Agilent Zorbax C8

Datasheet

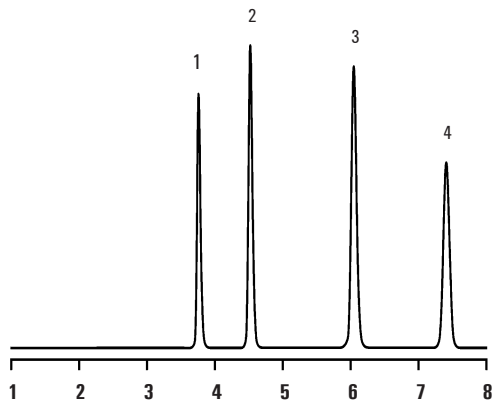
General Description

Zorbax C8 is a packing used for reversed-phase liquid chromatography. This packing is produced by chemically bonding n-octyldimethylsilane groups to Zorbax SIL silica particles followed by end-capping with trimethylchlorosilane. The C8 bonded phase is a monolayer coating produced by reacting a monofunctional silane with the Zorbax support. A true monolayer bonded phase gives the best lot-to-lot chromatographic reproducibility in columns because a more uniform and controllable surface coverage is achieved. The use of polyfunctional reagents (di- and tri-functional silanes) can yield uneven surface coverage that is difficult to reproduce from column-to-column. Because of monolayer technology, Zorbax C8 may have different sample selectivities than C8 columns from other manufacturers, and minor adjustments in mobile-phase composition may be necessary.

FIGURE 1
Zorbax C8 QC Chromatogram

OPERATING CONDITIONS
Column: Zorbax C8, 4.6 mm ID x 250 mm (5 µm)
Mobile Phase: 80% Methanol/20% Water
Flow Rate: 1.0 mL/min
Temperature: Ambient (23°C)
Detector: UV ABS. (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 C8 particles have a controlled pore size of 60Å. 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, reverse 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 C8 is compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the Zorbax C8 column and extend its useful lifetime.
- Avoid use of this column below pH 2.0 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 60°C.

  NOTE: Zorbax columns are designed for high stablity 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 and acetonitrile/water mixtures. Increasing the amount of organic component typically reduces the retention time of the sample. Mobile phase gradients can be used where the organic component is usually the secondary solvent. Hydrocarbon mobile phases have little utility with Zorbax C8 columns. However, for separating very lipophilic samples, an attractive technique called non-aqueous reversed phase (NARP) chromatography may be employed. In NARP chromatography, a mobile phase composed of a mixture of methanol or acetonitrile with methylene chloride or THF is used to obtain the desired separation. Increasing the amount of the less polar component (e.g., CH_2Cl_2) usually reduces sample retention.

Since some of the recommended mobile phases are relatively viscous, increased column efficiency may be achieved by elevating column temperatures to 40-60°C. Additional information on solvent selection may be obtained 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. L. Glajch, and J. J. Kirkland, (John Wiley & Sons, 1997).

Column Care

The inlet frit on these columns have a nominal porosity of 2 µm. Samples that contain particulate matter larger than 2 µm may plug the column inlet frit. Such samples should be filtered using syringe filters. Additionally, Zorbax C8 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 reversed-phase column, rinse 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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