**This Data Sheet Contains Important Information About The Product.**

# Ascentis® Express 2.0 µm HILIC Column Care & Use Sheet

### Ascentis Express 2.0 µm Description

Ascentis Express 2.0 µm HILIC is a high-speed, UHPLC column based on the 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 bare silica stationary phase of Ascentis Express 2.0 µm HILIC 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 reversed-phase mobile phases for hydrophilic interactive liquid chromatography (HILIC) of 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.
- A new column contains 100% acetonitrile. 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 Ascentis Express 2.0 µm HILIC columns.
- Ascentis Express 2.0 µm HILIC columns are best used at temperatures below 60 °C for maximum column life.
- Mobile phase pH for Ascentis Express 2.0 µm HILIC columns is best maintained in the range of pH = 1 to 8 for maximum column stability.
- Ascentis Express 2.0 µm HILIC 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 Ascentis Express 2.0 µm HILIC 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 50/50 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. Alternatively, a mixture (95/5 v/v) of dichloromethane and methanol is often effective at this task.

### 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/H2O) 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.

### 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 and very high levels of water are required in the mobile phase for adequate retention. Retention in HILIC is not well-understood but appears to be a combination of hydrophilic interaction, ion-exchange and some reversed-phase retention. The aqueous layer which forms on the surface of HILIC particles promotes interaction with polar solutes. Retention in HILIC as a function of the mobile phase is just opposite from that in RPC. The strongest mobile phase has a high concentration of water and the weakest has a high concentration of organic solvent. Therefore, for gradient separations, the initial mobile phase has a high concentration of organic solvent and the gradient is formed by increasing the aqueous concentration. Greatest retention for basic and acidic 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 60-65% 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 type can be varied to change retention and separation selectivity, much as in RPC. Solvent strength (from weakest to strongest) for HILIC generally is tetrahydrofuran < acetone < acetonitrile < isopropanol < ethanol < methanol < water, where water is the strongest elution solvent. To further increase retention in HILIC, replacing some of the water in the mobile phase with another polar solvent such as methanol or isopropanol sometimes is effective.

For optimum column efficiency and reproducibility, buffers in the range of 10-20 mM concentration or additives in the 0.5% range are used the mobile phase. Phosphate buffers are not recommended because of their poor solubility in high organic mobile phases and incompatibility with MS detection. Additives such as formic acid, trifluoroacetic acid and phosphoric acid at concentrations up to about 1% can be a part of the mobile phase. Volatile ammonium formate/formic acid buffers up to a final concentration of about 20 mM and pH 3 are especially effective for separating both basic and acidic compounds when using MS detection. (Acetonitrile/formate mobile phases seem to be a good starting point for many separations of both basic and acidic compounds.) Ammonium acetate at pH ~5 also have been used at concentrations of 5-20 mM, but are generally less effective for separating stronger basic and acidic compounds. Buffers or additives above pH 6 usually are not recommended because of slow dissolution of the 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). 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 <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 (40 points per second preferred).
- 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 I.D.) 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, call 800-325-5832 (312-286-8032) or send an email to techserv@sial.com You may also visit ***sigma-aldrich.com/express***



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