HALO® 160 Å C30, 2.7 µm Column Care & Use Sheet

Description

HALO® 160 Å C30 is a high-speed, high-performance liquid chromatography column based on the wider-pore 160 Å Fused-Core® particle design to provide adequate spacing for the long-chain C30 ligands. 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 endcapped triacontrylsilane stationary phase of HALO® 160 Å C30 provides a stable, reversed-phase packing that can be used for hydrophobic, long-chain, structural isomers, such as vitamins and lipids.

Column Characteristics

A printed QC report including the actual test chromatogram and performance results is enclosed with every column.
The Fused-Core® particle has a surface area of ~ 90 m^2/g and an average pore size of 160 Å. The Fused-Core® particles are 30% to 50% heavier than commercially 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 of 150 - 200 m^2/g.

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 acetonitrile and water. Initial care should be taken to avoid mobile phases that are immiscible with this mixture or could cause a precipitate.
- Water and all common organic solvents are compatible with HALO® 160 Å C30 columns.
- HALO® 160 Å C30 columns are best used at temperatures below 60 ºC for maximum column life.
- Mobile phase pH for HALO® 160 Å C30 columns is best maintained in the range of pH = 2 to 9 for maximum column stability.
- HALO® 160 Å C30 columns are warranted to be stable to operating pressures up to 600 bar (9000 psi).

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 HALO® 90 Å C30 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 100% of the organic component of the mobile phase in use. A mixture (95/5 v/v) of dichloromethane and methanol is often effective at removing lipidic contaminants and certain detergents. Extreme cases may require the use of very strong solvents such as dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

Column Storage

Long-term storage of silica-based, reversed-phase 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 flushing the column with the same mobile phase without the buffer. Before storing the column, the end-fittings should be tightly sealed with the end-plugs that came with the column.

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 chosen for use with the columns. Precautions should be taken to avoid contact and leaks.
- HPLC columns should be used in well-ventilated environments to minimize concentration of solvent fumes.

Applications

The HALO® 160 Å C30 bonded phase is nonpolar in nature. It is best utilized with mobile phases that are mixtures of methanol and water or acetonitrile and water. Higher levels of the organic solvent component will typically reduce the retention of the sample compounds. Separations based on shape selectivity generally show increased resolution at lower temperatures so an effective method development strategy will explore ambient and subambient conditions. Using elevated temperatures (e.g., 40 – 60 ºC) will reduce the viscosity of the mobile phase and allow the use of faster flow rates and lower column pressure for high sample throughput. Gradient elution techniques using 5 -10% organic component as the initial mobile phase and increasing to 100% organic component as the final mobile phase often can effect separations of complex sample mixtures in minimal time.

HALO® 160 Å C30 columns are highly suited for the reversed-phase separation of geometric and positional isomers. Ionizable compounds, such as acids and bases, are generally best separated with mobile phases buffered at pH of 2 to 3. The use of 20-50 mM buffers is always recommended for optimum results and long-term stability when separating ionizable compounds. Additional information on solvent selection and separation techniques can be found in Chapters Six, Seven, and Eight, *Practical HPLC Method Development,* Second Edition, L.R. Snyder, J.L. Glajch, and J.J. Kirkland, (John Wiley & Sons, 1997).

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 mass spectrometers. These low-volume columns 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 separations performed in 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 cell volumes should be < 2µl. To properly sense and integrate the very fast peaks that can elute from low-volume columns, the detector response time should be set to the fastest level (~ 0.1 second) to allow integration of signal by software of at least 20 points across the narrowest peak.
- Injector – The injection system should be of a low-volume design (e.g., Rheodyne Model 8125). Auto-samplers will often cause band-spreading, but may be used for convenience with the expectation of some loss in column efficiency.
- Connecting Tubing – The shortest possible lengths of connecting tubing with small internal diameters (≤ 0.005-inch, 0.12 mm ID) must be used to connect the column to the injector and the detector cell.
- Peak Retention – As retention is increased, the peak volume increases, decreasing extra-column band spreading caused by components of the instrument.
- Sample Injection – For isocratic separations, the volume of sample injected should be kept as small as possible (≤ 2 µl) in a solvent weaker than the mobile phase. Sample volumes are less critical for gradient separations, and a larger volume is possible if the sample is dissolved in a weak solvent.

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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