Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl Rapid Resolution High Definition Threaded Column

Data Sheet

General Description

Agilent ZORBAX Rapid Resolution High Definition (RRHD) threaded columns are specially designed for use with ultrahigh performance liquid chromatographs (UHPLCs) such as the Agilent 1290 Infinity LC and can be used up to an operating pressure of 1200 bar. Eclipse Plus Phenyl-Hexyl RRHD threaded columns are specially designed for higher pressure operation (up to 1200 bar) and are packed with a high-performance microparticulate phenyl-hexyl packing for high-speed and high resolution reversed phase HPLC. Eclipse Plus Phenyl-Hexyl columns are designed for superior peak shape with basic compounds and deliver high efficiency and excellent peak shape with all sample types. Eclipse Plus Phenyl-Hexyl packing is made by first chemically bonding a dense monolayer of dimethylphenylhexylsilane stationary phase to a specially prepared and improved ultra-high purity ≥ 99.995% SiO_2) ZORBAX Rx-SIL porous silica support. This special ZORBAX silica support (Type B) is designed to reduce or eliminate strong adsorption of basic and highly polar compounds. The bonded-phase packing is then endcapped using proprietary reagents and procedures to obtain maximum deactivation of the silica surface. Eclipse Plus Phenyl-Hexyl columns can be used for acidic and neutral samples, but are especially suited for separations of basic compounds that produce poor peak shapes on other columns. These columns can be used for a wide range of applications and over a pH range of 2 to 8, accommodating most popular mobile phases.

The uniform, spherical Eclipse Plus Phenyl-Hexyl particles are based on an improved ZORBAX Rx-SIL support that has a nominal surface area of 160 m^2/g and a controlled pore size of 95Å. Columns are loaded to a stable, uniform bed density using a proprietary high-pressure slurry-loading technique to give maximum column efficiency.

Column Characteristics

A typical quality control (QC) test chromatogram for an Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl 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 in the Column Performance Report enclosed with your column.

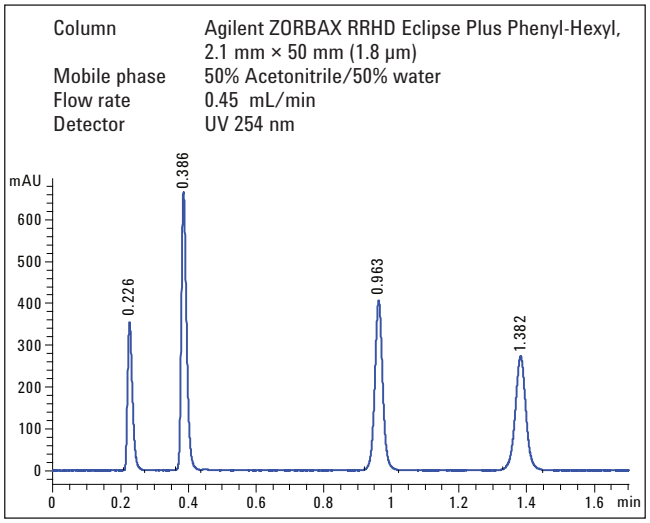

Figure 1. Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl QC chromatogram.

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. Maximum operating pressure is 1200 bar. Opening columns will compromise this pressure limit.
- Because of the small particle size, dry ZORBAX packings are respirable. Columns should only be opened in a well-ventilated area.

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Operational Guidelines

- The direction of flow is marked on the column.
- These columns are packed and assembled for high-pressure (up to 1200 bar) use. Disassembling the column will degrade column performance.
- Eclipse Plus Phenyl-Hexyl is compatible with water and all common organic solvents.
- Avoid use of this column below pH 2 or above pH 8.
- Maximum operating pressure is 1200 bar (17,000 psi).
- Maximum operating temperature is 60 °C.

  NOTE: Agilent ZORBAX RRHD Eclipse Plus Phenyl-Hexyl columns are designed for high stability over a wide pH range. 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 10 to 20 mM. 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 neutral or elevated pH, or at elevated temperature, when not in use.

Mobile Phase Selection

The phenyl-hexyl bonded phase can have unique selectivity that is strongly influenced by the choice of organic modifier in the mobile phase. The columns are best used with methanol/water or acetonitrile/water mobile phases, but methanol/ water mobile phases will enhance interactions with aromatic compounds.

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 to 60 °C; however, best column lifetime is achieved with operation at ≤ 40 °C. Gradient-elution techniques for this packing often use 5% methanol or acetonitrile as the initial solvent and 100% methanol or acetonitrile as the final solvent. Additional information on solvent selection can be found in chapters 6 and 7, Introduction to Modern Liquid Chromatography, Second Edition, L. R. Snyder and J. J. Kirkland, (John Wiley & Sons, 1979), and chapters 6, 7, and 8, Practical HPLC Method Development, Second Edition, L. R. Snyder, J. L. Glajch, and J. J. Kirkland, (John Wiley & Sons, 1997).

Applications

Eclipse Plus Phenyl-Hexyl can be used with basic, neutral, or acidic compounds. This column is an excellent choice when an alternate selectivity to the Eclipse Plus C18 is needed to enhance resolution. Resolution is especially enhanced with samples containing aromatic compounds in methanol-containing mobile phases.

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 backpressure), 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. 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.

Since columns have 3/8-in end nuts, use a short 3/8-in wrench 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. Additional care recommendations are included on the card in the box. Review these prior to using the column.

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 to 30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20 to 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 issue of corrosion from the salts is minimized.

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