Agilent Zorbax 300SB-C3

Datasheet

General Description

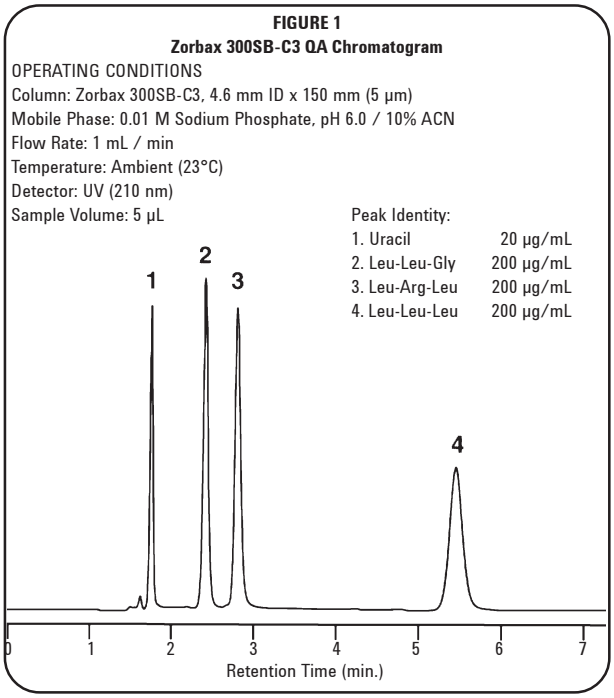
Zorbax 300SB-C3 reversed-phase chromatography columns are premium-quality products offered for bio-chemical separations. The Zorbax 300SB chromatography column series is specifically designed for the separation of biological macromolecules. This StableBond column packing material is prepared by covalently bonding a sterically protected organosilane to a ultra-high purity 300Å Zorbax silica support (300 Rx-SIL). This wide-pore bonded-phase silica is packed into columns using proprietary techniques to produce highly efficient and stable chromatography columns.

Product Description

Zorbax 300 Rx-SIL particles are porous silica microspheres with 300Å pores. This highly purified silica has extremely low levels of metal contaminants. This high-purity, together with a patented method of silica hydroxylation,[1] deactivates the silica, resulting in improved chromatographic peak shape, especially for basic compounds (less "tailing"). These features of the Zorbax 300SB packings are highly desirable for separations of proteins and peptides.[2-5]

The Zorbax 300SB-C3 bonded-phase is prepared by chemically bonding triisopropyl silane to the fully hydroxylated silica surface, using a method which results in high-density coverage. The silane reagent is of the sterically protected type, as described by Glajch and Kirkland.[6,7] Sterically protected silanes were developed to solve the problem of bonded-phase instability which is commonly encountered in reversed-phase separations of peptides and proteins.[4,5] Zorbax 300SB-C3 columns exhibit superior stability at low pH and can be operated at elevated temperatures. The monolayer bonded-phase provides reproducible separations, minimal resistance to mass transfer (i.e. high column efficiency), and reduced column equilibration time.

FIGURE 1
Zorbax 300SB-C3 QA Chromatogram



Column Characteristics

Zorbax 300SB-C3 packing materials undergo quality assurance analysis with rigorous specifications determined by separating small organic-molecule mixtures and peptide mixtures. An example of a typical quality assurance test is shown in Figure 1. This QA test is carried out with peptides at neutral pH, to assure very low silanol activity of Zorbax 300SB-C3. Periodic analyses are conducted using standard peptides and chromatographic conditions described by Mant and Hodges.[5,8]

Each column is tested chromatographically with inert small-molecule solutes before shipping. The actual QC test and performance 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 the small particle size, dry Zorbax packing is respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column.
- While not harmful to the column, reverse flow should be avoided, except to attempt removal of inlet pluggage (see "Column Care" section).
- A new column is shipped in a mixture of acetonitrile and water. Care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- The Zorbax 300SB-C3 is compatible with water all common organic solvents.
- The use of a guard column is recommended to protect the Zorbax 300SB-C3 column, and extend its useful lifetime.

- Avoid use of this column below pH 1 or above pH 8. Optimum column lifetime is obtained between the range of pH 2 to 7.
- Maximum operating pressure for these columns is 400 bar (6000 psi).
- Maximum recommended operating temperature is 80°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].

Applications

Zorbax 300SB-C3 is optimized for separating peptides and proteins. A useful and widely used method employs a gradient of acetonitrile in the presence of the solubilizing acid, trifluoroacetic acid (TFA). Typically, 0.05-0.2% TFA is employed, although higher concentrations are well tolerated. Alternative choices for acids include hydrochloric (0.001-0.010M), phosphoric acid (0.0025M and above), pentafluoropropionic acid (0.05-0.2%), and heptafluorobutyric acid (0.05-0.3%). Additional possibilities for selectivity manipulation include titration of these acidic solutions using triethylamine (TEA) or pyridine. As an organic modifier, acetonitrile is desirable because of its low UV absorbance and low viscosity. A typical protein or peptide analysis application uses a flow rate of 1 mL/min. (4.6 mm ID), a column temperature of 25-60°C, with a gradient from 0.1% TFA in water to 50% of 0.1% TFA in acetonitrile, increasing at 0.3% to 1% acetonitrile per minute. General recommendations for gradient elution are described in References 9 and 10.

Column Care

The inlet frit on these columns has a nominal porosity of 2 µm. Samples that contain particulate matter which is larger than 2 µm will plug the column inlet frit. A guard column and hardware kit are recommended for use with these samples.

If solvent flow appears to be restricted (high system backpressure), first check the system pressure 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 20 column volumes of mobile phase. 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 clean strongly retained materials from the column, flush with 20-40 column volumes of stronger (less polar) eluting 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. Steep gradients of 0.1% TFA in water to 0.1% TFA in acetonitrile or 0.1% TFA in 80% isopropanol/ 20% water also have been found useful.

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.

References

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