Agilent Zorbax Phenyl

Datasheet

General Description

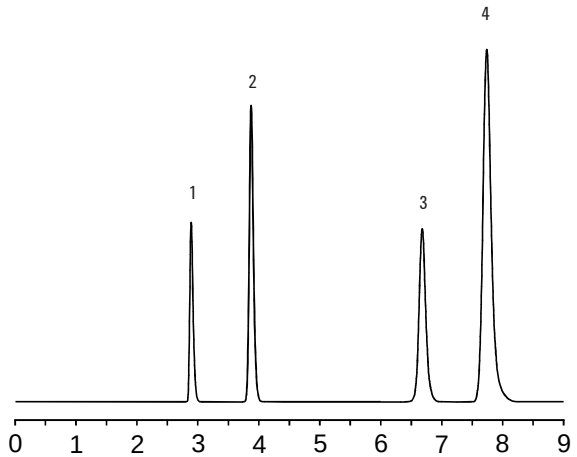
Zorbax Phenyl is a microparticulate packing used for reversed-phase high performance liquid chromatography. This packing is made by chemically bonding dimethylphenethylsilane to Zorbax SIL particles. The aromatic bonded phase is a monolayer coating produced by reacting a monofunctional silane with the Zorbax SIL support followed by end-capping with trimethylchlorosilane. A true monolayer bonded phase yields the best lot-to-lot chromatographic reproducibility in columns because a more uniform and controllable surface is achieved. The use of polyfunctional reagents (di- and tri-functional silanes) can yield uneven surface coverage with regions of bonded phase next to areas of bare silica on the support surface. This uneven surface coverage can result in mixed mechanisms of separation and is difficult to reproduce from column to column.

The uniform spherical Zorbax Phenyl particles have a controlled pore size of 70Å. Columns are loaded to a uniform bed density using a proprietary high-pressure slurry-loading technique to give optimum column efficiency.

FIGURE 1
Zorbax Phenyl QC Chromatogram

OPERATING CONDITIONS
Column: Zorbax Phenyl, 4.6 mm ID x 250 mm (5 µm)
Mobile Phase: 75% Methanol / 25% Water
Flow: 1.0 mL/min
Temperature: Ambient (23°C)
Detector: UV ABS (254 nm), 0.5 AUFS
Sample Volume: 5 µL
Peak Identity:

1. Uracil	5 µg/mL
2. Phenol	200 µg/mL
3. Toluene	850 µg/mL
4. 4-Chloro-nitrobenzene	25 µg/mL



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 the small particle size, dry Zorbax packings are respirable. Columns should only be opened in a well-ventilated area such as a hood.

Operational Guidelines

- The direction of flow is marked on the column.
- While it is 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. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- Zorbax Phenyl is compatible with water and all common organic solvents.
- The use of a Phenyl guard column is recommended to protect the Phenyl column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 8.
- Maximum operating pressure for columns up to 9.4mm ID 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 < 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 water/methanol or water/acetonitrile 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°-60°C. Gradient elution techniques are most suitable for this packing using water as the primary solvent and methanol or acetonitrile as the secondary 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 Phenyl is a less retentive reverse-phase packing than Zorbax C8 or ODS and can be used for highly polar as well as nonpolar samples. The aromatic nature of the bonded groups can result in significant selectivity differences for certain compounds compared to packings bonded with linear hydrocarbons such as ODS or C8. An example of this selectivity difference is seen in the QC Chromatogram in Figure 1 where the elution order of Toluene and 4-Chloro-nitrobenzene is reversed from that observed for ODS, C8, and TMS columns.

Zorbax Phenyl columns behave similarly to Zorbax C8 columns for nonpolar compounds, but have less retentivity. Lower concentration of methanol in a water/methanol mixture is required to obtain the same k' with a test compound on a Zorbax Phenyl column. However, the selectivity of Zorbax Phenyl for sample components may be different from the selectivity of either Zorbax ODS, C8, TMS, or CN. Thus, Zorbax Phenyl should be considered along with these columns when optimizing a separation by reversed-phase chromatography.

Column Care

The inlet frit on these columns has a nominal porosity of 2µm. Samples that contain particulate matter larger than 2µm will plug the column inlet frit. Zorbax Phenyl guard columns and 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 inlet 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 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 miscible solvent such as isopropanol.

Since columns have 1/16" terminations, a short 1/4" wrench should be used to assemble 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/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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