Agilent ZORBAX RRHD Bonus-RP Column

Data Sheet

General Description

Agilent ZORBAX Rapid Resolution High Definition (RRHD) 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. Bonus-RP columns are designed to reduce or eliminate strong interactions of basic and other highly polar compounds. Bonus-RP uses a unique combination of densely reacted, sterically protected diisopropyl-C14 groups covalently bonded through an "embedded" amide functionality to an ultra-pure (> 99.995% SiO_2; Type B) ZORBAX Rx-SIL silica support for superior peak shape. The embedded, highly polar, amide group of the stationary phase assists in reducing unwanted silanol interactions. Then this material is triple endcapped to further deactivate the chromatographic surface.

Bonus-RP shows different selectivity from totally alkyl or aryl stationary phases (for example, Eclipse Plus C18 or C8, and Eclipse XDB-Phenyl), and may be a preferred alternative to such phases for separating basic compounds by reversed-phase LC. This highly deactivated column packing can be used for all types of compounds, but it is especially suited for basic and other highly ionizable compounds that produce poor peak shapes with many reversed-phase columns. The steric protection provided by diisopropyl groups against hydrolysis of the bonded silane provides the packing with unusual stability in low pH applications. Therefore, Bonus-RP can be used in the pH range of 2 to 9, but is best suited for long-term operation at pH 2 to 8.

The uniform, spherical Bonus-RP particles are based on ZORBAX Rx-SIL silica support that has a surface area of 180 m^2/g and a controlled pore size of 80Å.

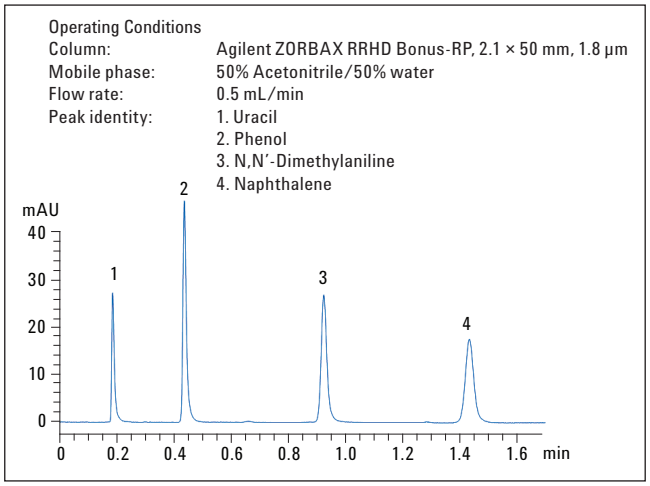

Figure 1. Agilent ZORBAX RRHD Bonus RP QC Chromatogram..

Column Characteristics

A typical quality control test chromatogram for an Agilent ZORBAX RRHD Bonus-RP 2.1 mm × 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 chromatographer.

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.

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- 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 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.
- These columns are packed and assembled for high-pressure (up to 1200 bar) use. Disassembling the column will degrade column performance.
- A new column contains a mixture of acetonitrile and water. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- Bonus-RP 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 2 to 8.
- The maximum recommended operating temperature is 60 °C.

NOTE: All silica-based column packings have some solubility in aqueous mobile phases when used at pH > 6. Maximum column lifetime is obtained by operating at lower 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].

Mobile Phase Selection

The bonded stationary phase 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. The more polar nature of Bonus-RP usually means that a lower concentration of organic mobile phase modifier is required for compound elution, compared to traditional long-chain alkyl stationary phases.

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. Other typical mobile phases are those with additives in the aqueous portion such as formic acid/ammonium formate, acetic acid or ammonium acetate, and trifluoroacetic acid (TFA, 0.05–1%).

Applications

Bonus-RP can be used with neutral, basic, or acidic components, and is especially suited for separating ionizable compounds that produce poor peak shapes with traditional C18, C8, or phenyl reversed-phase columns. The selectivity of Bonus-RP often is different from these alkyl or aryl bonded-phase columns, sometimes allowing separations that are not easily obtained with these separating media. Excellent peak shapes can be expected for highly polar compounds with Bonus-RP, as illustrated by the phenol and N,N'-dimethylaniline (DMA) peaks in Figure 1. Ionizable compounds (bases, acids) are especially suited for separation with Bonus-RP. These materials often are best separated at pH 2 and 3, but intermediate pH (4 to 8) applications also can be used to produce the desired selectivity (band spacings) while maintaining excellent peak shapes. Bonus-RP demonstrates superior lifetime to other "embedded polar group" columns because of the sterically protected bonded-phase groups and the unique endcapping that is used. For optimum results and long-term reproducibility, the use of 10 to 50 mM buffers is always recommended when separating ionizable compounds. Basic mobile phase modifiers such as triethylamine usually are not required for good peak shape for basic compounds.

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 plugged piece of tubing or filter. 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. 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. 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 has been 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 organic solvent. Before storing the column, the end fittings should be tightly capped with end plugs to prevent the packing from drying out.

Columns can be safely stored for short periods in most mobile phases. However, to protect equipment, it is best 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 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 chance of corrosion from the salts is eliminated.

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

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