

Analyze Challenging Polar Compounds with Confidence

Agilent InfinityLab Poroshell 120, ZORBAX and Polaris columns for Hydrophilic Interaction Chromatography (HILIC)



Rely on Agilent to Build a Robust and Reliable HILIC Workflow

Hydrophilic interaction chromatography (HILIC) is gaining popularity due to its ability to retain and separate small polar analytes using a standard LC system and common reversed-phase solvents. The advantages of this technique include better retention of polar analytes than reversed-phase methods as well as the ability to use MS-compatible eluents with an easy setup. This provides improved ionization and sensitivity in MS mode.

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Sample preparation is the correct starting point for all of your analyses. Whether you use simple filtration, solid-supported liquid extraction (SLE), catch and release cleanup, QuEChERS, or solid-phase extraction (SPE) - Agilent offers a high-quality product for your sample prep needs from award-winning Captiva EMR-Lipid to trusted Bond Elut SPE and QuEChERS kits.

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From routine analysis to cutting-edge research, the InfinityLab LC Series offers the broadest portfolio of HPLC and UHPLC solutions for any application and budget.

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3



Use Agilent InfinityLab supplies to increase throughput and minimize instrument downtime for your HILIC applications:

- Stay Safe caps help to avoid evaporation of harmful solvents and to keep your mobile phase concentration consistent
- Quick Connect and Quick Turn fittings for easy-to-use and hassle-free connections
- Filtration assembly and inline filters to remove particles from buffered mobile phases to extend your column lifetime
- Well plates for high throughput applications
- Smart supplies such as column ID tag, deuterium lamps and flow cells with RFID to enhance traceability and simplify documentation

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The InfinityLab Poroshell 120 portfolio offers three modern HILIC chemistries offering the highest separation efficiency, ruggedness, and reproducible chromatography for your polar LC/MS analyses. Challenged with analytes interacting with metal surfaces? Use Agilent's InfinityLab Deactivator Additive to inactivate metal surfaces within your system. Agilent's ultra-pure LC/MS solvents help minimize MS background.

www.agilent.com/chem/poroshell-HILIC

How do I select my HILIC column?

Agilent offers a broad portfolio of columns designed for modern HILIC separations. Three major hydrophilic interactions exist that enable retention and separation in HILIC mode: ionic interactions, hydrophilic dipol-dipol interactions, and hydrogen bonding. Even though it is advised to screen several stationary phases to identify the right stationary phase, the functional groups of your analyte and its possible interactions with the column can help to indicate a suitable HILIC column that might be a good starting point.

