

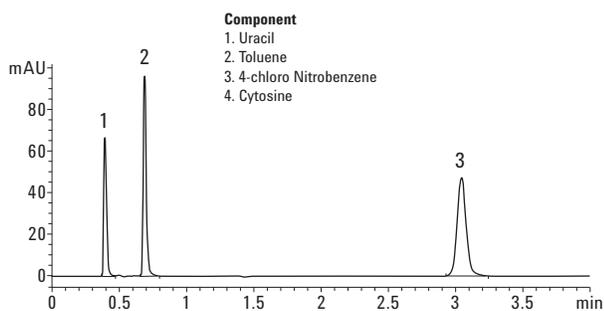
Agilent Poroshell 120 HILIC Threaded Column

Data Sheet

General Description

Agilent Poroshell 120 HILIC is a superficially porous microparticulate column packing. Superficially porous silica particles, such as Poroshell, have a solid silica core and a porous silica outer layer. Poroshell 120 HILIC columns are non-bonded silica columns made with Poroshell 120 silica and optimized for hydrophilic interaction chromatography (HILIC) separations. HILIC is typically used for the retention and separation of small, polar analytes. The Poroshell 120 HILIC columns ship containing acetonitrile:water and are ready to use in HILIC separations. HILIC columns require more equilibration than reversed-phase columns. More details are provided in the method development section of this data sheet.

The Poroshell 120 packing has a solid core of 1.7 μm with a porous outer layer 0.5 μm thick and a total particle size of 2.7 μm . The particles have a nominal surface area of 120 m^2/g and a controlled pore size of 120 \AA . The columns can be used up to an operating pressure of 600 bar (9,000 psi). The uniform, spherical particles are ultrahigh purity (>99.995% SiO_2) silica. This high purity silica is designed to reduce or eliminate strong adsorption of basic and highly polar compounds.



Column: Agilent Poroshell 120 HILIC, 2.1 \times 50 mm, 2.7 μm
Mobile phase: 94 % Acetonitrile/6 % pH3 100 mM ammonium acetate buffer
Flow rate: 0.3 mL/min

Figure 1. Agilent Poroshell 120 HILIC chromatogram.

Column Characteristics

A typical Quality Control test chromatogram for a Poroshell 120 HILIC, 2.1 \times 50 mm, 2.7 μm threaded 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. The efficiency reported on the Column 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 system volume. This allows a better evaluation of the packed column and assures a more consistent product for the chromatographer.

Safety Considerations

All points of connection in liquid chromatographic systems are potential sources of leaks. Users of LCs and UHPLCs should be aware of the toxicity or flammability of their mobile phases.

These Poroshell 120 columns are mechanically stable and have been tested to very high pressures to assure safe lab operation on a variety of LC and UHPLC instruments. The operating pressure limit for all 2.1-, 3.0-, and 4.6-mm id columns is 600 bar (9000 psi). While the 2.1- and 3.0-mm id columns are safe to 1,300 bar (20,000 psi) and the 4.6-mm id columns are safe to 1,000 bar (16,000 psi), chromatographic performance will be compromised if the 600 bar pressure limit is exceeded and the column may need to be replaced.

Because of its small particle size, dry Poroshell packings are respirable. Columns should only be opened in a well-ventilated area, and opening the column will compromise column performance.

Operational Guidelines

- The direction of flow is marked on the column. The column should only be operated in this direction.
- These columns are packed and assembled for use up to 600 bar (9,000 psi). Disassembling or over-tightening the column will degrade column performance.
- Poroshell 120 HILIC is compatible with water and all common organic solvents.
- The operating pH range of this column is pH 1 to 8.
- Maximum operating temperature is 40 $^{\circ}\text{C}$.



Agilent Technologies

Method Development with Poroshell 120 HILIC

The Agilent Poroshell 120 HILIC 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 Poroshell 120 HILIC 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

Poroshell 120 HILIC columns are designed for fast and high resolution separations of a wide range of small molecule analytes, including acidic, basic and neutral compounds. The unique, superficially porous particle and 2.7- μm particle size make this column ideal for fast separations at up to 40% to 50% lower pressures than sub-2 μm particles with similar (90% to 100%) efficiency. The columns can be used at high flow rates to achieve fast separations.

Poroshell 120 HILIC 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 that contain particulate matter may plug the column inlet frit and should be filtered before injection into the column. The inlet frit size on these columns is nominally 2 μm . Samples that contain particulate matter which is larger than 2 μm will plug the column inlet frit. The column should be operated only in the flow direction marked and should not be backflushed to remove particulates retained on the inlet frit.

To remove strongly retained materials from the column, flush the column with stronger (less polar) solvents. Solvents such as methanol, acetonitrile, isopropanol, or a 95/5 mixture of dichloromethane and methanol should remove most highly retained compounds. 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.

These columns have a 3/8-inch end nut and a short 3/8-inch wrench should be used 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.

Storage Recommendations

Acetonitrile:water (95%:5%) is recommended as the longterm storage solvent for the Poroshell 120 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).

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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Printed in the USA
September 5, 2012
Part No. 820301-001



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