Agilent PrepHT High Performance Preparative Cartridge Columns Datasheet

Introduction

Two different separation approaches can be applied for preparative LC isolations. One is to use relatively low-efficiency, large-diameter columns for the collection of large quantities of purified material. This approach normally utilizes columns packed with large-diameter particles, operated in a highly overloaded fashion, and requires large separation factors between the compounds of interest and the accompanying impurities. The second preparative goal is the isolation of modest amounts of highly purified materials from difficult matrices (e.g., complex biological samples, closely eluting impurities, etc.). This approach uses highly efficient, small-particle, large-diameter columns operated in a scale-up (non-overload) mode to achieve the required resolution and sample through-put (see Table1). Zorbax PrepHT cartridge columns are specifically engineered to perform the more difficult separations of high performance preparative liquid chromatography. However, they can also be used to isolate gram quantities of purified materials when resolution conditions permit sufficient sample overload.

TABLE 1

Typical Sample Capacities

Column ID	Normalized Flow Rate	Separation Type	
		Easy (α>1.5)	Difficult(α<1.2)
4.6 mm	1.0 mL/min.	2-10 mg	0.5-2 mg
9.4 mm	4.2 mL/min.	10-50 mg	2-10 mg
21.2 mm	21.0 mL/min.	50-200 mg	10-50 mg

PrepHT Cartridge Column Characteristics

Zorbax PrepHT Columns are 21.2 mm ID x 50, 100, 150 or 250 mm long, packed with Zorbax high-performance chromatographic packings. The nominal average particle size of the Zorbax packings used for PrepHT columns is either 5-microns or 7-microns with each column length and particle size combination chosen to produce columns with high separation performance and low operation pressures. The cross-sectional area of these columns is approximately 20 times that of 4.6 mm ID analytical columns. Therefore, in order to obtain equivalent separation times on a preparative column, relative to those obtained using analytical systems, the mobile phase flow rate must be increased twenty-fold.

The Zorbax packings used in the PrepHT columns are produced using the same particle and bonding technology employed in the production of analytical-scale Zorbax packings. The same thorough quality control procedures are used to monitor all Zorbax 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. This technology permits the direct scaleup of separations from analytical to preparative proportions with little or no modifications required in methodology.

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 Zorbax 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.

Operation 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 preparative cartridge is shipped dry; therefore, flush new cartridge columns with 200 mls of 100% organic solvent (e.g. Methanol or Acetonitrile) followed by at least 200 mls of mobile phase before use. This will avoid any equilibration problem and will ensure reproducible selectivity with new columns.
- Maximum operating pressure is 340 bar (5000 psi).
- Optimum pH range for maximum life of StableBond columns is 1.8 to 6 and for Eclipse columns 3 to 8. Maximum pH ranges are 1.8 to 8 for StableBond and 2 to 9 for Eclipse, with risk of reduced column lifetime.

The useful pH range for Extend-C18 columns is 2 to 11.5. Bonus-RP columns and traditional Zorbax columns (e.g. Zorbax ODS, Zorbax C8, etc.) should be operated in the pH range of 2 to 8.

- At low pH StableBond columns may be used up to 80°C. All other column types may be used up to 60°C. Maximum operating temperature is 40°C in all cases when using a mobile phase with pH ≥ 6.

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 preflushed with 200mls of 100% organic solvent (e.g. Methanol or acetonitrile) to elute potential contaminations.
- 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, recrystallization, 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 200-300 mls of a 50/50 mixture of methanol or acetonitrile and water, followed by 200-300 mls of the pure solvent. Before storing the column, column ends should be capped with the original caps 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.

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