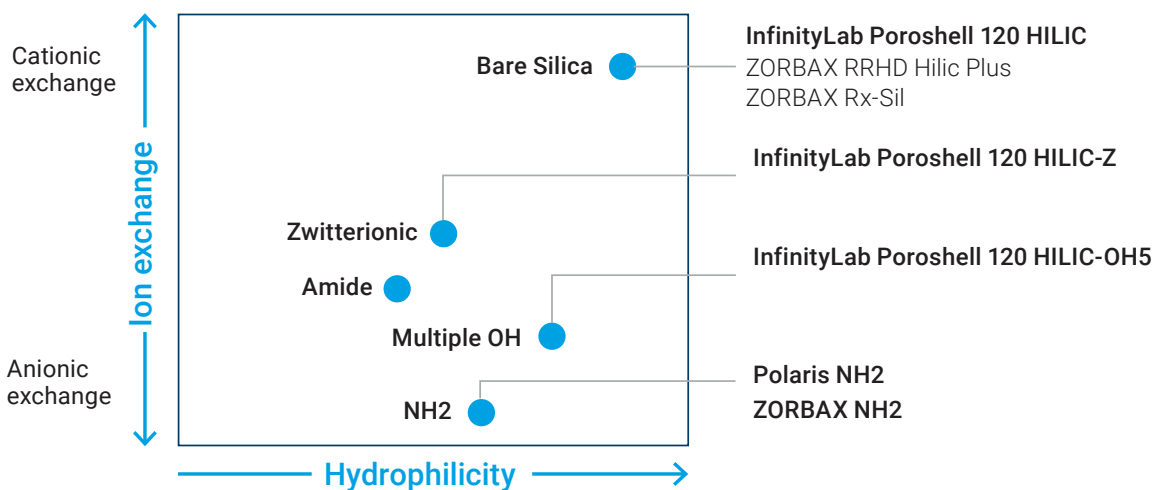


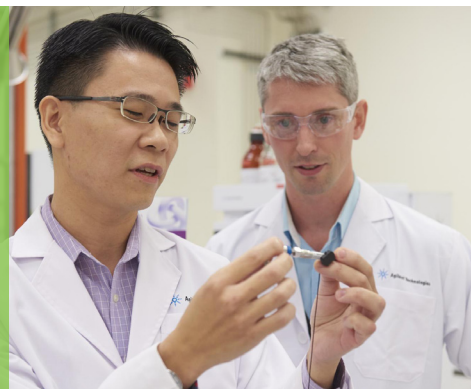
Figure 1. Qualitative map of hydrophilicity and ion exchange capabilities of different Agilent HILIC columns. Data measured according to Ibrahim et al., J.Chrom.A, 1260, (2012).

What is the right mobile phase for my HILIC separation?

Mobile phases are the second most important factor to vary selectivity in HILIC. The aqueous, strong mobile phase is often buffered to carefully select and control retention mechanisms, and prevent peak broadening as well as variations in selectivity due to small pH changes. Identically buffered acetonitrile is most often used as a weak, organic mobile phase.

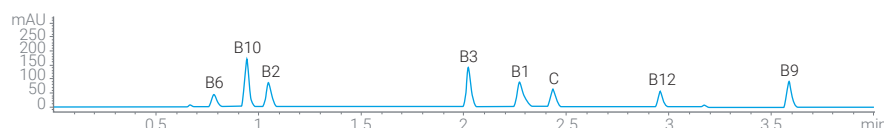
General Considerations	Typical Buffer concentration: 5-30 mM; most common: 10-20 mM Phosphates should be avoided as they have low solubility in high % ACN	
	Buffer and pH	Suitable Stationary Phase
Basic Analytes	Ammonium Formate, pH 3 Ammonium Acetate, pH 4-5	Poroshell 120 HILIC-Z Poroshell 120 HILIC-OH5 Poroshell 120 HILIC ZORBAX HILIC Plus
Mixture of Acidic and Basic Compounds	Ammonium Acetate, pH ~7	Poroshell 120 HILIC-Z Poroshell 120 HILIC-OH5 Poroshell 120 HILIC ZORBAX HILIC Plus
Acidic Analytes	Ammonium Formate, pH 9-10	Poroshell 120 HILIC-Z
Sugars	Ammonium Hydroxide, pH 10-11	Poroshell 120 HILIC-Z

Poroshell 120 HILIC Columns Allow for Fast, Reliable and Reproducible Chromatography

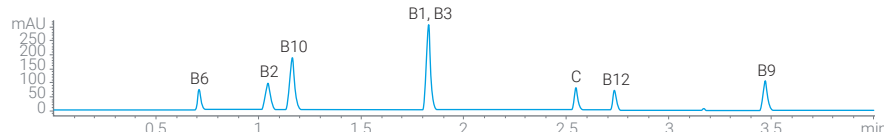


Meet the newest additions to the HILIC world! Agilent offers three unique, orthogonal HILIC phases: bare silica (HILIC), polyhydroxy fructan (HILIC-OH5), and zwitterionic (HILIC-Z). The robustness and reproducibility of HILIC separations on InfinityLab Poroshell columns help to switch from challenging, often MS-incompatible ion pair chromatography and normal phase applications.

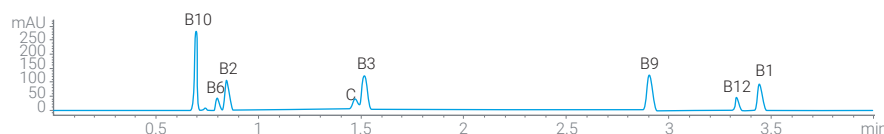
InfinityLab Poroshell 120 HILIC-OH5 2.1 x 100 mm, 2.7 µm



InfinityLab Poroshell 120 HILIC-Z 2.1 x 100 mm, 2.7 µm



InfinityLab Poroshell 120 HILIC 2.1 x 100 mm, 2.7 µm



Conditions:

Mobile phase: A: 100 mM ammonium acetate
+ 0.5% acetic acid (pH ~4.6)
in H₂O

B: CH₃CN 0.5 mL/min, 87% B
for 1 min, 87-50% B in 4 min,
3 min re-equilibration

Flow rate: 1 µL injection, 40 °C

Temperature: 40 °C

Detector: UV detection at 260 nm, 80 Hz

Sample: water-soluble vitamins

Figure 2. InfinityLab HILIC-Z and HILIC-OH5 columns offer a wide range of alternative selectivity for polar analyte separations. This separation of water-soluble vitamins demonstrates selectivity differences across HILIC phases using the same method conditions. Separations can be optimized on each column by altering the method conditions.

