This Data Sheet Contains Important Information About The Product.

Ascentis® Express OH5 Column Care & Use Sheet

Ascentis Express OH5 Description

Ascentis Express OH5 is a high-speed, high-performance liquid chromatography column based on a 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 Ascentis Express OH5 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 for traditional normal phase separations using non-polar, totally organic mobile phases (not discussed in this document) or for aqueous normal phase chromatography with the typical mobile phases for hydrophilic interactive liquid chromatography (HILIC) of polar basic, acidic, zwitterionic, or neutral compounds.

Column Characteristics

The Fused-Core particle has a surface area of ~ 150 m^2/g and an average pore size of 90Å. 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 90% acetonitrile/10% water.
- Water and all common organic HPLC solvents are compatible with Ascentis Express OH5 columns.
- Ascentis Express OH5 columns are best used at temperatures below 60 ºC for maximum column life.
- Mobile phase pH for Ascentis Express OH5 columns is best maintained in the range of pH = 2 to 9 for maximum column stability.
- Ascentis Express OH5 columns are stable to operating pressures up to at least 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 Ascentis Express OH5 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 10/90 methanol and deionized water. Extreme cases may require the use of very strong solvents such as 100% of the most polar component of the mobile phase in use, which is typically 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). To eliminate any concern about salt precipitation or corrosion from the salts, 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.

Applications

HILIC is a useful and complimentary method to reversed-phase chromatography (RPC) and is especially attractive in situations where compound retention is poor in RPC. Greatest retention for many analytes is found when using more than about 70% organic (e.g., acetonitrile) in acidic mobile phases. High organic concentrations are used in the mobile phases, therefore, HILIC is especially favorable for separations using mass spectrometry (MS) detection.

Acetonitrile is typically used as the weak organic solvent in the mobile phase. With this solvent, 95% is typically the upper limit and 50-60% the lower limit for adequate retention. At least 5% of the mobile phase should be the highly polar solvent such as water or methanol. Water should be the polar solvent if a buffer is included because of solubility limitations. The organic solvent can be varied to alter retention and selectivity. Solvent strength (from weakest to strongest) for HILIC generally is tetrahydrofuran < acetone < acetonitrile < isopropanol < ethanol < methanol < water, where water is the strongest elution solvent.

For optimum column efficiency and reproducibility, buffers in the range of 5 - 50 mM concentration or additives in the 0.1%-0.5% range are used as the mobile phase. Phosphate buffers are not generally recommended because of their poor solubility in high organic mobile phases and incompatibility with MS detection, although phosphoric acid at low concentrations can be employed, as long as sufficient water is present in the mobile phase. Additives such as formic acid, trifluoroacetic acid and acetic acid at concentrations up to about 1% can be a part of the mobile phase. HILIC separations can exhibit high load tolerance and efficiency, but with dependence on buffering capacity of the mobile phase. Injection from a suitable organic/water mixture is a must, when injection volume becomes significant. Ammonium formate/formic acid buffers up to a final concentration of about 50 mM and pH 3-5 are especially effective for analysis of basic and acidic compounds using MS detection with either ESI or APCI interfaces. (This volatile mobile phase buffer seems to be a good starting point for many separations of both basic and acidic compounds.) Ammonium acetate at pH 5-8 also have been used at concentrations of 1-20 mM, but are generally less favored for separating stronger basic and acidic compounds. Buffers or additives above pH 9-10 usually are not recommended, particularly at elevated temperatures because of slow dissolution of the underlying silica support.

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). 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 < 2 µL).
- 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).
- 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.
- 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 (less polar) than the 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.

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