BIOshell™
U/HPLC Columns

This Data Sheet Contains Important Information About The Product

Care & Use Sheet for 2.7 µm BIOshell Glycan Column

Description

BIOshell™ Glycan is a high speed, high performance liquid chromatography column based on a Fused-Core® particle design. The Fused-Core particle provides a thin porous shell of high-purity silica surrounding a solid silica core. This particle design exhibits very high column efficiency due to the shallow diffusion paths in the 0.5 micron thick porous shell and the small overall particle size of 2.7 microns. The BIOshell Glycan stationary phase is a highly polar ligand that possesses 5 hydroxyl groups tethered to the silica via novel, proprietary chemical linkage. This unique column chemistry is suitable for analysis using the typical mobile phases for hydrophilic interactive liquid chromatography (HILIC) of oligosaccharides, particularly for protein-linked glycans.

Column Characteristics

Each column is QC tested using small organic probe molecules. A printed test report including the actual test chromatogram and performance results for this column is enclosed. In addition, each lot of BIOshell Glycan material is tested for Quality Assurance by separation of a procainamide reducing end labeled glycan ladder of oligosaccharides of 2-25 glucose units (GU). The peaks for 5 and 10 GU must meet tight specifications for retention and peak widths before the lot is approved for BIOshell Glycan. The QA analysis chromatogram for the lot of material used for manufacture of this column is shown on the reverse side.

The Fused-Core particle has a surface area of ~135 m^2/g and an average pore size of 90 angstroms. The Fused-Core particles are 30% to 50% heavier than commerically available totally porous particles due to the density of the solid cores. Therefore, the effective surface area per column is similar to columns packed with totally porous particles having surface areas in the 225-300 m^2/g range.

Operation Guidelines

- The direction of flow is marked on the column label.
- Reversed flow may be used to attempt removal of inlet pluggage or contamination.
- A new column contains a mixture of 90% acetonitrile:10% water.
- Water and all common organic HPLC solvents are compatible with BIOshell Glycan columns.
- It is suggested that the column be equilibrated with mobile phase having a low concentration of acetonitrile (>50% water) before initial use.
- BIOshell Glycan columns are best used at temperatures below 65 °C for maximum column life.
- Mobile phase pH for BIOshell Glycan columns is best maintained in the range of pH = 2 to 9 for maximum column stability.
- BIOshell Glycan columns are stable to operating pressures up to at least 600 bar (9000 psi) and have been used at up to 1000 bar (14,500 psi).

Guidelines for Low-Volume Columns

High performance columns with small internal volumes (shorter lengths, internal diameters <3 mm) are being increasingly used for high speed separations, especially with specialty detection systems such as mass spectrometers. These low-volume columns generate peaks having considerably less volume than those eluting from columns of larger dimensions (e.g., 15 cm x 4.6 mm). All low-volume columns perform best when used with proper attention to the following factors.

- **Detector –** Flow cells should be of low-volume design (preferably <2 µL).
- **Detector –** To properly sense and integrate the often very fast peaks that elute from low-volume columns, the detector response time should be set to the fastest level (~0.1 second) and the integration software should sample the detector signal at least20 points per second.
- **Injector –** The injection system should be of a low-volume design.
- **Connecting Tubing –** The shortest possible lengths of connecting tubing with narrow internal diameters (at most 0.005 inch, 0.12 mm I.D.) should be used to connect the column to the injector and the detector cell.
- **Peak Retention –** As retention is increased, the volume of a peak increases, decreasing the effects on band spreading caused by components of the instrument.
- **Sample Solvent –** For isocratic separations, the sample should be dissolved in the mobile phase or in a solvent that is weaker (less polar) than the mobile phase.
- **Injection Volume –** For isocratic separations, the volume of sample injected should be kept as small as possible (typically 2 µL or less). Sample volumes are less critical for gradient separations, especially if the sample is dissolved in a weak solvent.

Column Care

To maximize column life, ensure that samples and mobile phases are particle-free. The use of guard columns or an in-line filter with 0.5 micron porosity between the sample injector and the column is highly recommended. The 2 micron porosity frits on BIOshell Glycan columns are less subject to pluggage than are the 0.5 micron frits typically used with other small-particle columns. Should the operating pressure of the column suddenly increase beyond normal levels, reversing the flow direction of the column may be attempted to remove debris on the inlet frit.