Table 1. Selected columns of the Poroshell 120 portfolio that are recommended for HILIC use.

Column Chemistry	Stationary Phase	Particle Size and Dimensions	Pressure Range	Recommended Use
InfinityLab Poroshell 120 HILIC-Z	Zwitterionic	Particle size: 1.9,2.7,4 µm ID: 2.1,3,4,6 mm Length: 50,100,150,250 mm	1300 bar (1.9µm) 600 bar (2.7, 4 µm)	Starting point for HILIC method development. High pH range. PEEK-lined version available.
InfinityLab Poroshell 120 HILIC-OH5	Polyhydroxy fructan phase	Particle size: 2.7 µm ID: 2.1,4,6 mm Length: 50,100,150 mm	400 bar (2.7 µm)	Alternative selectivity with reduced ionic exchange but strong hydrogen bonding character.
InfinityLab Poroshell 120 HILIC	Bare silica	Particle size: 1.9,2.7,4 µm ID: 2.1,3,4,6 mm Length: 50,100,150,250 mm	1300 bar (1.9µm) 600 bar (2.7, 4 µm)	Simple samples that do not contain fairly similar compounds. Ultra-low bleed.

Retain and separate highly charged compounds

InfinityLab Poroshell 120 HILIC-Z columns feature a novel zwitterionic stationary phase bonded to Poroshell 120 particles. Advantages include:

- A PEEK-lined column option for excellent peak shape and recovery of challenging compounds
- High pH stability up to pH 12 at 35 °C; temperature stability to 80 °C at pH 7
- Compatible with MS-friendly buffers (<10 mM salt content)

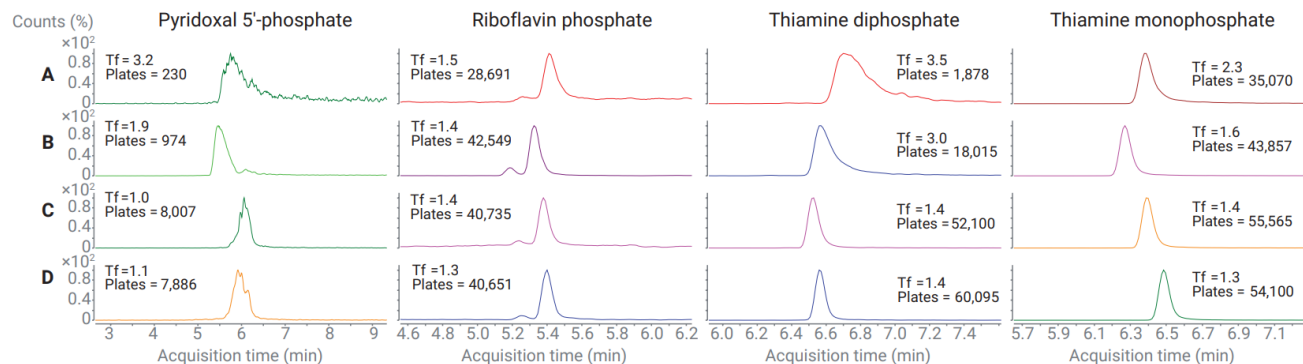


Figure 3. Interactions of phosphorylated metabolites with steel: before and after wash. A) Before system wash; B) After system wash, HILIC-Z column; C) After system wash, HILIC-Z column, with deactivator; D) After system wash, HILIC-Z PEEK-lined column, with deactivator.

Separate polar compounds with alternative selectivity

Separate polar compounds with alternative selectivity: InfinityLab Poroshell 120 HILIC-OH5–Poly-hydroxy fructan phase bonded to Poroshell 120 particles:

- Fast, high-efficiency separations with excellent retention of polar compounds
- Offers alternative selectivity to Poroshell 120 HILIC and HILIC-Z phases

