Agilent Prep-C18 Columns

Datasheet

General Description

Agilent Prep-C18 utilizes an ultra-pure (Type B) silica support with high specific surface area (400 m^2/gram) and 100Å pore size in two particle sizes – 5-microns and 10-microns. A proprietary bonding process gives high carbon loading (24%) and a highly inert surface. The Agilent Prep-C18 columns have high sample loadability and give symmetrical peaks even for strongly basic compounds. The columns are stable at pH 2-10. Agilent Prep-C18 is especially useful for the preparative purification of acidic, basic, and other highly polar compounds by reversed-phase liquid chromatography. This densely covered, deactivated, column packing can be especially beneficial for preparative separation of basic compounds that produce poor peak shapes and lower loadability on other columns. The extra-dense coating of bonded phase and exhaustive endcapping simultaneously deactivate the silica surface from deleterious interactions with samples, and also protects the silica support from dissolution in intermediate and higher pH environments. 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

The Agilent Prep-C18 Columns have two formats: cartridge format for 21.2 mm ID columns and conventional fixed end-fitting formats for 30 and 50 mm ID columns. For the cartridge columns, a special fitting kit is needed to connect the columns with an instrument. The kit can be ordered from Agilent and the Agilent P/N is 820400-901. The nominal average particle size of the packings used for Agilent Prep-C18 columns is either 5-microns or 10-microns with each column length and particle size combination chosen to produce columns with high separation performance and low operating pressures. In general, in order to obtain equivalent separation on a preparative column, relative to those obtained using analytical columns of the same packing, the mobile phase flow rate must be adjusted proportional to the square of the ratio of the column internal diameters (see Table 1).

All larger-diameter columns (20 mm or greater) are susceptible to cross-sectional thermal gradients where the interior core of the column becomes warmer during use compared to the area of the column near the column wall. This thermal gradient, while small during room-temperature operation, can cause observable band-broadening when the column is operated under non-overload sample conditions. When operating the column using typical preparative sample-overload conditions, these temperature effects are seldom important and can be ignored in most cases. The cross-sectional temperature variations are caused by the frictional heating of the mobile phase as it is forced under pressure through the packed bed of the column and the small-diameter tubing of the instrumentation. The heat near the column walls is more easily dissipated through the heat-conductive steel walls of the column while the heat in the center of the column is insulated by the relatively non-heat-conductive silica packing material. This thermal band-broadening increases if the column is being operated at temperatures higher than ambient. To avoid thermal effects, it is recommended that large-diameter columns, especially the higher mass 30 and 50 mm ID x 250 mm long columns, be heated in a water bath at 30°C or higher, if desired. It is important to also maintain the mobile phase at the same temperature as the column to avoid thermal mis-matches which may result in distorted peaks.

The 10µm packings used in the Agilent Prep-C18 columns are produced using the same particle and bonding technology employed in the production of 5µm Agilent Prep-C18 scalar packings in analytical size columns. The same thorough quality control procedures are used to monitor all Agilent Prep-C18 products, including the measurement of surface area, pore size, and particle size of the base silica packing as well as elemental analysis of all bonded phases. Sensitive chromatographic tests are also performed on all packings to confirm lot-to-lot reproducibility. This technology permits the direct scale-up of separations from analytical to preparative proportions with little or no modifications required in methodology.

Table 1
Typical Sample Capacities

Column ID	Normalized Flow Rate	Separation Type (small molecules)	
		Easy (alpha > 1.5)	Difficult (alpha=1.2-1.5)
4.6 mm	1.0 mL/min	3-15mg	0.5-3 mg
21.2 mm	20 mL/min	70-400 mg	20-70 mg
30 mm	40 mL/min	140-800 mg	40-140 mg
50 mm	100 mL/min	400-2000 mg	100-400 mg

Safety Considerations

The following points with respect to the safe use of preparative columns should be considered:

- Because of the larger volumes of mobile phase used with preparative columns, special awareness of solvent toxicity and flammability hazards is recommended.
- Maximum operating pressure limit for Preparative Columns is 340 bar (5000psi). Since liquid chromatographic columns are totally hydraulic in nature, little stored energy is present in these columns during use. Should a column be over-pressurized and a tubing or fitting failure occur, the major result will be a large flow leak of mobile phase. Special caution is required in this regard for flammable or toxic solvents.

Operational Guidelines

- The direction of flow is marked on the column.
- While generally not harmful to the column, reversing flow should be avoided except to attempt removal of inlet pluggage.
- A new 21.2 mm ID preparative cartridge is shipped dry; therefore, flush new cartridge columns with 10 column volumes of 100% organic solvent (e.g. Methanol or Acetonitrile) followed by at least 10 column volumes of mobile phase before use. This will avoid any equilibration problem and will ensure reproducible selectivity with new columns.
- New 30 and 50 mm ID columns should be flushed with at least 10 column volumes of mobile phase before use.
- Maximum operating pressure is 340 bar (5000 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 0.02M. 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].
- While the useful pH range is 2-10, caution should be taken when the columns are used at the pH limits. Extensive exposure to high or low pH mobile phase may reduce the lifetime of the columns.

Preparative Strategies

A detailed discussion of how to conduct preparative liquid chromatography is beyond the scope of this data sheet. However, a few helpful guidelines can be given.

- Prior to initial start-up of the preparative column or for start-up after prolonged storage (e.g., greater than 5 days), it is recommended that the column be pre-flushed with 10 column volumes of 100% organic solvent (e.g. Methanol or acetonitrile) to elute potential contaminants.
- Use larger sample volumes of dilute solutions to avoid column overload at the inlet. However, sample volume generally should not exceed one-third the volume of the earliest eluting peak of interest.
- Method development is best accomplished by employing analytical-scale HPLC techniques. Once the optimum mobile phase/stationary phase system has been established using these approaches, the separation can be scaled up to the preparative system with only minor adjustments.
- To prevent the deposition of strongly retained sample components on the preparative column, precautions such as sample filtration and pre-fractionation of the sample using gravity-feed chromatography columns, re-crystallization, distillation, etc., should be taken to maximize column life and sample throughput. Use of a guard column is highly recommended.
- The interested reader is referred to the book "Preparative Chromatography" B.A. Bidlingmeyer, ed., Elsevier Publishing (Volume 38 in the "Journal of Chromatography Library Science Series") for a good compendium on strategies for successful preparative separations.

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 5 to 10 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 2 column volumes of the pure solvent. Before storing the column, column ends should be capped with the original caps or end-plugs used for shipping the columns to prevent contamination or damage to the threaded column ends.

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 (e.g. 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 or to order our products, visit our Agilent Technologies home page on the World Wide Web at: www.Agilent.com/chem/supplies

For Technical Support in the US and Canada, call 1-800-227-9770 or call your local Agilent sales office.

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