

Agilent Zorbax 300SB-C18

Datasheet

General Description

Zorbax 300SB-C18 is a microparticulate column packing designed specifically for reversed-phase HPLC of peptides and proteins. The StableBond packing is made by chemically bonding a sterically-protected octadecyl stationary phase to a specially prepared, ultra-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-octadecyl stationary phase is chemically stable and gives longer column life. As a result, Zorbax 300SB-C18 is a stable, reversed-phase packing at low pH that can be used for peptide mapping, and separation of synthetic and natural peptides and proteins. It is particularly well suited for use with aggressive mobile phases (e.g., pH < 2, high ionic strength, ion-pair additives, TFA, etc.) since the steric protection of the bonded phase resists degradation caused by such mobile phases. This

characteristic is particularly important for use in methods that need long-term stability and reproducibility. Zorbax 300SB-C18 is well suited to applications that utilize high-sensitivity detectors that require low backgrounds (e.g., mass spectrometers). The uniform, spherical, Zorbax 300SB-C18 particles have a controlled pore size of 300Å. 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 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.

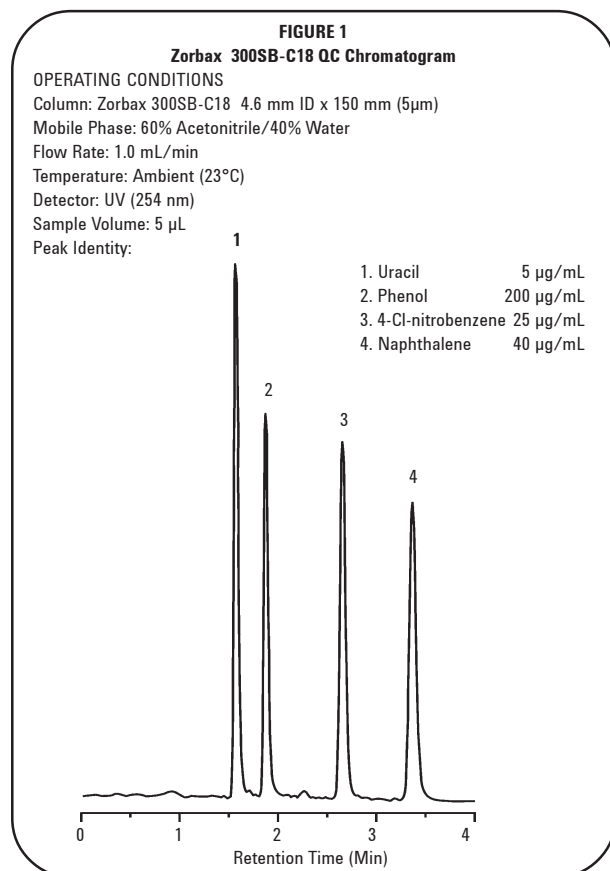
Safety Considerations

- 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 generally not harmful to the column, reversed flow should be avoided except to attempt removal of inlet pluggage (see "Column Care" section).
- 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.
- Zorbax 300SB-C18 is compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the Zorbax 300SB-C18 column and extend its useful lifetime.
- Avoid use of this column below pH 0.8 or above pH 8.
- 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 < 4). 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 solvent component typically 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. When separating peptides or proteins, gradient elution with acetonitrile and water with 0.1%TFA (trifluoroacetic acid) is typically used. 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 300SB-C18 HPLC columns are specifically designed for reversed-phase separation of synthetic and natural peptides and proteins, and peptide fragments from enzymatic digests (peptide mapping). The wide-pore (300Å) packing used in the Zorbax 300SB-C18 columns is highly recommended for solutes with molecular sizes greater than 4,000 daltons. Peptides and proteins generally show more retention on this column than the Zorbax 300SB-C8, 300SB-C3, and 300SB-CN columns. The Zorbax300SB-C18 column may also provide band spacing (selectivity) different from these other columns. Small organic molecules (less than 1,000 daltons) will have less retention on the Zorbax 300SB-C18 compared to the Zorbax SB-C18 (80Å pore size).

Column Care

The inlet frit on these columns have a nominal porosity of 2 µm. Samples that contain particulate matter which is larger than 2 µm will plug the column inlet frit. Zorbax 300SB-C18 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 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 immiscible solvent such as isopropanol. Separations involving samples rich in cellular debris may rapidly foul the column. Solubilization of such materials may be achieved by the use of 6 M guanidine HCl/50 mM Tris HCl, pH 7.5, pumped at 1 mL/min for 20-30 min. The use of this solvent requires complete flushing of the system with about 200 mL of water.

Since columns have 1/16" terminations, a short 1/4" wrench should be used in assembling fittings in order 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/H₂O 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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