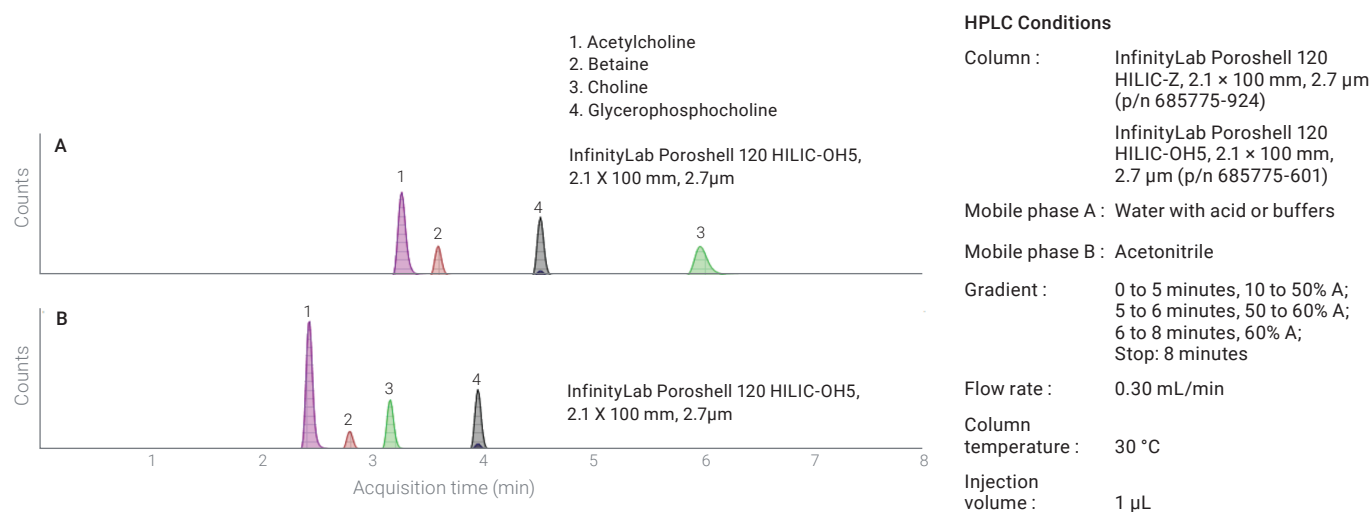
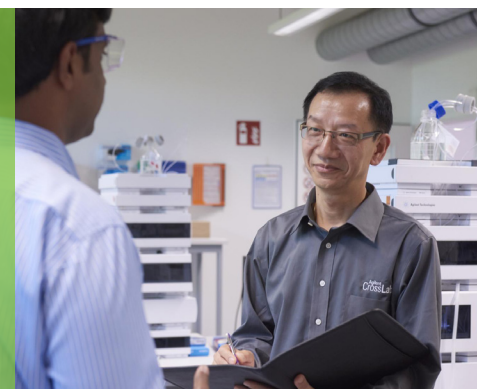
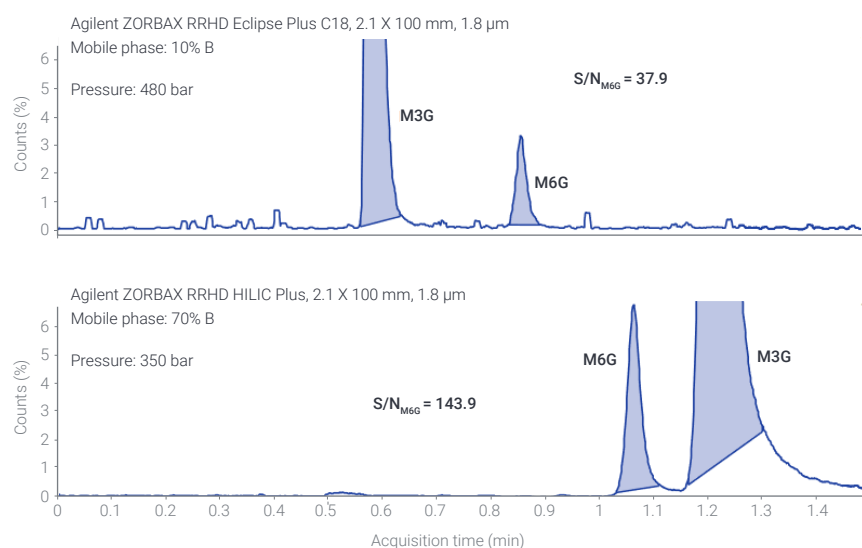


Figure 4. Analysis of Choline Metabolites on Poroshell 120 HILIC-OH 5 and HILIC-Z using LC/MS/MS.

Reliable, Totally Porous Materials for Your HILIC Separations



Agilent ZORBAX and Polaris HILIC columns are designed for hydrophilic interaction chromatography (HILIC) and are a traditional, fully porous alternative for your HILIC method development. Choose from traditional bare silica (Hilic Plus and Rx-Sil) phases as well as from a robust, reliable amino phase (Polaris NH2).



