Agilent Eclipse XDB-C18 RRHT Threaded Column

Datasheet

General Description

Eclipse XDB-C18 RRHT threaded columns are specially designed for higher pressure operation (up to 600 bar) and are packed with a high performance microparticulate C18 packing for high-speed reversed phase HPLC. Eclipse XDB-C18 uses the technologies of eXtra-Dense Bonding (XDB) of organo-silane ligands and double endcapping to protect the ultra-pure (Type B) silica support from dissolution in mobile phases of intermediate and higher pH. Eclipse XDB-C18 is especially useful for the separation of acidic, basic, and other highly polar compounds by reversed-phase liquid chromatography. Eclipse XDB-C18 packing is made by first chemically bonding a dense monolayer of dimethyl-n-octadecylsilane stationary phase to a specially prepared, 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. This densely covered, deactivated, column packing can be used for acidic and neutral samples, but is especially suited for separating basic compounds that produce poor peak shapes on most 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.

The uniform, spherical Eclipse XDB-C18 particles are based on ZORBAX Rx-SIL silica support that has a nominal surface area of 180 m^2/g and a controlled pore size of 80Å. Columns are loaded to a stable, uniform bed density using a proprietary, high-pressure slurry-loading technique to give maximum column efficiency.

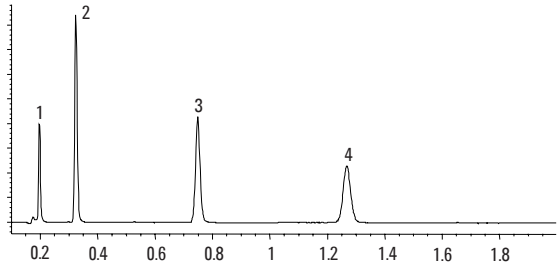
FIGURE 1

Eclipse XDB-C18 RRHT QC Chromatogram

Operating Conditions

Column: Eclipse XDB-C18 RRHT 4.6 mm ID x 50 mm (1.8 µm) threaded column
Mobile Phase: 60% Acetonitrile/40% Water
Flow Rate: 2.0 mL/min
Temperature: Ambient (23°C)
Detector: UV (254 nm)
Sample Volume: 2 µL
Peak Identity:

1. Uracil	5 µg/mL
2. Phenol	200 µg/mL
3. 4-Cl-nitrobenzene	25 µg/mL
4. Naphthalene	40 µg/mL



Column Characteristics

A typical Quality Control test chromatogram for a 1.8 µm Eclipse XDB-C18 RRHT 4.6-mm ID × 50-mm 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.

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 RRHT 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 20,000 psi (1300 bar) operation and 4.6-mm ID columns will support 16,000 psi (1000 bar) operation. Opening columns may compromise these pressure limits. Chromatographic performance has not been tested above 600 bar.
- 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.
- While it is not harmful to the column, reverse flow should be avoided except to attempt removal of inlet pluggage (see “Column Care” section).
- These columns are packed and assembled for high pressure (up to 600 bar) use. Disassembling the column will degrade column performance.
- Eclipse XDB-C18 is compatible with water and all common organic solvents.

- Avoid use of this column below pH 2 or above pH 9; optimum lifetime and performance are obtained at pH 3–8.
- Maximum operating pressure is 600 bar (9000 psi).
- Maximum operating temperature is 60 °C.

  NOTE: Eclipse XDB 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 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].
- Columns should not be maintained at neutral or elevated pH or 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. 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.L. Glajch, and J.J. Kirkland, (John Wiley & Sons, 1997).

Applications

Eclipse XDB-C18 can be used with basic, neutral or acidic compounds. Ionizable compounds (basic, acidic) generally are best separated at pH~3 with this column. However, Eclipse XDB-C18 is especially suited for separating basic compounds when 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. For many basic compounds, basic modifiers such as triethylamine will not be required to achieve efficient separations with symmetrical peaks. For very strongly basic compounds, the use of 10–20-mM triethylamine or 5–10-mM dimethyloctylamine is recommended when using any silica-based reversed-phase column, especially when highly robust, long-term, reproducible methods are required.

Column Care

Samples that contain particulate matter may plug the column inlet frit and should be filtered before injection into the column.
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 column has the restriction, there may be particulate matter on the inlet frit. An attempt should be made to remove any inlet debris by back-flushing 25–30-mL of mobile phase through the column. If this fails to return the column to near its original back pressure, the column should be replaced.
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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