HALO® 90 Å C18, 2.7 µm Nano/Capillary Column Care & Use Sheet

Description

HALO® 90 Å C18 is a high-speed, 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. 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 densely bonded, extensively endcapped dimethyloctadecyl stationary phase of HALO® 90 Å C18 provides a stable, reversed-phase packing that can be used for basic, acidic, or neutral compounds.

Column Characteristics

The Fused-Core® particle has a surface area of ~ 135 m^2/g and an average pore size of 90 Å. A printed report including the actual test chromatogram and performance results is enclosed with every column.

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 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 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® 90 Å C18 columns.
- HALO® 90 Å C18 columns are best used at temperatures below 60 ºC for maximum column life.
- Mobile phase pH for HALO® 90 Å C18 columns is best maintained in the range of pH = 2 to 9 for maximum column stability.

HALO® Nano/Capillary Columns	
ID (microns)	Max Pressure (bar)
75 - 100	600
200 - 500	400

Column Care

To maximize column life, ensure that samples and mobile phases are particle-free. The use an in-line filter with 0.5-micron porosity between the pump and sample injector is highly 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 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 this task. 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 (e.g., when using 60/40 ACN/buffer, flush the column with 60/40 ACN/H_2O) to eliminate any danger from corrosion from the salts while providing rapid re-equilibration of the column with the original mobile phase.

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.

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® 90 Å C18 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. 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® 90 Å C18 columns are highly suited for the reversed-phase separation of basic, neutral, or acidic compounds. 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 < 0.5 mm) are being increasingly used for high sensitivity and 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., 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:

- LC/MS – Most nano/capillary columns are utilized with the Mass Spec as the detector. Spray tips should be of low-volume design (preferably ~20nL or less) to minimize band spreading.
- UV Detector – Flow cells should be of low-volume design (preferably ~20nL or less) to minimize band spreading. 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 least 20 points per second.
- Injector – The injection system should be of a low-volume design (nano). The volume of sample injected should be kept as small as possible. It is highly recommended that a concentration trap cartridge is used to reduce injection volume and remove unwanted salts.
- Connecting Tubing – The shortest possible lengths of connecting tubing with narrow internal diameters (at most 50µm ID) should be used to connect the column to the injector and the detector cell. The tubing must have flat ends and should bottom out inside all fittings. Zero-dead-volume fittings should always 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 (more polar) 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.

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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