HALO® 90 Å Glycan, 2.7 µm Column Care & Use Sheet

Description

HALO® 90 Å Glycan is a high-performance liquid chromatography column based on a new Fused-Core® particle design. The Fused-Core® particle provides a thin porous shell of high-purity silica surrounding a solid silica core. The HALO® 90 Å Glycan stationary phase is a highly polar ligand that possesses 5 hydroxyl groups tethered to the silica via novel proprietary linkage chemistry. This high performance material provides a column that can be used with the typical mobile phases for hydrophilic interactive liquid chromatography (HILIC) of oligosaccharides and particularly for protein-linked glycans.

Column Characteristics

Each column is QC tested by the use of small organic molecules. A printed test report including the actual test chromatogram and performance results for this column is enclosed. In addition, each lot of HALO® 90 Å 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 Glycan columns. The QA 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 Å. Fused-Core® particles are 30% to 50% heavier than commercially available totally porous particles making the effective surface area per column 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 90% acetonitrile/10% water.
- Water and all common organic solvents are compatible with HALO® 90 Å Glycan columns.
- IT IS SUGGESTED THAT THE COLUMN BE EQUILIBRATED WITH MOBILE PHASE HAVING A LOW CONCENTRATION OF ACETONITRILE (>50% WATER) BEFORE INITIAL USE.
- HALO® 90 Å Glycan columns are best used at temperatures below 65 ºC.
- Mobile phase pH for HALO® 90 Å Glycan columns is best maintained in the range of pH = 2 to 9 for maximum column stability.
- HALO® 90 Å 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).

Column Care

Ensure that samples and mobile phases are particle-free. The use of guard columns between the sample injector and the column is recommended. 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/water or 100% 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 ACN/buffer, flush the column with 90/10 ACN/H_2O) then 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 reduced flow rate at room temperature overnight in 50% acetonitrile/water mixture.

Safety

- **HPLC columns are for laboratory use only. Not for drug, household, or other use.**
- Users of HPLC columns should be aware of the toxicity or flammability of the mobile phases. Precautions should be taken to avoid contact and leaks.
- HPLC columns should be used in well-ventilated environments to minimize concentration of solvent fumes.
- Stable retention may require hydration of the highly polar surface by flushing the column with > 50% water before use or when routinely using acetonitrile concentrations at or above 90%.

Applications

HILIC is especially attractive in situations where compound retention is poor in reversed phase chromatography (RPC), which includes protein-linked glycans and many oligosaccharides. Retention in HILIC seems to be 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, the strong mobile phase has high concentration of water and the weakest 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 formed by increasing the aqueous concentration.

Protein N-linked and O-linked glycans can be usefully studied employing HILIC separations methods[1,2]. 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[3,4], and for QA of the HALO® 90 Å 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 HALO® 90 Å 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 ºC, 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. 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[5]. 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.

Guidelines for Low-Volume Columns

Low-volume columns (e.g., short columns, internal diameters ≤ 3 mm) generate peaks having considerably less volume than those eluting from columns of larger dimensions (e.g., 4.6 mm x 150 mm). The efficiency of low-volume columns is highly dependent on the HPLC system having components designed to minimize band spreading. 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 < 2µl).
- Detector – To properly sense and integrate the 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 least 20 points per second.
- Injector – The injection system should be of a low-volume design. Auto-samplers may cause band spreading with low-volume columns but may be used for convenience.
- Connecting Tubing – Short lengths of connecting tubing with narrow internal diameters (at most 0.005-inch, 0.12 mm ID) should be used to connect the column to the injector and the detector cell. The tubing must have flat ends and bottom out inside all fittings. Zero-dead-volume fittings should be used where required.
- 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 than the mobile phase. For gradient separations, the sample should be dissolved in the initial mobile phase or in a solvent substantially weaker than the final mobile phase
- Injection Volume – For isocratic separations, the volume injected should be kept as small as possible (2 µl or less). Sample volumes are less critical for gradient separations, especially if the sample is dissolved in a weak solvent (higher organic concentration). Volumes of 10 µl or more may be tolerated.

References

1. Anamula, K.R. and Dhume, S.T. (1998) Glycobiol. 8, 685-694.
2. Wada, et al., (2007) Glycobiol. 17, 411-422.
3. Harvey, D.J. (2011) J. Chromatogr. B 879, 1196-1225.
4. Klapoetke, S., Zhang, J., Becht, S., Gu, X., and Ding, X. (2010) J. Pharm. Biomed. Anal. 53, 315-324.
5. Practical HPLC Method Development, Second Edition, L.R. Snyder, J.L. Glajch, and J.J. Kirkland, (John Wiley & Sons, 1997), Chapters 6, 7, 8, and 11.

Ordering Information

For ordering information or for technical support on this product, please contact your local HALO® distributor at advanced-materials-tech.com

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