Agilent ZORBAX Eclipse PAH Columns

Data Sheet

General Description

ZORBAX Eclipse polynuclear aromatic hydrocarbon (PAH) columns are designed for the high-efficiency separation of polynuclear aromatic hydrocarbons. The ZORBAX Eclipse PAH column can be used for these separations and others that require high shape selectivity in a bonded phase.

The ZORBAX Eclipse PAH column packing is a polymeric C18 bonding designed to provide the high level of selectivity needed for PAH separations. This bonded phase is put on a specially prepared, improved ultra-high purity (> 99.995% SiO_2) ZORBAX Rx-SIL porous support. This special silica support (Type B) is designed to reduce or eliminate strong adsorption of basic and highly polar compounds.

ZORBAX Eclipse PAH columns are designed for separations of PAHs and are tested with a mixture of 16 PAHs to verify performance. The ZORBAX Eclipse PAH column can also be used for other samples that benefit from bonded phases that improve shape selectivity or as an alternate type of C18 column. Therefore these columns can be used for a variety of applications and over a pH range of 2 to 8.

The uniform, spherical, ZORBAX Eclipse PAH 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 95 Å. 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 assurance (QA) test chromatogram for a 4.6 mm × 100 mm, 5-µm column is shown in Figure 1. The quality control (QC) test with the performance of your column is shown in the Column Performance Report enclosed with your column. You will also find a specific batch QA test with a PAH sample in the report.

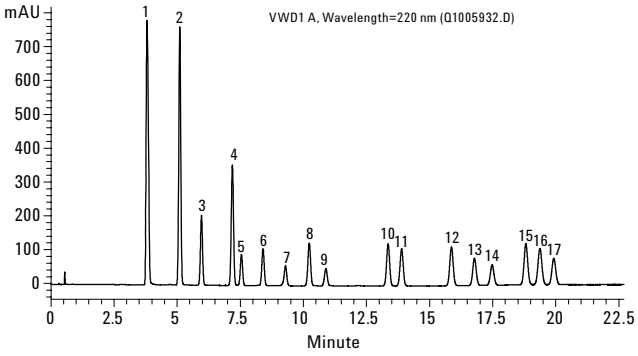

Figure 1. ZORBAX Eclipse PAH QA chromatogram.

Operating Conditions

Column:	Eclipse PAH 4.6 × 100 (5 micron)
Mobile phase:	Gradient elution

Time	Percent B
0.00	40
0.66	40
20.00	100
25.00	100
27.00	40
30.00	40

Solvent A:	Water
Solvent B:	Acetonitrile
Flow rate:	2 mL/min
Injection volume:	1 microliter
Detector:	UV; 220 nm, 4 nm bandwidth: reference off
Temperature	25 °C
Standard:	PAH mixture; Agilent part number 8500-6035

1. Acetone (from sample solvent)
2. Naphthalene
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benzo(a)anthracene
11. Chrysene
12. Benzo(b)fluoranthene
13. Benzo(k)fluoranthene
14. Benzo(a)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(g,h,i)perylene
17. Indeno(1,2,3-cd)pyrene

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic instruments 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 blockage (see "Column Care").
- A new column contains a mixture of acetonitrile and water. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- ZORBAX Eclipse PAH columns are compatible with water and all common organic solvents.
- The use of a guard column is recommended to protect the ZORBAX Eclipse PAH column and extend its useful lifetime.
- Avoid use of this column below pH 2 or above pH 8.
- Maximum operating pressure for columns with compression fittings up through 9.4 mm id is 400 bar (6,000 psi). For higher pressure operation use a ZORBAX Rapid Resolution High Throughput Eclipse PAH threaded column.
- Maximum operating temperature is 60 °C below pH 6.*

*ZORBAX Eclipse PAH columns are designed for high stability over a wide pH range. However, all silica-based packings have some solubility in pH > 6 aqueous containing mobile phases. Therefore, when using silica-based columns under conditions of pH > 6, maximum column lifetime is obtained by operating at low temperatures (< 40 °C) using low buffer concentrations in the range of 10 to 20 mM. The typical acetonitrile:water mobile phase for separations of PAHs is one that has a pH > 6, so these precautions are important.

- The column should not be maintained at an elevated pH or temperature when not in use.

Mobile Phase Selection

Most PAH separations are performed using a gradient method with a mobile phase of acetonitrile and water. This mobile phase can be used on the ZORBAX Eclipse PAH with excellent results. The bonded stationary phase is nonpolar in nature and for other applications 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 samples.

Gradient-elution techniques for PAHs on ZORBAX Eclipse PAH columns often use 30% to 40% acetonitrile as the initial solvent and 100% acetonitrile as the final solvent. For other gradient elution applications, a mobile phase of 10% acetonitrile or methanol may be used 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).

Column temperature may also be changed to improve separations of PAHs. It may be necessary to cool the column down to 15 to 20 °C for improved resolution. Shape selectivity can be enhanced at cool temperatures. Column temperatures can also be elevated with the ZORBAX Eclipse PAH column, up to 60 °C; however, best column lifetime is achieved with operation at < 40 °C.

Applications

ZORBAX Eclipse PAH columns are designed for the rapid and efficient separation of the 16 priority pollutant PAHs. The ZORBAX Eclipse PAH column can also be used for other PAH separations, such as the 15-component European Union Smoked Food PAH standards. Beyond PAHs, the polymeric C18 column can be used for separations of other analytes that are better separated on a shape-selective bonded-phase column. These include analytes such as carotenoids, retinols, and tocopherols as potential analytes. This type of C18 column can also be used for a variety of other general applications. This column is suited to the analysis of basic, as well as acidic and neutral, compounds. For optimum results and long-term reproducibility, the use of 10- to 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. ZORBAX Eclipse PAH guard columns and a hardware kit are recommended for use with such samples.

If solvent flow appears to be restricted (high column backpressure), 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 backflushing 25 to 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. A 100% acetonitrile solvent should be the first choice for flushing a ZORBAX Eclipse PAH column to remove strongly retained material because acetonitrile is typically used in the mobile phase. In addition, solvents such as methanol, isopropanol, or a 95%/5% mixture of dichloromethane and methanol should remove most highly retained compounds. In extreme cases, dimethyl sulfoxide (DMSO) or dimethylformamide (DMF) 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 to 30 column volumes of a 50/50 mixture of methanol or acetonitrile and water, followed by 20 to 30 column volumes of the pure solvent. Before storing the column, the end fittings should be tightly capped with the end plugs provided with the column in order to prevent the packing from drying out.

In general, columns may be safely stored for short periods in most mobile phases. The ZORBAX Eclipse PAH column can be safely stored for longer periods of time in the acetonitrile:water mobile phase typically used at the end of the gradient analysis or in 60% acetonitrile or more. 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 a buffer (for example, 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.

Agilent Ordering Information

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

For technical support in the U.S. and Canada, call 1-800-227-9770 or your local Agilent sales office.

www.agilent.com / chem

Printed in the USA

August 8, 2007

Part No. 820108-018

Agilent Technologies