

# Agilent Altura Poroshell HILIC-Z Columns

Zwitterionic HILIC columns with  
Ultra Inert technology



## Introduction

Agilent Altura Poroshell HILIC-Z columns with Ultra Inert technology are designed and manufactured for normal-phase separation of small, polar analytes such as amino acids and organic acid metabolites. The zwitterionic phase bonded onto superficially porous silica particles allows for fast, efficient, and reproducible separations of small, charged molecules. The coated stainless-steel column hardware ensures an inert surface for maximum recovery of anionic analytes, with the robustness and consistency of stainless steel.

## Getting started

Hydrophilic interaction liquid chromatography (HILIC) is a normal-phase chromatography method where the stationary phase is polar and the mobile phase is predominantly nonpolar. Therefore, water is considered the "strong" solvent in a HILIC method. More hydrophobic compounds elute earlier, with more polar or hydrophilic compounds eluting later.

A column performance report, including a column-specific QC test chromatogram and a batch-specific amino acid separation, is enclosed with every Altura Poroshell HILIC-Z column. The Agilent QC test system has been modified from a standard system to minimize dead volume, so it may vary from the system used in your lab. This modification enables a better evaluation of the column efficiency and assures a more consistent product. An optimized LC system will generate similar results to the chromatogram on your column performance report. Individual column performance reports may be found by searching by part and serial number at [www.agilent.com/chem/cop](http://www.agilent.com/chem/cop).

### Important safety considerations

- All points of connection in an LC system are potential sources of leaks. Users of liquid chromatography instruments should be aware of the potential toxicity or flammability of their mobile phases.
- Do not remove the column end fittings.

## Using your column

### LC instrument passivation

Anionic small molecules are sensitive to the presence of metals that may chelate with the analytes. An inert LC is recommended, but whichever type of LC you use, you can improve both peak shape and sensitivity by implementing a passivation procedure before running samples. We suggest running 90:10 acetonitrile:water with 0.5% (v/v) phosphoric acid overnight through channel B of the LC, as well as through the column and the nebulizer of the mass spectrometer. Be sure to remove the nebulizer from the mass spectrometer and direct it to a waste container to avoid sending unnecessary flow to the instrument.

### Installation

Remove both end plugs and ensure that the direction of your system's flow matches the arrow on the column. Do not use the column with the flow in the reverse direction. Do not overtighten fittings. They should be swaged to the correct depth to avoid damage and provide maximum efficiency.

Agilent InfinityLab Quick Connect fittings (part number 5067-5957 for a 0.12 × 105 mm assembly) or Agilent InfinityLab Quick Turn fittings (part number 5067-5966) produce simple and reliable connections to your HPLC or UHPLC instrument. No tools are needed to make leak-free connections that fit the column perfectly.

### Column conditioning

Columns are shipped in acetonitrile:water. Like other normal-phase columns, HILIC columns require more equilibration than reversed-phase columns to achieve reproducible separations. We recommend conditioning the column with 30 to 40% water in acetonitrile before use. Thorough equilibration may require up to 50 column volumes.

### Instructions for use

Altura Poroshell HILIC-Z columns are compatible with aqueous buffers and all common organic solvents. When changing or mixing eluents, always take into account the viscosity and risk of salt precipitation.

Prepare buffers freshly using high-purity components and ultrahigh-purity water, such as Milli-Q or Nanopure. Use a 0.2 or 0.45 µm filter to remove particulates and help reduce the risk of microbial growth, which will damage the column and your HPLC or UHPLC system.

For best peak shape, prepare samples in conditions as close to the initial mobile phase conditions as possible, including matching pH and ionic strength. Ideally, if starting with 100% mobile phase B, dilute or prepare samples in mobile phase B. Make sure that samples are completely dissolved, and filter or centrifuge before injection.

Altura Ultra Inert LC column hardware is robust under a wide range of conditions, and as a result, the stationary phase determines the operating parameters of the column.