To remove strongly retained materials from the column, flush the column in the reverse direction with very strong solvents such as 10:90 methanol and deionized water. Extreme cases may require the use of very strong solvents such as 100% of the most polar component of the mobile phase in use, which is typically water.

Column Storage

Long-term storage of silica-based columns is best in 100% acetonitrile. Columns may be safely stored for short periods (up to 3 or 4 days) in most common mobile phases. However, when using buffers, it is best to remove the salts to protect both the column and the HPLC equipment by first flushing the column with the same mobile phase without the buffer (e.g., when using 90:10 acetonitrile:buffer, flush the column with 90:10 acetonitrile:water). To eliminate any concern about salt precipitation or corrosion from the salts, flush the column with 100% acetonitrile for storage. Before storing the column, the end-fittings should be tightly sealed with the end-plugs that came with the column to prevent the packing from drying. After storing the column in 100% acetonitrile for a prolonged period of time, the packing material may become dehydrated, necessitating a rehydration treatment. We recommend flushing the column at a reduced flow rate at room temperature overnight in 50% acetonitrile:water mixture.

Applications

HILIC is especially attractive in situations where compound retention is poor using reversed phase chromatography (RPC), which includes protein-linked glycans and many oligosaccharides. Retention in HILIC is a combination of hydrophilic interaction, ion-exchange and hydrophobic retention. The aqueous layer which forms on the surface of HILIC particles promotes interaction with polar solutes. Retention in HILIC is a function of the mobile phase opposite to that in RPC. In HILIC, a strong mobile phase has a high concentration of water and a weak mobile phase has a high concentration of organic solvent. For gradient separations, the initial mobile phase has a high concentration of organic solvent and the gradient is generated by increasing the aqueous concentration.

Protein N-linked and O-linked glycans can be usefully studied employing HILIC separations methods. Protein-linked glycans are released by various chemical or enzymatic methods, followed by isolation from protein and reactants by several methods, including gel filtration, HILIC or RPC Solid Phase Extraction, or selective solvent precipitation. Glycan resolution using HILIC can use either native glycans, often with MS detection (negative ions), or a suitable chromophore or fluorophore can be attached at the reducing terminus to render the glycan detectable using absorbance or fluorescence detectors. Reductive amination labeling is common and for QA of the BIOshell Glycan column, procainamide is employed for terminal alditol labeling via Schiff's base reaction. Procainamide allows MS (positive ion), fluorescence (Ex 330 nm/Em 380 nm), or absorbance detection (300 nm). Separations of glycan mixtures using the BIOshell Glycan column typically use acetonitrile and aqueous ammonium formate buffer (50 mM, pH 4.4) to form a gradient of increasing water content during elution. The aqueous buffer is prepared with 50 mM ammonium formate solutions, adjusting to final pH with formic acid, then diluting to volume.

Separations usually have a column temperature of 50-60 °C, with limited resolution improvement from further temperature increase. The column length, flow rate, initial gradient conditions, gradient program time and final gradient composition can be varied to manipulate resolution of glycan mixtures. The highest resolution is obtained with longer columns and gradient times. Columns of 2.1 mm ID are operated in the range of 0.2 – 0.6 mL/min, and other column I.D. flow rates can be calculated using this range. A typical condition for a relatively simple mixture of protein-linked glycans is detailed in the column QA chromatogram, but complex mixtures for "glycomic" experiments or complex glycoproteins, may require longer elution at decreased flow rates with longer columns. For samples of smaller glycans, initial acetonitrile of 80% or greater (20% aqueous) may be needed, and larger glycans (20+ GU) may require final % acetonitrile to be less than 60% (>40% aqueous). Shallow gradients increase separation selectivity of closely eluting glycans.

Larger volume injection of samples with high water content can result in poor peak shape, early elution, or even elution in the void volume. However, oligosaccharides exhibit limited solution solubility in high organic content that is sample concentration and temperature dependent (lower temperatures promote precipitation). A reasonable compromise is to dilute samples to 50-65% acetonitrile at room temperature prior to injection, and to be aware that concentrated oligosaccharides (or any carbohydrate) may precipitate at organic concentrations above 50% acetonitrile.

Technical Support

For technical support on this product, contact your local Sigma-Aldrich office, designated distributor in your country, or visit **sigma-aldrich.com/BIOshell**

Trademarks

BIOshell — Sigma-Aldrich Co. LLC
Fused-Core — Advanced Materials Technology, Inc.

References

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