Supelco®

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Care & Use Guide for 2.7 µm Supel™ Carbon LC Column

Description

Supel™ Carbon LC is a high-speed, ultra-high performance liquid chromatography column based on 200 Å porous graphitic carbon (PGC) particle technology. The PGC particle provides a 100% carbon plane for the analytes to interact with via hydrophobic interactions and through ionic interactions via the polar retention effect on graphite (PREG) effect. This particle design exhibits high column efficiency for polar compounds and enhanced retention of compounds typically requiring hydrophilic interaction liquid chromatography (HILIC) to retain. Through the use of patent-pending synthetic procedures, a narrow particle size distribution is prevalent with this technology, further enhancing the chromatographic performance that this column can display.

Column Characteristics

The opposite side of this Care & Use Guide displays the Quality Control (QC) test chromatogram which also includes the Batch Quality Assurance Test. The PGC particle has a surface area of 155 m^2/g and an average pore size of 200 Å.

Operation Guidelines

- The direction of flow is marked on the column label.
- Reversed flow may be used to attempt removal of inlet blockage or contamination.
- A new column contains a mixture of acetonitrile and water (80:20 v:v). Initial care should be taken to avoid mobile phases that are immiscible with this mixture or could cause precipitation.
- Supel™ Carbon LC columns have been application tested using mobile phases with pH values ranging from 1–14 with no reduction in lifetime with prolonged use. In addition, the unique nature of the PGC particle permits the use of elevated temperatures (up to 250 °C) without any decline in column lifetime. It is recommended to use a pre-column heater/post-column cooler when running applications employing elevated temperatures to minimize temperature gradients from forming in the column and to reduce the risk of detector damage from high temperature effluent entering the detector cell.
- Supel™ Carbon LC columns are stable to operating pressures up to 9,000 psi (620 bar). The column can be operated up to 11,500 psi (800 bar) though some decrease in column lifetime may be observed with prolonged use at this pressure.
- Separations of multi-component mixtures, with compounds having a wide range of polarities, should be separated by using gradient elution.
- Common mobile phases used with this column include, in order of increasing elution strength, water, methanol, acetonitrile, isopropanol, and tetrahydrofuran (THF).

Column Care

To maximize column lifetime, 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. 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. In addition, a mixture of 95:5 (v/v) THF: Isopropanol has been shown to remove lipid contaminants, detergents, ion pairing reagents, and strongly adsorbed, planar compounds. In extreme situations, the column can be flushed overnight with dimethylformamide (DMF) or dimethylsulfoxide (DMSO).

Column Storage

Supel™ Carbon LC should be stored in 80:20 (v/v) acetonitrile: water for long-term storage. Columns may be safely stored for short periods (up to 3–4 days) in most common mobile phases. However, when using buffers, it is best practice to remove the salts to protect both the column and the U/HPLC equipment by flushing the column with the same mobile phase without the buffer. Before storing the column, the end fittings should be tightly sealed with the end-plugs that came with the column.

Safety

- UHPLC columns are for laboratory use only. Not for drug, household, or other use.
- Users of UHPLC columns should be aware of the toxicity or flammability of the mobile phases chosen for use with the column. Precautions should be taken to avoid contact and leaks.
- UHPLC columns should be used in well-ventilated environments to minimize concentration of solvent fumes.

Applications

Supel™ Carbon LC columns are commonly operated by running a gradient from low (0–5%) to high (95–100%) organic solvent at low or high pH. The use of elevated temperature can increase sample throughput and can, in some cases, improve selectivity. The PGC-based nature of the column allows for long term operation of the phase at low and high pH and at high temperature. To minimize some of the adsorptive nature of the phase, mobile phase modifiers, like trifluoroacetic acid (TFA), difluoroacetic acid (DFA), or formic acid, can be employed in the mobile phase at concentrations ranging from 0.01–0.1% (v/v). These acids exhibit desirable, low UV transparency, volatility, and ion-pairing properties with compounds.

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 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 U/HPLC system having components designed to minimize band spreading. All low volume columns perform best when used with proper attention being paid to the following factors:

- **Detector:** Flow cell volumes should be <2 µL. To properly sense and integrate the fast peaks that can elute from low volume columns, the detector response time should be set to the fastest level (~0.1 s) to allow integration of signal by software of at least 20 points across the narrowest peak.
- **Injector:** The injection system should be of a low volume design. Autosamplers will often cause band spreading, but may be used for convenience with the expectation of some loss in column efficiency.
- **Connection Tubing:** The shortest possible lengths of connection tubing with small internal diameters (<0.005 in, 0.12 mm I.D.) must be used to connect the column to the injector and the detector cell.
- **Peak Retention:** As retention is increased, the peak volume increases, decreasing extra column band spreading caused by components of the instrument.
- **Sample Solvent:** 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.

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