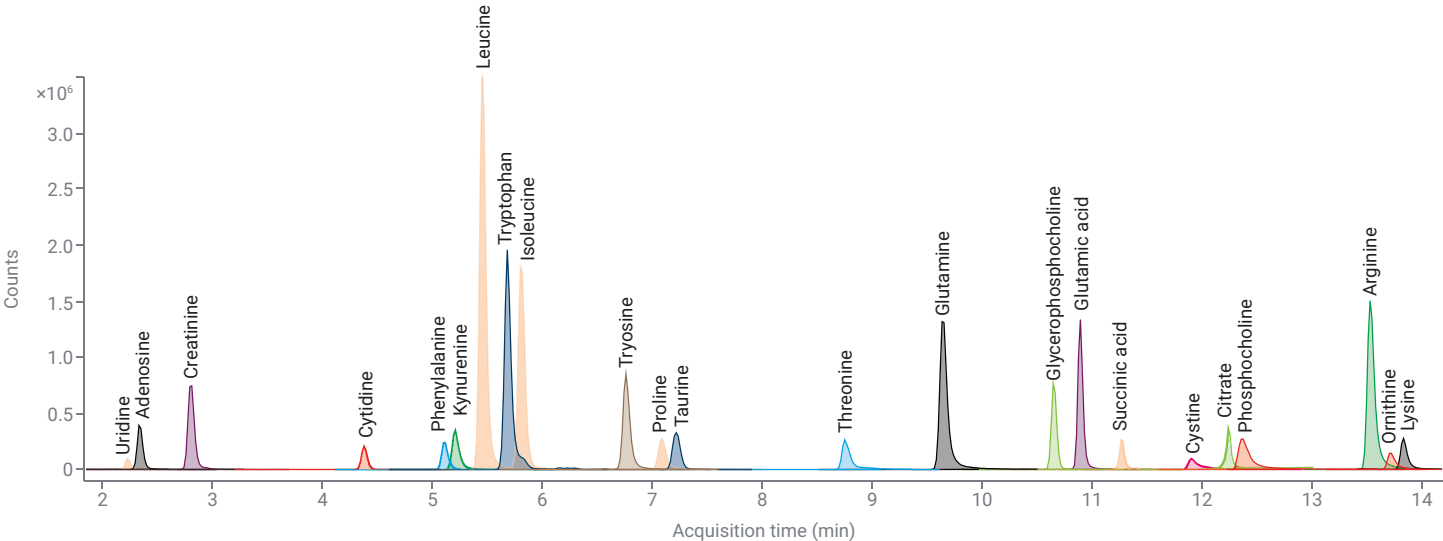
**Table 1.** Operating parameters.

Parameter	Value
Moble-phase Compatibility	<b>Aqueous buffers and acetonitrile</b> <ul style="list-style-type: none"> <li>– Aqueous buffers with high and low salt can be used.</li> <li>– Methanol may be used to alter selectivity.</li> <li>– Avoid exposing the column to 100% aqueous conditions.</li> </ul>
pH Stability	3 to 11
Operating Temperature	30 °C (recommended), 80 °C (maximum)
Maxumum Preasure	600 bar (9,000 psi)

**Note:** Working at extremes of the operating parameters is likely to reduce column lifetime.

### Sample method

LC Conditions	
Column	Agilent Altura Poroshell HILIC-Z, 2.1 × 150 mm, 2.7 µm (p/n 227215-924)
Column Temperature	15 °C
Injection	4 µL
Autosampler	5 °C
Mobile Phase	A) 20 mM ammonium acetate, pH 9.3 + 5 µM medronic acid in H <sub>2</sub> O B) Pure ACN
Flow Rate	0.400 mL/min
Gradient Program	Time (min)      %B
	0                    90
	1.00               90
	8.00               78
	12.00              60
	15.00              10
	18.00              10
	19.00              90
	23.00              90



**Figure 1.** Metabolites analyzed using an Agilent Altura Poroshell HILIC-Z column, Agilent 1290 Infinity III Bio LC, and Agilent 6495D triple quadrupole LC/MS operated in polarity switching mode.

## Column care

An increase in backpressure and decrease in performance may occur over time. If the pressure has increased, first identify if the increase is due to the instrument or columns. If a system component, such as tubing or a filter, is causing the increase, then replace it and retest.

### Column cleaning instructions

Where performance has deteriorated below the acceptance criteria, the column may be regenerated by flushing with 50:50 acetonitrile:10 mM ammonium acetate at 20% of normal operating flow rates for at least 3 hours. The 10 mM ammonium acetate does not need to be pH adjusted. Equilibrate the column with mobile phase.

### Column storage

Whenever a column is not installed on the LC, seal both ends of the column with the removable end plugs supplied with the column. For long-term storage, we recommend 90:10 acetonitrile:water. For short-term storage, most HILIC mobile phase solvents may be used as long as the pH is < 6. All silica particles are prone to dissolution above pH 6. However, to protect equipment, remove salts from the instrument and column by purging the system with the same solvents without the buffer additives (for example, use 90:10 ACN:H<sub>2</sub>O to remove a 90:10 ACN:10 mM formate buffered mobile phase). Re-equilibration is faster with the original mobile phase when using this approach, but three to six injections should be made to verify column equilibration.

## Ordering details

Description	Part Number
Altura Poroshell HILIC-Z 2.7 µm, 2.1 × 50 mm	227205-924
Altura Poroshell HILIC-Z 2.7 µm 2.1 × 100 mm	227210-924
Altura Poroshell HILIC-Z 2.7 µm 2.1 × 150 mm	227215-924

[www.agilent.com/columns/altura](http://www.agilent.com/columns/altura)

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