Ascentis® Express RP-Amide Column Care & Use Sheet

Ascentis Express Description

Ascentis Express RP-Amide 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 µm thick porous shell and the small overall particle size of 2.7 µm. Ascentis Express provides a stable, reversed-phase packing that can be used for basic, acidic, or neutral compounds. The one-step bonded, endcapped, amide-based, polar-embedded stationary phase provides a stable, reversed phase packing with decreased hydrophobic character. Ascentis Express RP-Amide can be used for basic, acidic, or neutral compounds with orthogonal selectivity to C18 or C8.

Column Characteristics

The fused core particle has a surface area of ~ 150 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.
- 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 Ascentis Express RP-Amide columns.
- Ascentis Express RP-Amide columns are best used at temperatures below 60 °C for maximum column life.
- Mobile phase pH for Ascentis Express RP-Amide columns is best maintained in the range of pH = 2 to 9 for maximum column stability.
- The polar-embedded Ascentis Express RP-Amide columns are compatible with 100% aqueous mobile phases without suffering from phase collapse.

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 µm porosity between the sample injector and the column is highly recommended. The 2 µm porosity frits on Ascentis Express columns are less subject to pluggage than are the 0.5 µm 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.

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 by removing 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.

Applications

The Ascentis Express RP-Amide 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 °C) 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 affect separations of complex sample mixtures in minimal time.

Ascentis Express RP-Amide 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).

Ascentis Express RP-Amide will have similar retention but different selectivity for some compounds compared to alkyl phases. Typically, bases move to shorter retention compared to C18 columns.

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., 15 cm x 4.6 mm I.D.). 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.
- 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 0.010-inch, 0.25 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 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.

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