZORBAX Carbohydrate Analysis, 4.6 mm ID x 150 mm (5 µm)
Mobile Phase:75/25 Acetonitrile/Water
Flow Rate: 1.4 mL/min
Temperature: 30° C
Detector: HP1100 RID, 30°C
Sample Volume: 3 µL in 50/50 Acetonitrile/Water

Peak Identity:		
	1. Fructose	3.2 mg/mL
	2. Glucose	12 mg/mL
	3. Sucrose	12 mg/mL
	4. Maltose	20 mg/mL
	5. Lactose	20 mg/mL



Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users should be aware of the hazards from such leaks due to the toxicity or flammability of the chosen mobile phases.
- Because of the small particle size, dry ZORBAX packings are respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column.
- While generally not harmful to the column, reversing flow should be avoided except to attempt removal of inlet pluggage (see "Column Care" section).
- ZORBAX Carbohydrate Analysis Columns are shipped containing acetonitrile. As the column is to be used with an aqueous mobile phase, flush it first with acetonitrile/water (75/25) then, with at least 20 column volumes of the chosen mobile phase.
- ZORBAX Carbohydrate Analysis Columns are compatible with water and all common organic solvents.
- Use of a guard column is recommended to protect the ZORBAX Carbohydrate Analysis Column and extend its useful lifetime (see Part Numbers).
- Maximum operating pressure is 400 bar (6000 psi).
- Maximum operating temperature is 60 °C.
- **NOTE:** ZORBAX columns are designed for high stability at low pH (e.g., pH < 5). However, all silica-based packings have some solubility in pH > 6 aqueous mobile phases. Therefore, when using silica-based columns under conditions of pH > 6, maximum column lifetime is obtained by operation at low temperature (< 40 °C) using low buffer concentrations in the range of 0.01 to 0.02M. Column stability at pH > 6 is also enhanced by avoiding phosphate and carbonate buffers [ref.: H.A. Claessens, M.A. van Straten, and J.J. Kirkland, *J. Chromatogr.*(A), 728 (1996) 259].
- Avoid exposure to aldehydes and methyl ketones, which may form Schiff's bases with the bonded phase.
- The column should be filled with 100% acetonitrile for long term storage.

Sample Preparation

The leaner the sample to be analyzed, the longer the column life. Care should be taken to remove lipids and ionic contaminants from the sample before injection onto the column. This will prolong column lifetime. Routine filtering of samples should be considered if there is the possibility that the samples contain particulate matter (see Part Numbers for syringe filters).

Mobile Phase Selection

As organic content (e.g. acetonitrile) of the mobile phase increases, the resolution of the sugars may improve, with an accompanying increase in analysis time. The higher the aqueous content that is used, the more soluble are the sugars in the mobile phase, resulting in decreased retention. The typical range for the mobile phase is 60% to 75% acetonitrile. Retention time of the sugars may decrease with extended column use. By increasing the amount of acetonitrile in the mobile phase, retention can be adjusted to the appropriate time.

Temperature

Control of temperature is important for maintaining retention time reproducibility and for maintaining refractometer baseline stability.

Resolution may be improved when column temperature is above room temperature. Operation at 30 °C or 35 °C may give better resolution of a carbohydrate mixture and; therefore, temperature should be investigated for optimizing resolution.

Small changes in temperature has a noticeable effect on baseline stability of the Refractive Index Detector (RID). Therefore, it is important to shield the HPLC column and system from temperature fluctuations. Use of a thermostated column compartment and use of a RID with temperature stabilization is highly recommended for best performance.

Flow Rate

Adjusting flow rate is appropriate for minimizing analysis time. Typical flow rates are 1 to 1.5 mL/min. The column should not be operated above the back pressure limit (400 bar).

Column Care

The inlet frit on these columns has a nominal porosity of 2 µm. Samples that contain particulate matter larger than 2 µm may plug the column inlet frit and should be filtered before injection into the column. ZORBAX guard columns and a hardware kit are recommended for use to extend column life (see Part Numbers).

If solvent flow appears to be restricted (high column-back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the column is the source of the high back-pressure, there may be particulate matter on the inlet frit. An initial attempt should be made to clear any inlet debris by back-flushing 25-30 mL of mobile phase through the column. If this fails to return the column to near its original back-pressure, the inlet frit should be changed. To remove the frit, loosen the nut at the column inlet, taking care not to turn the end fitting itself. Then remove the fitting, taking care not to disturb the column bed. The frit should drop out when the fitting is tapped sharply on a hard surface. Install a new frit and carefully tighten the fitting.

The columns are shipped in acetonitrile. Equilibrate the column with the desired mobile phase (e.g. 75/25 acetonitrile/water by volume) until a steady baseline is obtained on the detector. Many column volumes of mobile phase may be necessary for equilibration to occur after changes in mobile phase composition. The number of column volumes required depends on the system in use.

Since columns have 1/16" terminations, a short 1/4" wrench should be used in attaching the column to the HPLC to prevent overtightening. Overtightening the fittings can damage the fitting and necessitate replacement.

Ordering Information	Agilent Part No.
Carbohydrate Analysis Columns (5 µm)	
4.6 mm ID x 150 mm	843300-908
4.6 mm ID x 250 mm	840300-908
Guard Column	
4.6 mm ID x 12.5 mm (4 Pack)	820950-908
Guard Column Hardware Kit	820888-901
Syringe Filters for Aqueous Solutions (100 Pack)	
Regenerated Cellulose (0.45 µm)	
13 mm for 1-10 mL sample size	5061-3365
20 mm for 1-50 mL sample size	5061-3364

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