Agilent HC-C18(2) and TC-C18(2) Columns

Data Sheet

General Description

The Agilent Technologies family of HC-C18(2) and TC-C18(2) columns are used both for developing new LC methods and for routine testing of finished products. These columns utilize an ultra-pure (Type B) silica support with high specific surface area (290 m^2/gram) and 170 Å pore size in a 5 µm particle. A proprietary bonding and exhaustive endcapping process gives high carbon loading and an inert surface. These columns have high loading capacity and give symmetrical peaks even for strongly basic compounds. The columns are stable at pH 2-8 (9). These columns are useful for the analysis of acidic, basic, and other highly polar compounds by reversed-phase liquid chromatography. These densely covered, deactivated columns can be especially beneficial for applications where high sample loads in a strong solvent are required. Columns are loaded to a stable, uniform bed density using a proprietary, high-pressure slurry-loading technique to give maximum column efficiency and maintain column bed stability.

Column Characteristics

Thorough quality control procedures are used to monitor all Agilent Technologies LC packing materials, including the measurement of surface area, pore size, and particle size of the base silica. Sensitive chromatographic tests are also performed on all packings to confirm lot-to-lot reproducibility. The Agilent HC-C18(2) has the highest carbon load (17%) and is highly endcapped. The Agilent TC-C18(2) has lower carbon loading (12%) but is still thoroughly endcapped. This phase is the most appropriate for applications requiring high aqueous mobile phases.

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.
- Because of the small particle size, dry packings are respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column
- While not harmful to the column, reverse flow should be avoided except to attempt removal of a plugged inlet.
- A new column contains a mixture of methanol and water. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- The Agilent HC-C18(2) and TC-C18(2) columns are compatible with water and all common organic solvents. It is generally not recommended to use the HC-C18(2) with mobile phases containing more than 95% water.
- Avoid use of these columns below pH 2 or above pH 8-9 HC-C18(2).
- Maximum operating pressure of these columns is 400 bar (6000 psi).
- Maximum operating temperature is 60 °C.
- Maximum column lifetime is obtained by operation at low temperatures (< 40 °C) using low buffer concentrations in the range of 0.01 to 0.02 M. Column stability at pH > 6 is also enhanced by avoiding phosphate and carbonate buffers [ref.: H. A. Claessens, M. A. van Straten, and J. J. Kirkland, *J. Chromatogr.* (A), 728 (1996) 259].
- Columns should not be maintained at elevated pH or elevated temperature when not in use.

Method Development

Column Choices

When developing reversed-phased LC methods, it is advisable to begin with the Agilent TC-C18(2) phase. This phase chemistry has the highest compatibility with high aqueous mobile phases. The 250 mm length will provide the highest efficiency. Optimum flow rates are about 1 mL/min for the 4.6 mm id columns. If there is not sufficient retention, change to the Agilent HC-C18(2).

Mobile Phase Choices

It is always advisable to use a buffered aqueous mobile phase. Buffer concentrations should be in the range of 20-50 mM. If the pKa of the compound is known, choose a pH that is one pH unit above or one pH unit below the pKa. If the sample pKa is not known, start with a pH 3 buffer. As a second choice, try a pH 7 buffer. Always be sure the buffers will be compatible with your detector and soluble with the organic component of the mobile phase.

A good choice for the organic portion of the mobile phase is methanol. It is inexpensive but will produce higher pressure than other common solvents. Acetonitrile and tetrahydrofuran (THF) can also be investigated if methanol is not suitable.

Temperature

For the most reproducible results use a controlled temperature that is within the temperature range of the column. Increasing column temperature can change the selectivity and may improve the separation.

Storage Recommendations

Long term storage of silica-based, bonded phase columns should be in a pure organic solvent, preferably an aprotic liquid such as 100% acetonitrile. If the column has been previously used with a buffered mobile phase, the buffer should first be removed by purging the column with 20–30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20–30 column volumes of the pure solvent. Before storing the column, the end-fittings should be tightly capped with end-plugs to prevent the packing from drying out.

Columns may be safely stored for short periods in most mobile phases. However, to protect equipment, it is desirable to remove salts from the instrument and column by purging the column with the same mobile phase without the buffer (for example, using 60/40 ACN/H_2O to remove a 60/40 ACN/0.02 M phosphate buffered mobile phase). Re-equilibration is rapid with the original mobile phase when using this approach, and any danger of corrosion from the salts is eliminated.

Agilent Ordering Information

For more information on our products and services, visit our Web site at: www.agilent.com/chem/supplies.

For Technical Support in China, call 1-800-820-3278.

Printed in the USA
January 31, 2008
Part No. 820695-001

Agilent Technologies