Agilent ZORBAX Eclipse Plus C18

Data Sheet

General Description

Eclipse Plus C18 columns are designed for superior peak shape with basic compounds and deliver high efficiency and excellent peak shape with all sample types. Eclipse Plus C18 is especially useful for the separation of acidic, basic, and other highly polar compounds by reverse-phase liquid chromatography. Eclipse Plus C18 packing is made by first chemically bonding a dense monolayer of dimethyl-n-octadecylsilane stationary phase to a specially prepared, an improved ultra-high purity (>99.995% SiO_2), ZORBAX Rx-SIL porous silica support. This special 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. Eclipse Plus C18 columns can be used for acidic and neutral samples, but are especially suited for separating basic compounds that produce poor peak shapes on other columns. These columns can be used for a wide range of applications and over a pH range of 2–9, accommodating most popular mobile phases.

The uniform, spherical, Eclipse Plus C18 particles are based on an improved ZORBAX Rx-SIL support that has a nominal surface area of 160 m^2/g and a controlled pore size of 95Å. Columns are loaded to a stable, uniform bed density using a proprietary high-pressure slurry-loading technique to give maximum column efficiency.

Column Characteristics

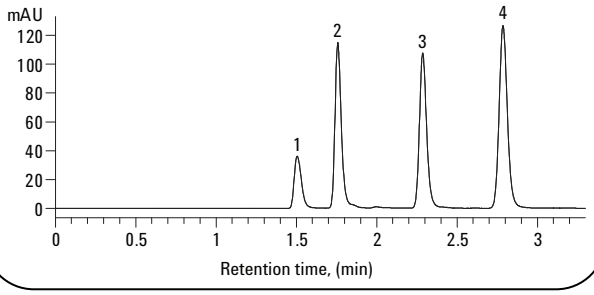
A typical Quality Control test chromatogram for a 4.6 mm × 150 mm column is shown in Figure 1. The actual QC test and performance of your column is described on the Column Performance Report enclosed with your column.

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. User of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.

Figure 1
Eclipse Plus C18 QC Chromatogram

Operating Conditions		
Column:	Eclipse Plus C18, 4.6 mm × 150 mm, (5 µm)	
Mobile phase:	85% Methanol/15% water	
Flow rate:	1.0 mL/min	
Temperature:	Ambient	
Injection:	5 µL	
Detector:	UV 254 nm	
Peak identity:	1. Uracil	5 µg/mL
	2. Phenol	200 µg/mL
	3. 4-Chloronitrobenzene	25 µg/mL
	4. Toluene	850 µg/mL



- Because of the 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 blockage (see “Column Care”)
- 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.
- Eclipse Plus C18 is compatible with water and all common organic solvents
- The use of a guard column is recommended to protect the Eclipse Plus C18 column and extend its useful lifetime

- Avoid use of this column below pH 2 or above pH 9
- Maximum operating pressure for columns up through 9.4 mm id is 400 bar (6000 psi)
- Maximum operation temperature is 60 °C

  NOTE: Eclipse Plus columns are designed for high stability over a wide pH range. 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 10 to 20 mM. 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].
- Columns should not be maintained at a neutral or elevated pH, or at elevated temperature, when not in use

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 viscocity 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 technique for this packing often uses 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. L. Glajch, and J. J. Kirkland, (John Wiley & Sons, 1997).

Applications

Eclipse Plus C18 can be used with basic, neutral or acidic compounds. Ionizable compounds (basic, acidic) generally are best separated at about pH 3 with this column. However, Eclipse Plus C18 is especially suited for separating basic compounds when an 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.

Column Care

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

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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 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 operating pressure consider replacing the column.

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 (for example, using 60/40 ACN/H_20 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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