HALO® 90 Å C8, 2 µm Column Care & Use Sheet

Description

HALO® 90 Å C8 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 0.4-micron thick porous shell and the small overall particle size of 2-microns. The densely bonded, extensively endcapped dimethyloctyl stationary phase of HALO® 90 Å C8 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 ~ 120 m^2/g and an average pore size of 90 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 in the 225-300 m^2/g range.

Operation Guidelines

- The direction of flow is marked on the column label. Columns should NOT be operated in a reverse-flow direction. (See discussion in Column Care Section below.)
- 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 C8 columns.
- HALO® 90 Å C8 columns are best used at temperatures below 60 ºC for maximum column life.
- Mobile phase pH for HALO® 90 Å C8 columns is best maintained in the range of pH = 2 to 9 for maximum column stability.
- HALO® 90 Å C8 columns are stable to operating pressures up to 1000 bar (14,500 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 1-micron porosity frits on HALO® 90 Å C8 columns are less subject to pluggage than are the 0.2-0.5-micron frits typically used with other small-particle columns, but these frits may allow a small number of packing particles to escape if the column is run in the reverse-flow direction. The column direction is indicated on the label and it should only be reverse flushed if other measures are not successful to remove inlet blockage. To remove strongly retained materials from the column, flush the column 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 protect both the column and the HPLC equipment and remove the salts 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 C8 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 C8 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 < 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., 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 cells should be of low-volume design (preferably < 2µl).
- 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 least 20 points per second.
- Injector – The injection system should be of a low-volume design (e.g., Rheodyne Model 8125). Auto-samplers will often cause band spreading with low-volume columns 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 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 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.
- 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.

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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