HALO® 400 Å C4, 3.4 µm 1.0 mm
Column Care & Use Sheet

Description
HALO® 400 Å C4 is a high-speed, high-performance liquid chromatography column based on a new wide-pore (400 Å) 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 0.2-micron thin porous shell and the small overall particle size of 3.4-microns. The densely bonded, extensively endcapped dimethylbutyl stationary phase of HALO® 400 Å C4 provides a stable, reversed-phase packing that can be used for separating high molecular weight compounds such as proteins.

Column Characteristics
A printed report including the actual QC chromatogram and performance results is enclosed with every column. Also included with each column is a QA test report for the specific batch of packing contained in the column. The Fused-Core® particle has a surface area of ~ 15 m^2/g and an average pore size of 400 Å.

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 100% acetonitrile. Initial care should be taken to avoid mobile phases that are immiscible with this solvent or could cause a precipitate.
- Water and all common organic solvents are compatible with HALO® 400 Å C4 columns.
- HALO® 400 Å C4 columns are best used at temperatures below 90 ºC for maximum column life.
- Mobile phase pH for HALO® 400 Å C4 columns is best maintained in the range of pH = 1 to 9 for maximum column stability.
- HALO® 400 Å C4 columns are stable to operating pressures up to 275 bar (4000 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® 400 Å C4 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 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® 400 Å C4 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 – 90 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® 400 Å C4 columns are highly suited for the reversed-phase separation of high molecular weight compounds such as proteins with MW of 400 to 500 kDa. 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).
HALO® 400 Å C4 columns use a densely bonded, extensively endcapped dimethylbutyl bonded phase that can withstand the low pH and high temperature mobile phases that are used for protein separations. The use of low pH mobile phase modifiers, such as trifluoroacetic acid (TFA) at concentrations of 0.01-0.1% is recommended for protein separations without mass spectrometry (MS) detection. Under MS operating conditions, the use of formic acid at similar concentrations with the addition of 10-20 mM ammonium formate is suggested.

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). 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 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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