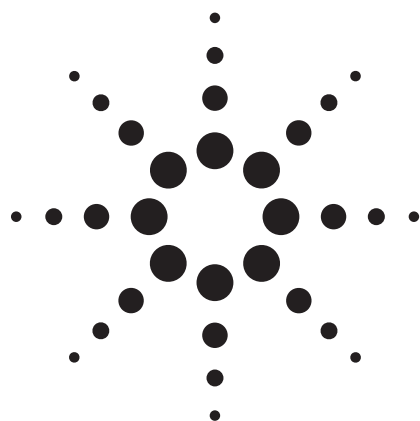




Agilent ZORBAX HILIC Plus HPLC Columns

Data Sheet

General Description

Agilent ZORBAX HILIC Plus HPLC columns are non-bonded silica columns made with ZORBAX Eclipse Plus silica (high-purity, Type B silica, specially treated for good peak shape) and optimized for hydrophilic interaction chromatography (HILIC) separations. HILIC is typically used for the retention and separation of small, polar analytes. The ZORBAX HILIC Plus columns ship containing acetonitrile:water and are ready to use for HILIC separation. HILIC columns require more equilibration than reversed-phase columns and more details on this are provided in the method development section of this data sheet.

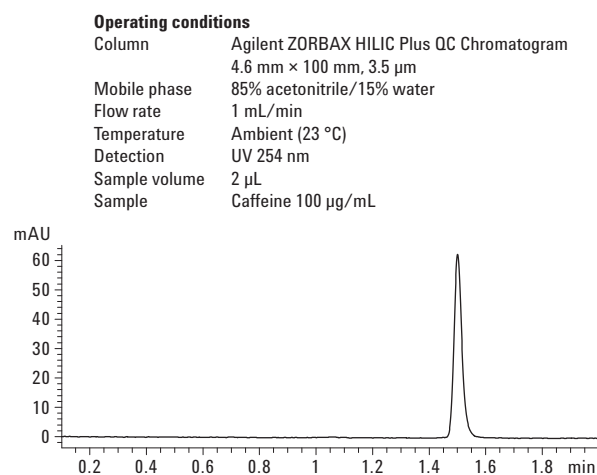


Figure 1. ZORBAX HILIC Plus QC chromatogram.

Column Characteristics

A typical quality control (QC) chromatogram for a 4.6 mm × 100 mm, 3.5 µm ZORBAX HILIC Plus column is shown in Figure 1. The QC test with the performance of your column is shown on the Column Performance Report enclosed with your column.

Safety Considerations

- All points of connection in a liquid chromatographic system are potential sources of leaks. Users of liquid chromatographic instruments should be aware of the potential 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 generally not harmful to the column, reversing flow should be avoided except to attempt to remove contamination from the inlet frit.
- ZORBAX HILIC Plus columns are shipped containing acetonitrile and water.
- ZORBAX HILIC Plus columns are compatible with water and all common organic solvents.
- Maximum operating pressure is 400 bar.
- Maximum operating temperature is 40 °C.
- The operating pH range of this column is pH 1 to 8.

Note: HILIC Plus columns are silica-based columns with no bonded phase. All silica has some solubility in pH > 6 aqueous mobile phases. Solubility of the silica is also increased at elevated temperatures. Therefore, using the HILIC Plus column above pH 6 and 40 °C will reduce the column lifetime.



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Method Development with HILIC Plus

The ZORBAX HILIC Plus column is best used for separations of polar analytes inadequately retained on typical reversed-phase columns. For the HILIC mechanism to work effectively, the column must be equilibrated with water to create a water layer on the silica sorbent. Therefore it is best to equilibrate the column with 30 to 40% water in acetonitrile before use. The column must be equilibrated with 20 to 50 column volumes before use. Several injections should be done to verify that the column is properly equilibrated.

A typical mobile phase for the HILIC Plus column will be acetonitrile:water with an acetate or formate buffer. This will most commonly be ammonium acetate or formate to achieve compatibility with an MS detector. To optimize retention for HILIC methods, increase the percent acetonitrile in the mobile phase and decrease the aqueous/buffer to increase retention. In addition, it is critical to optimize pH and buffer strength for the best results. A recommended starting buffer concentration is 5 to 10 mM and increase up to 20 mM for improved peak shape and retention. A typical pH range for HILIC separations will be pH 2 to 7 using formate and acetate buffers.

Applications

ZORBAX HILIC Plus columns can be used for typical HILIC applications. The most common HILIC applications are for the retention of small, polar, and/or basic analytes. HILIC is considered a good alternative to ion-pair chromatography and the use of bonded phases with polar groups in the bonded phase. These applications typically require additives and high aqueous conditions that are less compatible with MS detectors; therefore, HILIC may be a preferred column choice. HILIC can also be a preferred alternative to normal-phase chromatography techniques.

Some typical analytes may include melamine and other polar analytes, including such compounds as acrylamide, nucleosides, metformin, and other compounds in the U.S. EPA 1694 Method.

Column Care

Samples that contain particulate matter may plug the column inlet frit and should be filtered before injection

into the column. If solvent flow appears to be restricted (unusually 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 backflushing 25 to 30 mL of mobile phase through the column. If this fails to return the column to near its original back-pressure, the column should be replaced.

To remove strongly-retained materials from the column, flush the column with a strong solvent, which is water in the HILIC mode.

Storage Recommendations

Acetonitrile:water (95%:5%) is recommended as the long-term storage solvent for the HILIC Plus column. It may be necessary to flush the column with 60% acetonitrile:40% water to remove strongly-retained compounds prior to switching to the storage solvent. Before storing the column, the end fittings should be tightly capped with the end plugs to prevent the packing from drying out.

Columns may be safely stored for short periods in most HILIC mobile phases. However, to protect equipment, it is best to remove salts from the instrument and column by purging the column with the same mobile phase without the buffer (for example, using 90/10 ACN/H₂O to remove a 90/10 ACN/0.01 M formate buffered mobile phase). Re-equilibration is faster with the original mobile phase when using this approach, but several (3 to 6) injections should be made to verify column equilibration.

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

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