

Agilent ZORBAX Rapid Resolution High Definition HILIC Plus Threaded Column

Data Sheet

General Description

Agilent ZORBAX Rapid Resolution High Definition (RRHD) HILIC Plus threaded columns are specially designed for use with ultra-high performance liquid chromatographs (UHPLCs) such as the Agilent 1290 Infinity LC and can be used up to an operating pressure of 1200 bar. Agilent ZORBAX HILIC Plus HPLC columns are nonbonded silica columns made with Agilent 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. More details are provided in the method development section of this data sheet.

Column Characteristics

A typical quality control (QC) chromatogram for an Agilent ZORBAX RRHD HILIC Plus 2.1 mm × 50 mm, 1.8 μm column is shown in Figure 1. The QC test with the performance of the column is shown on the Column Performance Report enclosed with the column. The efficiency found on the QC Performance Report may be higher than the efficiency found in your laboratory. The QC test system may vary from the LC used in your lab, and has been modified from a standard system to minimize volume. This allows a better evaluation of the column and assures a more consistent product for the chromatographer.

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.
- These RRHD columns are mechanically stable and have been tested to very high pressures to ensure safe lab operation on a variety of LC instruments. Maximum operating

pressure is 1200 bar. Opening columns will compromise this pressure limit.

- 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.
- These columns are packed and assembled for high-pressure (up to 1200 bar) use. Disassembling the column will degrade column performance.
- Agilent ZORBAX RRHD HILIC Plus columns are shipped containing acetonitrile and water.
- Agilent ZORBAX RRHD HILIC Plus columns are compatible with water and all common organic solvents.
- Maximum operating pressure is 1,200 bar (17,000 psi). Optimal lifetime is achieved when operating up to 1000 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. Using the HILIC Plus column above pH 6 and 40 °C will reduce the column lifetime.

Method Development with HILIC Plus

The Agilent ZORBAX RRHD 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.



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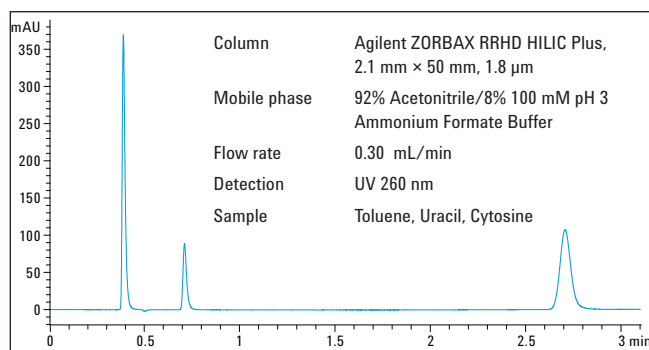


Figure 1. Agilent ZORBAX RRHD HILIC Plus QC chromatogram.

A typical mobile phase for the RRHD 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 RRHD 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 Method 1694.

Column Care

Samples should be filtered before injection into the column. The column inlet frit is nominally 0.5 μm and samples should be filtered through a 0.2 μm sample filter. 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 restriction is prior to the column, replace the appropriate piece of tubing or filter that is plugged. If the column is plugged, do not backflush the column. Replace the column.

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

Since columns have 3/8-inch end nuts, use a short 3/8-inch wrench to attach the columns to the instrument to avoid any additional tightening of the end fittings. Over-tightening the end fittings will cause damage and require column replacement. Additional care recommendations are included on the card in the box. Review these prior to using the column.

Storage Recommendations

Acetonitrile:water (95%:5%) is recommended as the long-term storage solvent for the RRHD 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, tightly cap the end fittings 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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