Agilent Zorbax SB-C18

datasheet

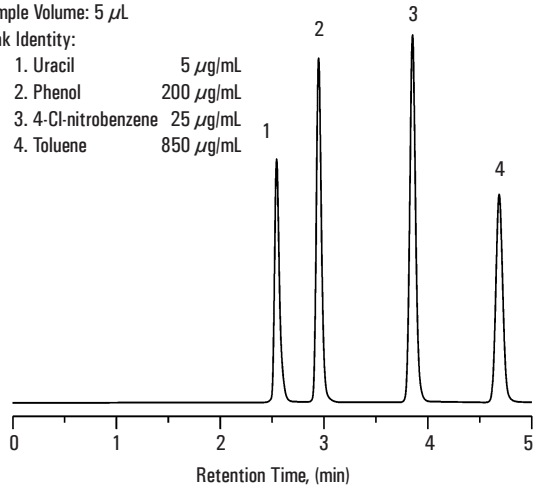
General Description

Zorbax SB-C18 is a unique microparticulate C18 packing used for reversed-phase high performance liquid chromatography. The StableBond packing is made by chemically bonding a sterically-protected C18 stationary phase to a specially prepared, high purity Zorbax porous silica microsphere. The special Zorbax silica support is designed to reduce or eliminate strong adsorption of basic compounds. The densely covered, sterically protected, diisobutyl n-octadecylsilane stationary phase is chemically stable and gives longer column life. As a result, Zorbax SB-C18 is a stable, reversed-phase packing that can be used for basic, neutral, or acidic samples. It is particularly well suited for use with aggressive mobile phases (e.g., pH < 2, high ionic strength, ion-pair additives, etc.) since the steric protection of the bonded phase resists degradation caused by such mobile phases. This material has also been shown to be stable when operated at temperatures up to 90°C. These characteristics are particularly important for use in methods that need long-term stability and reproducibility. Zorbax SB-C18 is especially suited in applications that utilize high-sensitivity detectors that require low backgrounds (e.g., mass spectrometers).

FIGURE 1
Zorbax SB-C18 QC Chromatogram

OPERATING CONDITIONS
Column: Zorbax SB-C18, 4.6 mm ID x 250 mm, (5 μm)
Mobile Phase: 85% MeOH/15% H_2O
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. 4-Cl-nitrobenzene	25 μg/mL
4. Toluene	850 μg/mL



The uniform, spherical, Zorbax SB-C18 particles have a controlled pore size of 80Å. Columns are loaded to a uniform bed density using a proprietary, high-pressure, slurry-loading technique to give optimum column efficiency.

Column Characteristics

A typical Quality Control test chromatogram for a 4.6 mm ID x 250 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 generally 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. Care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- Zorbax SB-C18 is compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the Zorbax SB-C18 column and to extend its useful lifetime.
- Avoid use of columns below pH 0.8 or above pH 8.0.
- Maximum operating pressure for columns up to 9.4 mm ID is 400 bar (6000 psi).
- Maximum operating temperature is 90°C.

  NOTE: StableBond columns are designed for high stability at low pH (e.g., pH < 5). 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 0.02M. 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 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 40-65°C. Gradient elution techniques for this packing often use 5% methanol or acetonitrile in water 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

Zorbax SB-C18 is similar to Zorbax ODS and Zorbax Rx-C18 in retention of acidic and neutral compounds. However, Zorbax SB-C18, like Zorbax Rx-C18 provides better chromatographic performance with basic compounds, using the same buffers and organic modifiers employed in reversed-phase chromatography. For many basic compounds, it will normally not be necessary to use basic modifiers, such as triethylamine, to achieve efficient, symmetrical peaks. However, very basic compounds may require the addition of basic modifiers such as 10-20 mM dimethyl-octylamine or 20-30 mM triethylamine. Such samples are often best chromatographed with mobile phases of pH ≤ 3. One highly recommended mobile phase for very basic compounds is 0.1% trifluoroacetic acid adjusted to pH = 3 with triethylamine and an appropriate concentration of methanol or acetonitrile.

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 and should be filtered before injection into the column. Zorbax 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, loosen the nut at the column inlet, taking care not to turn the end fitting itself. Then 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 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.

Since columns have 1/16" terminations, a short 1/4" wrench should be used in assembling fittings to prevent overtightening the ferrules. Overtightening the fittings can damage the fitting and necessitate 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 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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