Agilent Zorbax Bonus-RP Datasheet

General Description

ZORBAX 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. This material is then triple endcapped. This special silica support is designed to reduce or eliminate strong interaction of basic and other highly polar compounds. Bonus-RP shows different selectivity from totally alkyl or aryl stationary phases (e.g., Eclipse XDB-C8, XDB-C18 and XDB-Phenyl), and may be a preferred alternative to such phases for separating basic, acidic and other highly polar compounds by reversed-phase liquid chromatography. The embedded, highly polar, amide group of the stationary phase assists in deactivating unwanted silanol interactions, while proprietary endcapping procedures complete the process to deactivate the chromatographic surface. The more polar nature of this stationary phase usually means that a lower concentration of organic mobile phase modifier is required for compound elution, compared to traditional long-chain alkyl stationary phases. This highly deactivated column packing can be used for neutral 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 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 2-9, but is best suited for long-term operation at pH 2-8. The dense coating of bonded phase and exhaustive endcapping simultaneously deactivate the silica support surface from deleterious interactions with samples and protects the silica from dissolution (and resulting column degradation) in intermediate and higher pH environments. Bonus-RP combines excellent peak shape and column efficiency with unusual column stability to provide a superior solution for separating highly polar and ionizable compounds by reversed-phase liquid chromatography.

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.

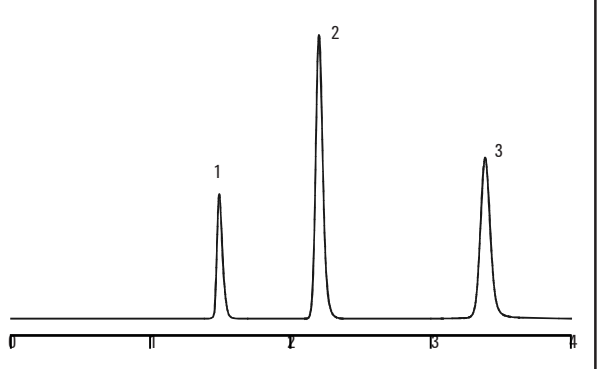
FIGURE 1

Zorbax Bonus-RP QC Chromatogram

OPERATING CONDITIONS

Column: Zorbax Bonus-RP 4.6 mm ID x 150 mm (5 µm)
Mobile Phase: 70% Methanol / 30% Water
Flow Rate: 1.0 mL/min
Temperature: Ambient (23°C)
Detector: UV (254 nm)
Sample Volume: 5 µL

Peak Identity:	1. Uracil	5 µg/mL
	2. Phenol	200 µg/mL
	3. N,N-Dimethylaniline	10 µg/mL



Retention Time (min.)

Column Characteristics

A typical Quality Control test chromatogram for a 4.6 mm ID x 150 mm 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.
- 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.
- While it is not harmful to the column, reversing flow should be avoided except to attempt removal of inlet pluggage (see "Column Care" section).
- A new column contains a mixture of methanol 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 all common organic solvents.
- Use of a Bonus-RP guard column is recommended to protect the Bonus-RP column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 9; optimum lifetime and performance are obtained at pH 2-8.
- Maximum operating pressure for columns up to 9.4 mm ID is 400 bar (6000 psi).

- 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 0.02M. 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.

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.J. Kirkland, and J.L. Glajch, (John Wiley & Sons, 1997).

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 2-3, but intermediate pH (4-8) 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 - 50 mM buffers is always recommended when separating ionizable compounds. Organic buffers are best for intermediate pH applications to maintain long column lifetime (see H. A. Claessens, M. A. van Straten and J. J. Kirkland, *J. Chromatogr. A*, 728 (1996) 259). Basic mobile phase modifiers such as triethylamine usually are not required for good peak shape for basic compounds.

Column Care

The inlet frit on these columns has a nominal porosity of 2 µm. Samples that contain particulate matter larger than 2 µm may plug the column inlet frit. Bonus-RP guard columns and a hardware kit are recommended for use with such samples.

If solvent flow appears to be restricted (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 initial 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 inlet frit should be changed. To remove the frit, carefully loosen the nut at the inlet, taking care not to turn the end fitting itself. Then, carefully remove the fitting, taking care not to disturb the column bed. The frit should drop out when the fitting is tapped sharply on a hard surface. Install a new frit and carefully tighten the fitting.

To remove strongly retained materials from the reversed-phase 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 dimethyl-formamide 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.

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 20-30 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 (e.g. 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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