

Agilent Eclipse XDB-Phenyl

Datasheet

General Description

Eclipse XDB-Phenyl utilizes the technologies of Extra-Dense Bonding (XDB) of organo-silane ligands and double endcapping to protect the ultra-pure (Type B) silica support from dissolution in mobile phases of intermediate pH. Eclipse XDB-Phenyl provides different selectivity from XDB-C8 and XDB-C18 and is an alternative to those phases for the separation of acidic, basic, and other highly polar compounds by reversed-phase liquid chromatography. Eclipse XDB-Phenyl packing is made by first chemically bonding a dense monolayer of dimethyl-phenethylsilane stationary phase to a specially prepared, ultra-high purity ($\geq 99.995\%$ SiO_2), Zorbax Rx-SIL porous silica support. This special Zorbax silica support (Type B) is designed to reduce or eliminate strong adsorption of basic and highly polar compounds. The bonded-phase packing is then doubly endcapped using proprietary reagents and procedures to obtain maximum deactivation of the silica surface. This densely covered, deactivated, column packing can be used for acidic and neutral samples,

but is especially suited for separating basic compounds that produce poor peak shapes on most columns. Eclipse XDB-Phenyl can be used in the pH range of 2-9, but is best suited for long-term operation at pH 3-8. The extra-dense coating of bonded phase and exhaustive endcapping simultaneously deactivate the silica surface from deleterious interactions with samples, and also protects the silica support from dissolution in intermediate and higher pH environments.

The uniform, spherical Eclipse XDB-Phenyl particles are based on Zorbax Rx-SIL silica support that has a surface area of $180 \text{ m}^2/\text{g}$ and a controlled pore size of 80 \AA .

Column Characteristics

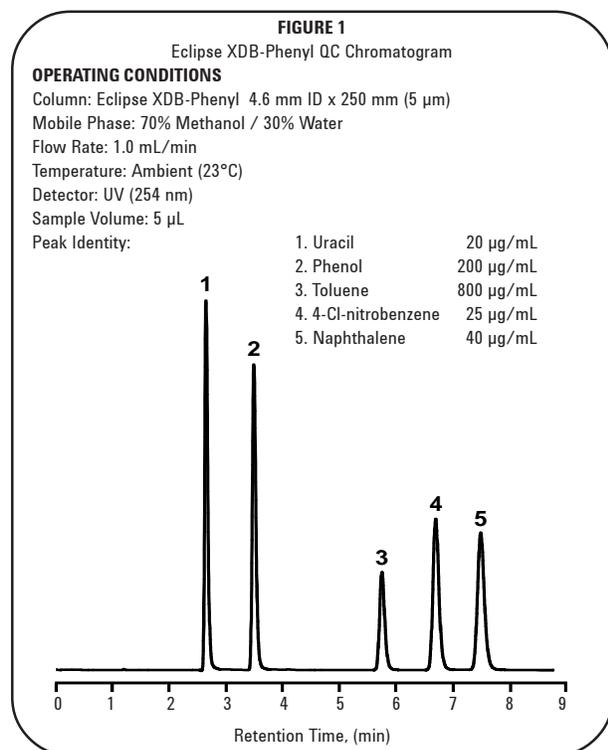
A typical Quality Control test chromatogram for a 4.6 mm ID x 250 mm column is shown in Figure 1. The actual QC test and performance of your column is described on the Column Performance Report enclosed with your column.

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 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 or acetonitrile and water. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- Eclipse XDB-Phenyl is compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the Eclipse XDB-Phenyl column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 9; optimum lifetime and performance are obtained at pH 3-8.



- Maximum operating pressure for columns up to 9.4 mm ID is 400 bar (6000 psi).
- Maximum recommended operating temperature is 60°C.

NOTE: All silica-based column packings have some solubility in aqueous mobile phases when used at pH > 6. Maximum column lifetime is obtained by operating at lower temperatures (<40°C) using low buffer concentrations in the range of 0.01 to 0.05M. 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 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; however, best column lifetime is achieved with operation at ≤ 40°C. Gradient-elution techniques for this packing often use 5% methanol or acetonitrile as the initial solvent and 100% methanol or acetonitrile as the final 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

Eclipse XDB-Phenyl can be used with basic, neutral or acidic compounds and will exhibit similar retention characteristics as XDB-C8. However, the selectivity of XDB-Phenyl may differ from aliphatic bonded phases such as XDB-C8 or XDB-C18 as observed in the QC chromatogram (Figure 1). In the figure, toluene and 4-Cl-nitrobenzene elute in reverse order compared to the aliphatic phases when run under similar conditions. Ionizable compounds (basic, acidic) generally are best separated at pH ~3 with this column. However, Eclipse XDB-Phenyl is especially suited for separating basic compounds when intermediate pH (4-8) must be used to maintain compound stability or to obtain desired band spacing (selectivity). For optimum results and long-term reproducibility, the use of 10 - 50 mM buffers is always recommended when separating ionizable compounds. For many basic compounds, basic modifiers such as triethylamine will not be required to achieve efficient separations with symmetrical peaks. For very strongly basic compounds, the use of 10 - 20 mM triethylamine or 5 - 10 mM dimethyloctylamine is recommended when using any silica-based reversed-phase column, especially when highly robust, long-term, reproducible methods are required.

Column Care

The inlet frit on these columns has a nominal porosity of 2 µm. Samples that contain particulate matter larger than 2 µm may plug the column inlet frit. 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 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.

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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