Conditions:

Mobile phase: A: 10 mM ammonium formate pH 3.2 in water
 B: Acetonitrile/100 mM ammonium formate pH 3.2 in water (9:1)
 Flow rate: 0.4 mL/min
 Elution: Isocratic
 Injection: 2 μ L of 1 μ L/ml each of morphine-3- β -D-glucuronide, and morphine-6- β -D-glucuronide
 Columns: 2.1 x 100 mm, 1.8 μ m
 Temperature: 25 $^{\circ}$ C
 MS Source: ESI+, 200 V, 250 $^{\circ}$ C, 11 L/min, 30 psi, 4000 V
 SIM: 462, Frag 170 V
 Agilent publication number 5991-0245

Figure 5. Comparison of RP and HILIC mode using ZORBAX RHHD HILIC Plus. HILIC mode allows for more efficient spraying and desolvation in the ESI-MS source compared to RPLC, generating less baseline noise and a more intense MS signal and improved sensitivity.

Table 2. Selection of the ZORBAX and Polaris columns recommended to be used in HILIC mode.

Column Chemistry	Stationary Phase	Particle Size and Dimensions	Pressure Range	Applications
ZORBAX HILIC Plus	Bare silica	Particle size: 1.8 , 3.5 μ m ID: 2.1, 4.6 mm Length: 50, 100 mm	1200 bar (1.8 μ m) 400 bar (3.5 μ m)	Universal bare silica material for samples with low complexity.
ZORBAX Rx-Sil	Bare silica	Particle size: 1.8, 5 μ m ID: 2.1 ,3, 4.6 mm Length: various (50-250 mm)	600 bar (1.8 μ m) 400 bar (5 μ m)	Bare silica material with higher retention and different selectivity.
Polaris NH2	Amino	Particle size: 3, 5 μ m ID: 2.1 , 4.6 mm Length: 50, 150, 250 mm	400 bar (3 μ m) 400 bar (5 μ m)	Alternative selectivity with strong anionic exchange properties for acidic compounds.

"Before we obtained the Poroshell 120 HILIC-Z, we encountered often issues with a competitor's predecessor. Poor peak shapes and RT shifts were not uncommon. As soon as we developed in-house methods with HILIC-Z, our peak shapes improved rapidly as well as more stable RTs were obtained. Also, a better limit of quantitation was obtained due to sharp peaks and better separation of the analytes and matrix interferences. When analyzing polar pesticides with this column, analysts feel more comfortable and confident than ever before!"

– **Elisa Platjouw**,
Analytical Scientist, Eurofins Netherlands



Selected applications that highlight Agilent's HILIC columns

Application	Market	Column Used	Application Note Title	Publication Number
A comparison of RP-LC and HILIC	All markets	Multiple	Retaining and separating Polar Molecules—a detailed investigation of when to use HILIC versus a reversed-phase LC column.	5994-1137EN
Beginner's guide to HILIC method development	All markets	Poroshell 120 HILIC Poroshell 120 HILIC-OH5 Poroshell 120 HILIC-Z	Hydrophilic interaction. Chromatography method. Development and troubleshooting.	5991-9271EN
Overview of HILIC used in LC/MS/MS analyses	All markets	Poroshell 120 HILIC-Z ZORBAX RRHD HILIC Plus	Application of Novel HILIC Column configurations to improve LC/ESI/MS sensitivity of metabolites.	ASMS 2018 Poster WP-536
Underivatized amino acids in complex matrix	Food and Agriculture	Poroshell 120 HILIC-Z	Analysis of amino acids in animal feed matrices using the Ultivo Triple Quadrupole LC/MS System.	5994-0586EN
Water-soluble vitamins	Food and Agriculture	Poroshell 120 HILIC-OH5	Analysis of water-soluble vitamins on an Agilent InfinityLab Poroshell 120 HILIC-OH5 Column.	5991-8780EN
Separations of nucleotides	Biopharma	Poroshell 120 HILIC-Z (PEEK-lined)	HPLC-DAD analysis of nucleotides using a fully inert flow path.	5994-0680EN

More application notes can be found with [Agilent LC Application finder](#).

Reliable efficient, always innovating for your best result



You can rely on Agilent InfinityLab LC instruments, columns, and supplies to deliver rugged quality and robust analytical results. But our promise to you does not stop there. Every component of the Agilent InfinityLab LC family is uniquely designed to work together and to help you continuously improve your workflow, for efficiency gains that help you get more done and reduce operational costs.

Learn more about the InfinityLab Poroshell portfolio at

www.agilent.com/chem/Poroshell-HILIC

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[If applicable, place proper regulatory statement here]

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