Agilent Zorbax NH_2

Datasheet

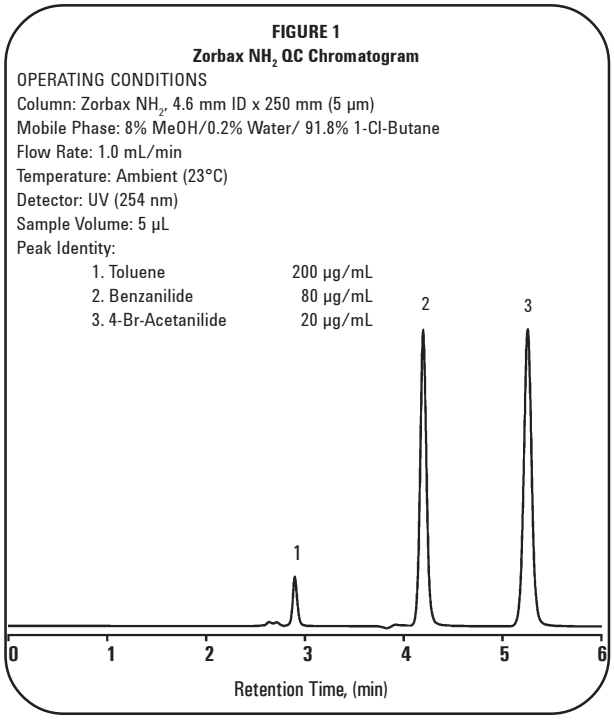
General Description

Zorbax NH_2 is a polar bonded-phase packing used for normal-phase, reversed-phase, or anion-exchange high performance liquid chromatography. This packing is produced by reacting 3-aminopropyltriethoxysilane with Zorbax SIL particles. If not strictly controlled, the use of a polyfunctional reagent can yield uneven surface coverage, difficult to reproduce from column to column. The reaction conditions used to produce Zorbax NH_2 were specifically developed to minimize reagent polymerization and to maximize surface coverage with a monolayer bonded phase. The uniform, spherical, Zorbax NH_2 particles in 4.6 and 9.4 mm ID columns are about 5 µm in diameter, and have a controlled pore size to give optimum column efficiency. The 21.2 mm ID column contains particles of about 7 µm diameter. Columns are packed to a uniform bed density using a proprietary, high-pressure, slurry-loading technique.

Column Characteristics

Zorbax NH_2 columns come in a variety of diameters and lengths to meet your performance requirements. Typical chromatographic performance for a 4.6 mm ID x 250 mm column is shown in Figure 1. The actual performance of your column is described in the enclosed Column Performance Report.

FIGURE 1
Zorbax NH_2 QC Chromatogram



Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users should be aware of the hazards from such leaks due to the toxicity or flammability of the chosen mobile phases.
- Because of the small particle size, dry Zorbax packings are respirable. Columns should only be opened in a well-ventilated area. Material Safety Data Sheets are available upon request.

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).
- Zorbax NH_2 columns are shipped containing hexane. If the column is to be used with an aqueous mobile phase, flush it first with isopropanol.
- Zorbax NH_2 is compatible with water and all common organic solvents.
- The use of an NH_2 guard column is recommended to protect the Zorbax NH_2 column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 7.5.
- Maximum operating pressure for columns up to 9.4 mm ID columns is 400 bar (6000 psi).
- Maximum operating temperature is 60°C.

  NOTE: Zorbax 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].
- Avoid exposure to aldehydes and methyl ketones, which may form Schiff's bases with the bonded phase.

Mobile Phase Selection

The aminopropyl-bonded stationary phase is polar and is used in the normal-partition mode with relatively nonpolar mobile phases such as methylene chloride/hexane or isopropanol/hexane mixtures. Increasing the amount of the polar component in these mixtures reduces the retention time of the sample. This packing can also be used with highly polar mobile phases such as water/methanol or water/acetonitrile

mixtures. Buffers, such as phosphate or borate, may be used (below pH 7.5) to operate Zorbax NH_2 columns in an anion-exchange mode. Mobile-phase pH, ionic strength, and counterions can be adjusted to affect sample retention and selectivity. Additional information on solvent selection may be found in *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

In the normal-phase mode, Zorbax NH_2 can often be used to separate compounds that have in the past been separated on silica columns. However, with Zorbax NH_2 the selectivity often is different due to the chemical nature of the amino moiety. In addition, since primary amines can function as weak anion-exchanger sites, Zorbax NH_2 can be used with buffered mobile phases to separate ionic solutes. Typical applications include the separation of aromatic amines, carbohydrates (sugars), pesticides, pharmaceuticals, and many additional compounds of biological interest.

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 and should be filtered before injection into the column. Zorbax guard columns and a hardware kit are recommended for use with such samples (see Part Numbers). 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, 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.

Many column volumes of mobile phase are necessary for equilibration to occur after changes in mobile phase composition. The number of column volumes required depends on the system in use and can be as high as 50 to 100 column volumes. This reduces the convenience of gradient-elution chromatography since, as is the case with silica adsorbents, long equilibration times are necessary between sample analyses.

It is recommended that a particular column only be used with a given mode of operation (e.g., normal phase).

Buffer solutions should not be left in Zorbax NH_2 columns during long-term storage. If the column is being used with aqueous buffers as the mobile phase, flushing the column with distilled water, then acetonitrile prior to storage is recommended.

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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