Agilent ZORBAX Rapid Resolution HD Eclipse Plus C8 Threaded Column

Data Sheet

General Description

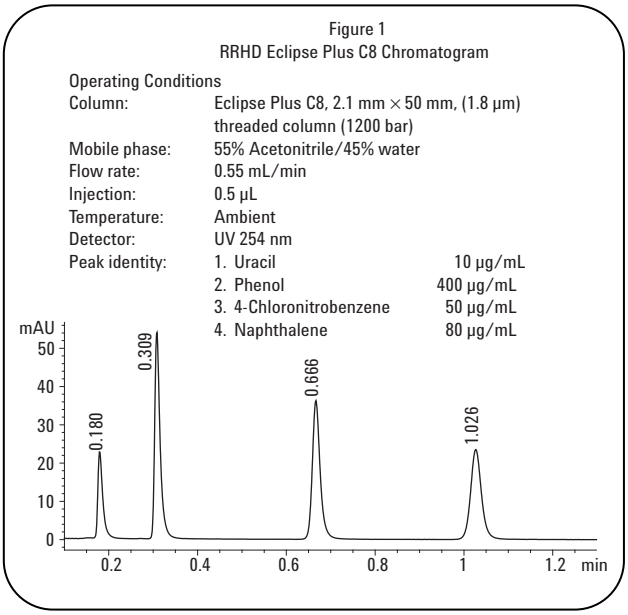
Agilent ZORBAX Rapid Resolution High Definition (RRHD) Eclipse Plus C8 threaded columns are specially designed for use with ultra-high performance liquid chromatographs (UHPLCs) such as the Agilent 1290 Infinity LC and can be used up to an operating pressure of 1200 bar. They are packed with a high performance 1.8-µm microparticulate C8 packing for high-speed reverse phase HPLC. Eclipse Plus C8 columns are designed for superior peak shape with basic compounds and deliver high efficiency and excellent peak shape with all sample types. Eclipse Plus C8 packing is made by first chemically bonding a dense monolayer of dimethyl-n-octyl silane stationary phase to a specially prepared, 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 doubly endcapped using proprietary reagents and procedures to obtain maximum deactivation of the silica surface. Eclipse Plus C8 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 over a pH range of 2–8, accommodating most popular mobile phases.

The uniform, spherical Eclipse Plus C8 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 test chromatogram for a Rapid Resolution HD Eclipse Plus C8, 2.1 × 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 reported on the Column 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 the system volume. This allows a better evaluation of the packed column and assures a more consistent product for the chromatographer.

Figure 1
RRHD Eclipse Plus C8 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 assembled columns are mechanically stable and have been tested to very high pressures to assure safe lab operation on a variety of LC instruments. The 2.1- and 3.0-mm id columns will support 17,000 psi (1200 bar) operation. Opening columns may compromise these pressure limits.
- Because of the 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. These columns should only be operated in the marked flow direction.
- These columns are packed and assembled for high pressure (up to 1200 bar) use. Disassembling the column will degrade column performance.
- Eclipse Plus C8 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: Eclipse Plus C8 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 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 40–60 °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.

Applications

Eclipse Plus C8 can be used with basic, neutral or acidic compounds. Ionizable compounds (basic, acidic) generally are best separated at about pH 3 with this column. However, Eclipse Plus C8 is especially suited for separating basic compounds when an intermediate pH (4–8) must be used to maintain compound stability or to obtain desired band spacing (selectivity). For optimum results and long-term reproducibility, the use of 10–50 mM buffers is always recommended when separating ionizable compounds.

Column Care

Samples that contain particulate matter may plug the column inlet frit and 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. 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-inch end nuts, a short 3/8-inch 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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