

Agilent Zorbax Eclipse AAA

Datasheet

General Description

The Zorbax Eclipse AAA column contains batch qualified reversed-phase material that, when used according to the protocol described in Technical Note 5980-1193 (see Ordering Information Section), enables the user to separate the AA's commonly found in protein/peptide hydrolyzates. Because it uses a different mobile phase, this column is not a substitute for the column used in the AminoQuant Method.

Zorbax Eclipse AAA utilizes the technologies of eXtra-Dense Bonding (XDB) to protect the ultra-pure (Type B) silica support from dissolution in mobile phases of intermediate and higher pH. Eclipse AAA is specially prepared using 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.

The uniform, spherical Eclipse AAA particles are based on Zorbax Rx-SIL silica support that has a nominal surface area of $180 \text{ m}^2/\text{g}$ and a controlled pore size of 80\AA . Columns are packed to a stable, uniform bed density using a proprietary, high-pressure slurry-loading technique to give maximum column efficiency.

Column Characteristics

A typical batch test chromatogram for a 4.6 mm ID x 150 mm, $3.5 \mu\text{m}$ 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.

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.
- Eclipse AAA is compatible with water and all common organic solvents.

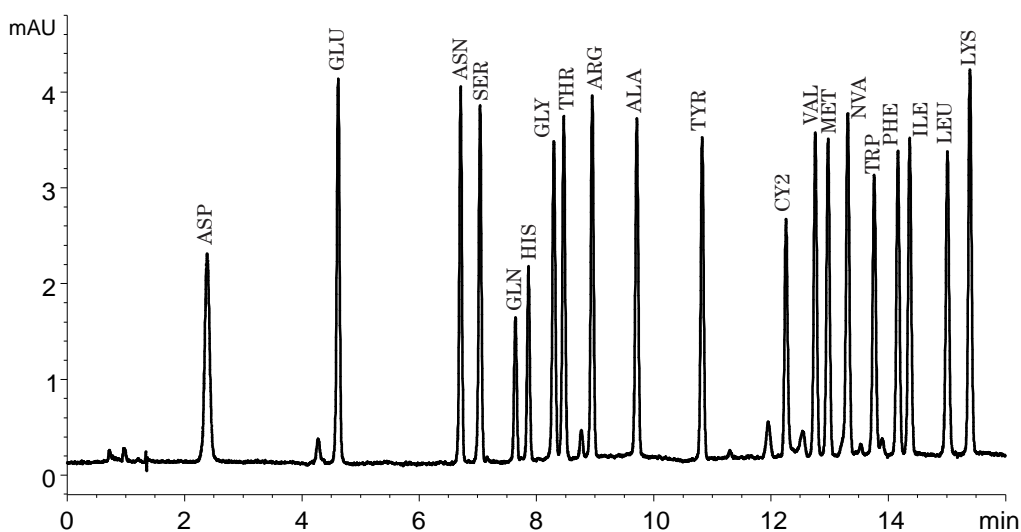


Figure 1: Batch Test Analysis, for Zorbax Eclipse AAA protocol column, using 4.6 x 150 mm, $3.5 \mu\text{m}$.

- The use of a Eclipse AAA guard column is recommended to protect the Eclipse AAA 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 4.6 mm ID columns is 400 bar (6000 psi).

Applications

This column is specifically manufactured for use with the Eclipse AAA protocol described in Technical Note 5980-1193 (see Ordering Information). This protocol uses the combination of the well-known OPA and FMOc chemistries which enables a fast pre-column derivatization of amino acids (AA's) for chromatographic analysis. The reaction mixture is buffered at pH 10.2, which allows direct analysis of acid hydrolyzed protein/peptide samples. The primary AA's are reacted first with OPA using 3-mercaptopropionic acid (3-MPA). The secondary AA's, which do not react with OPA, are then derivatized using FMOc. The incorporation of 3-MPA into the indoles decreases their hydrophobicity and as a result the products elute chromatographically before the FMOc derivatives. Excess FMOc and its degradation products elute after the last of the secondary AA's and do not interfere with the analysis. Using the well established OPA and FMOc chemistries, amino acid analysis of protein and peptide hydrolysates can be separated in ten minutes using the Zorbax Eclipse AAA column (4.6 mm x 75 mm) and the Agilent 1100 HPLC.

The choice of column depends upon the analysis speed and resolution desired:

- Zorbax Eclipse AAA, 4.6 x 75 mm (3.5 μ m), for routine sensitivity, high-throughput work
- Zorbax Eclipse AAA, 4.6 x 150 mm (5 μ m), for routine sensitivity, high-resolution work
- Zorbax Eclipse AAA, 4.6 x 150 mm (3.5 μ m), for high sensitivity, high resolution work

Using the Agilent 1100 autosampler to automate the pre-column derivatization procedure results in a speedy and reproducible reaction with minimal operator intervention. Using the DAD collecting two wavelengths, or with programmed wavelength switching, this method can be used for routine analysis of primary and secondary amino acids. For high sensitivity work, the fluorescent detector is required. See Technical Note, 5980-1193, for specific HPLC system parameters.

Column Care

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

Eclipse AAA guard columns and a hardware kit are recommended for use with such samples (see Ordering Information).

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. Since the column is 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 acetonitrile and water, followed by 20-30 column volumes of the pure acetonitrile. 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. This can be achieved using 100% mobile phase B (45/45/10, ACN/MeOH/H₂O) from the Eclipse AAA protocol. Equilibration is rapid without the need to make a special mobile phase, and any danger of corrosion from the salts is eliminated.

Agilent Ordering Information

For more information on our products, visit our Agilent Technologies home page on the World Wide Web at: www.agilent.com/chem/supplies

For Technical Support in the US and Canada, call 1-800-227-9770 or call your local Agilent sales office.



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