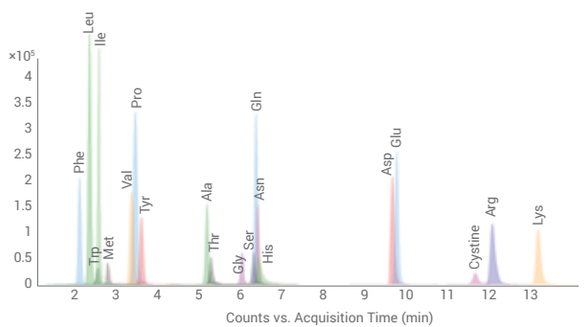
High pH, negative ion mode MS detection	
Column	Agilent AdvanceBio MS Spent Media column, 2.1 x 100 mm, part number 675775-901
Column temp	30 °C
Injection Volume	1.0 µL
Mobile phase	A = 10% 100 mM ammonium acetate in water pH 9, 90% water B = 10% 100 mM ammonium acetate in water pH 9, 90% acetonitrile *Final salt concentration is 10 mM.*
Flow rate	0.5 mL/min
Gradient program	Time (min) % B 0 100 15 80 15.5 100 20 100
Sample	Cell culture media, diluted 5-fold with Mobile Phase B
Detection	Agilent 6230 time-of-flight LC/MS



Operating parameters	
Mobile-phase compatibility	Aqueous buffers and acetonitrile *Aqueous buffers with high and low salt can be used. Methanol may be used to alter selectivity. Avoid exposing the column to 100% aqueous conditions.*
pH stability	3 to 11
Operating temperature	30 °C (recommended), 80 °C (maximum)
Maximum pressure	600 bar (9,000 psi)
Working at extremes of the operating parameters is likely to reduce column lifetime.	

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_2O 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
AdvanceBio MS Spent Media column 100 Å, 2.1 x 50 mm, 2.7 µm	679775-901
AdvanceBio MS Spent Media column 100 Å, 2.1 x 100 mm, 2.7 µm	675775-901
AdvanceBio MS Spent Media column 100 Å, 2.1 x 150 mm, 2.7 µm	673775-901

www.agilent.com/chem/advancebio

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Agilent AdvanceBio MS Spent Media column

User guide

Hydrophilic interaction liquid chromatography columns for analysis of amino acids and metabolites from cell culture media

Agilent AdvanceBio MS Spent Media columns are designed and manufactured for normal phase separation of amino acids and small, polar metabolites in cell culture media. The zwitter ionic phase bonded onto superficially porous silica particles allows for fast, efficient, and reproducible separations of small, charged molecules. The PEEK-lined hardware ensures an inert surface for maximum recovery of anionic analytes.

Getting started

Hydrophilic interaction liquid chromatography, HILIC, is a normal phase chromatography method, where the stationary phase in 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 Agilent AdvanceBio MS Spent Media 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.

If you have specific questions, contact Technical Support at **www.agilent.com/chem/techsupport**.

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 passivation

Anionic small molecules are sensitive to the presence of metals that may chelate with the analytes. A bio-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.

Avoid damage to PEEK-lined columns during installation. Combined compression and rotation may cause damage, so fittings without ferrules, such as PEEK finger-tight fittings, are not recommended. 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 x 105 mm assembly) or 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 reverse-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

Agilent AdvanceBio MS Spent Media 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.

Recommended Starting Conditions

Low pH, positive, ion mode MS detection		
Column	Agilent AdvanceBio MS Spent Media column, 2.1 x 150 mm, part number 675775-901	
Column temp	30 °C	
Injection Volume	1.0 µL	
Mobile phase	A = 10% 200 mM ammonium formate in water pH 3, 90% water B = 10% 200 mM ammonium formate in water pH 3, 90% acetonitrile *Final salt concentration is 20 mM.*	
Flow rate	0.5 mL/min	
Gradient program	Time (min) 0 15 15.5 20	% B 100 80 100 100
Sample	Cell culture media, diluted 5-fold with Mobile Phase B	
Detection	Agilent 6230 time-of-flight LC/MS