Agilent ZORBAX Rapid Resolution High Definition 300SB-C8 Threaded Column

Data Sheet

General Description

ZORBAX Rapid Resolution High Definition (RRHD) 300SB-C8 threaded columns are specially designed for use with ultra-high performance liquid chromatographs (UHPLCs) such as the Agilent 1290 Infinity LC and can be used up to an operating pressure of 1200 bar, but continued operation close to the maximum operating pressure may result in a reduction in column lifetime. They are packed with a high-performance microparticulate 300Å C8 packing for high-speed and high resolution reversed phase HPLC. The StableBond packing is made by chemically bonding a sterically-protected C8 stationary phase to a specially prepared, high-purity ZORBAX porous silica microsphere. The special ZORBAX silica support is designed to reduce or eliminate strong adsorption of basic compounds. The densely covered, sterically protected, diisopropyl n-octadecylsilane stationary phase is chemically stable and gives longer column life. As a result, Agilent ZORBAX 300SB-C8 is a stable, reversed phase packing that can be used at low pH for intact protein analysis and peptide mapping. It is particularly well suited for use with aggressive mobile phases (for example, pH < 2, high ionic strength, ion-pair additives, etc.) since the steric protection of the bonded phase resists degradation caused by such mobile phases. This material has also been shown to be stable when operated at temperatures up to 80 °C. These characteristics are particularly important for use in methods that need long-term stability and reproducibility. ZORBAX 300SB-C8 is especially suited in applications that use high-sensitivity detectors that require low backgrounds (for example, mass spectrometers). The uniform, spherical, ZORBAX 300SB-C8 particles have a controlled pore size of 300Å. Columns are loaded to a uniform bed density using a proprietary, high-pressure, slurry-loading technique to give optimum column efficiency.

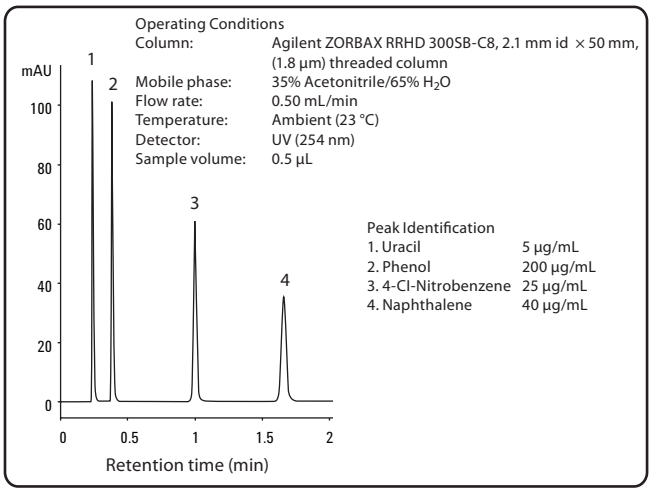

Figure 1. Agilent ZORBAX RRHD 300SB-C8 QC chromatogram.

Column Characteristics

A typical quality control test chromatogram for a ZORBAX RRHD 300SB-C8 2.1 mm id × 50 mm, 1.8 µm threaded column is shown in Figure 1. The actual QC test and performance of your column is described on the Column Performance Report enclosed with your column. The efficiency found on the QC Performance Report may be higher than the efficiency found in your laboratory. The QC test system may vary from the LC used in your lab and has been modified from a standard system to minimize system volume. This allows a better evaluation of the column and assures a more consistent product for the chomatographer.

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.
- These RRHD columns are mechanically stable and have been tested to very high pressures to ensure safe lab operation on a variety of LC instruments. The 2.1 mm id columns will support 1200 bar operation. Opening columns will compromise these pressure limits.
- Because of its small particle size, dry ZORBAX packings are respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column.

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- These columns are packed and assembled for high pressure (up to 1200 bar) use. Disassembling the column will degrade column performance.
- ZORBAX 300SB-C8 is compatible with water and all common organic solvents.
- Avoid use of columns below pH 0.8 or above pH 8.0.
- Maximum operating pressure is 1200 bar (17,000 psi). Optimal lifetime is achieved when operating up to 1000 bar.
- Maximum recommended operating temperature is 80 °C, although higher temperatures will be tolerated, at the cost of column longevity.

  NOTE: StableBond columns are designed for high stability at low pH (for example, pH < 5). However, all silica-based packings have some solubility in pH > 6 aqueous mobile phases. Therefore, when using silica-based columns under conditions of pH > 6, 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].
- Column should not be maintained at neutral or elevated pH or at elevated temperature when not in use.

Mobile Phase Selection

The bonded stationary phase is nonpolar in nature and is best used with mobile phases such as methanol/water or acetonitrile/water mixtures. Increasing the amount of organic component usually reduces the retention time of the sample. Due to the relatively high viscosity of recommended mobile phases, increased efficiency can be achieved with the use of column temperatures in the range of 40–65 °C. When separating peptides and proteins, gradient elution with acetonitrile and water with 0.1% TFA (trifluoracetic acid) is typically used. Additional information on solvent selection may be found in Chapters Six and Seven, *Introduction to Modern Liquid Chromatography*, Second Edition, L. R. Snyder and J. J. Kirkland, (John Wiley & Sons, 1979), and Chapters Six, Seven, and Eight, *Practical HPLC Method Development*, Second Edition, L. R. Snyder, J. J. Kirkland, and J. L. Glajch, (John Wiley & Sons, 1997).

Applications

Zorbax 300SB-C8 is designed for reversed phase separation of synthetic and natural peptides and proteins, and peptide fragments from enzymatic digests (peptide mapping). The wide pore (300Å) packing used in the Zorbax 300SB-C8 columns is recommended for solutes with molecular size greater then 4,000 Daltons.

Column Care

Samples should be filtered before injection into the column. The column inlet frit is nominally 0.5 µm and samples should be filtered through a 0.2 µm sample filter. If solvent flow appears to be restricted (unusually high column back-pressure), check first to see that solvent flow is unobstructed up to the column inlet. If the restriction is prior to the column, replace the appropriate piece of tubing or filter that is plugged. If the column is plugged, do not backflush the column. It will be necessary to replace the column.

To remove strongly retained materials from the column, flush the column with stronger (less polar) solvents. Solvents such as methanol, acetonitrile, or a 95/5 mixture of dichloromethane and methanol should remove most highly retained compounds. In extreme cases, dimethyl sulfoxide or dimethylformamide at low flow rates may also be used for this purpose. When switching between solvents with vastly different polarities, it may be necessary to first purge the column with a mutually miscible solvent such as isopropanol.

Since columns have 3/8 in end nuts, a short 3/8 in wrench should be used to attach the columns to the instrument to avoid any additional tightening of the end fittings. Over-tightening the end fittings will cause damage and require column replacement.

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 was 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.

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

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