

Agilent ZORBAX Rapid Resolution High Definition 300Å HILIC Wide Pore (WP) Threaded Column

Data Sheet

General Description

Agilent ZORBAX Rapid Resolution High Definition (RRHD) 300-HILIC 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. RRHD 300-HILIC UHPLC columns are nonbonded silica columns made with Agilent ZORBAX 300Å, 1.8 µm 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, but this column with the larger pore size is specifically designed for the separation of glycopeptides. The RRHD 300-HILIC columns ship containing acetonitrile:water and are ready to use for HILIC separations. HILIC columns require more equilibration than reversed-phase columns. More details are provided in the method development section of this data sheet.

Column Characteristics

Figure 1 shows a typical quality control (QC) chromatogram for an RRHD 300-HILIC, 2.1 × 50 mm, 1.8 µm column. The Column Performance Report enclosed with the column shows the QC test with the performance of 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. The maximum operating pressure is 1200 bar. Opening columns will compromise this pressure limit.

- Because of the small particle size, dry ZORBAX packings are respirable. Open columns only 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 degrades column performance.
- RRHD 300-HILIC columns are shipped containing acetonitrile and water.
- RRHD 300-HILIC columns are compatible with water and acetonitrile, which are most commonly used, but other organic solvents can be used.
- Maximum operating pressure is 1,200 bar (17,000 psi). Optimal lifetime is achieved when operating up to 1,000 bar.
- Typical operating temperature is 40 °C. Higher temperatures can be used but may shorten column lifetime.
- The operating pH range of this column is pH 1 to 8.

Note: RRHD 300-HILIC 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 RRHD 300-HILIC column above pH 6 and 40 °C reduces the column lifetime.

Method Development with HILIC Plus

The RRHD 300-HILIC column is best used for separations of glycopeptides. For the HILIC mechanism to work effectively, the column must be equilibrated with water to create a water layer on the silica sorbent.



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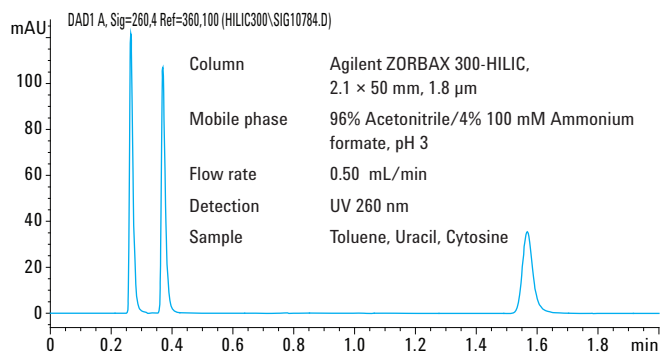


Figure 1. Agilent ZORBAX RRHD 300-HILIC QC chromatogram.

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, 3 to 6, may be done to verify that the column is properly equilibrated.

A typical mobile phase for the ZORBAX RRHD 300-HILIC column is 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 50 mM for improved peak shape and retention. A typical pH range for HILIC separations is pH 2 to 7 using formate and acetate buffers.

Applications

ZORBAX RRHD 300-HILIC columns can be used for typical HILIC applications. These columns are designed for the separation of glycopeptides and provide an alternative selectivity to reversed-phase. The separation uses the difference in the glycosylation to achieve resolution.

Column Care

Filter samples 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 the solvent flow appears to be restricted (unusually high column back-pressure), first check 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, use the following sequence to regenerate, 20-30 CV water, 20-30 CV 0.5 M NaCl, 20-30 CV water, and then re-equilibrate in eluent. A strong solvent, is water in the HILIC mode.

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 300-HILIC 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). For short term storage, re-equilibration is faster when the column is stored in 80% ACN/20% 5 mM ammonium formate, but several (3 to 6) injections should be made to verify column equilibration.

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