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Application of Novel HILIC Column Configurations to Improve LC/ESI/MS Sensitivity of Metabolites

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Introduction

Abstract

Hydrophilic interaction chromatography (HILIC) is known in liquid chromatography for its ability to retain and separate polar analytes. In HILIC mode, organic solvents make up the weak mobile phase, while water is the strong eluting solvent. The resulting mobile phase is organic-rich and more volatile than RPLC mobile phase, giving HILIC an advantage with LC/ESI/MS. More efficient spraying and desolvation in the ESI/MS source generates taller analyte peaks and reduces baseline noise for a more sensitive analysis.

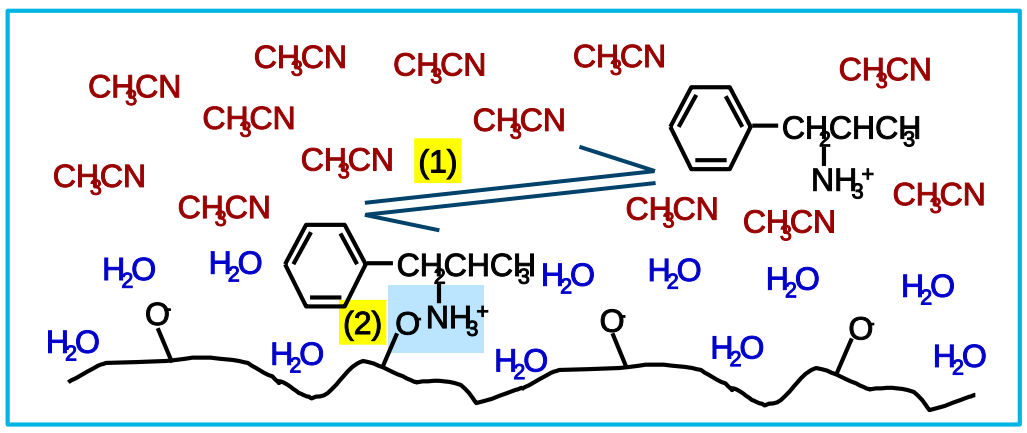
Unique HILIC column configurations provide additional improvements to LC/MS. Novel HILIC phases on superficially porous particles provide high efficiency and orthogonal selectivity to resolve difficult compounds, while inert PEEK-lined stainless steel hardware ensures more sensitivity and reproducibility for sticky analytes under UHPLC conditions.

Novel Aspect

Novel HILIC phases on superficially porous particles give fast separation of metabolites, including isobars, while improving method sensitivity over RPLC.

How HILIC Works

Possible Retention Mechanisms on a Silica-Based HILIC Column:



- A water layer is adsorbed onto the polar silica surface, creating a liquid/liquid extraction system
- Polar analytes can partition into and out of the water layer, with more polar analytes having a stronger interaction (1)
- Charged polar analytes can also undergo ion exchange with the silica surface (2)
- Elution is typically from least to most polar, opposite of RPLC
- Solvent strengths in HILIC mode are:
  - THF < acetone < acetonitrile < isopropanol < ethanol < methanol < water

Experimental

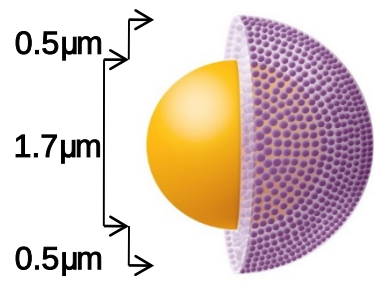
Methods

LC/ESI/MS analyses of various metabolites are used to systematically compare MS sensitivity with RPLC and HILIC. Additional MS sensitivity is gained with the use of a PEEK-lined HILIC column on an inert LC system. A UHPLC system with a triple quadrupole mass spectrometer was used. Several superficially porous particle columns are used, including two novel HILIC stationary phases: a zwitterionic phase and a poly-hydroxy fructan phase. The importance of selecting the correct stationary phase is demonstrated by resolving several isobaric pairs.

Columns

Superficially porous particle HILIC columns are used throughout this work.

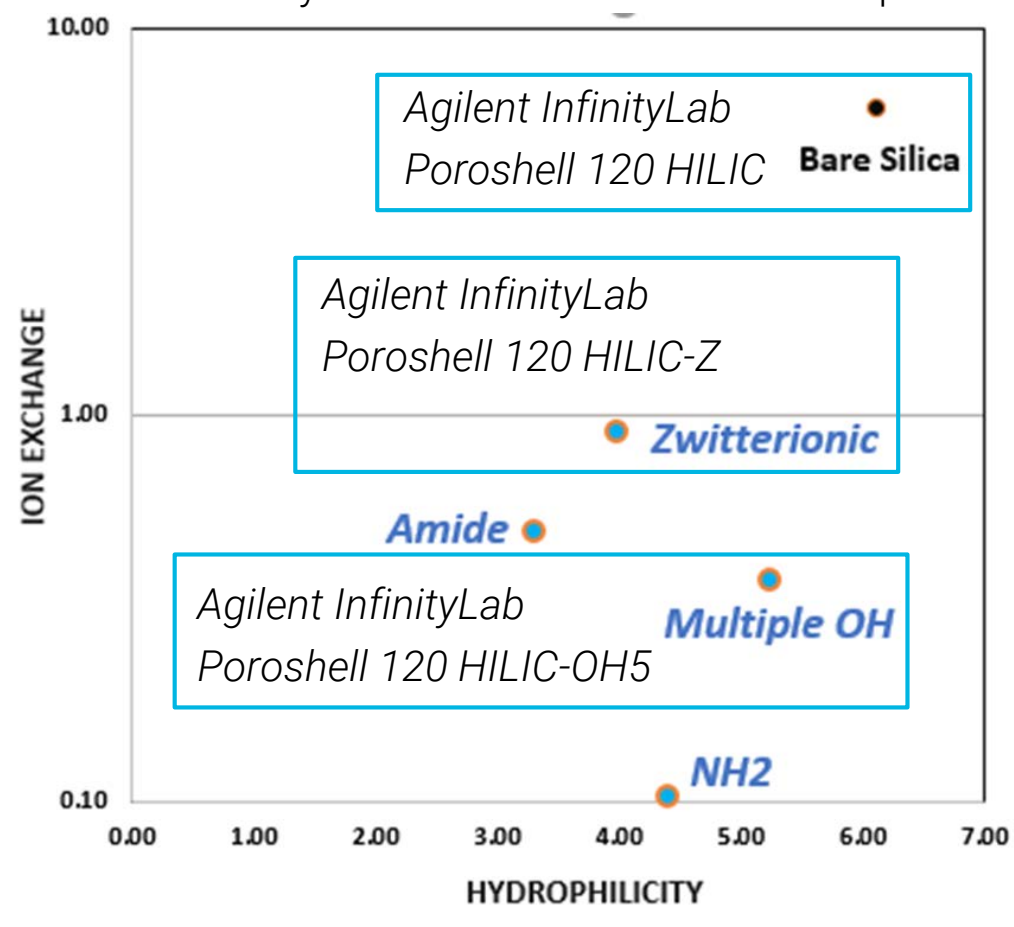
Structure of a 2.7 µm Agilent InfinityLab Poroshell 120 particle:



- Efficiency is 90% of sub-2-µm totally porous particle columns
- Pressure is 50% of sub-2-µm totally porous particle columns
- 2 µm inlet frit to reduce clogging with dirty samples

Agilent offers three HILIC phases on 2.7 µm InfinityLab Poroshell 120 particles.

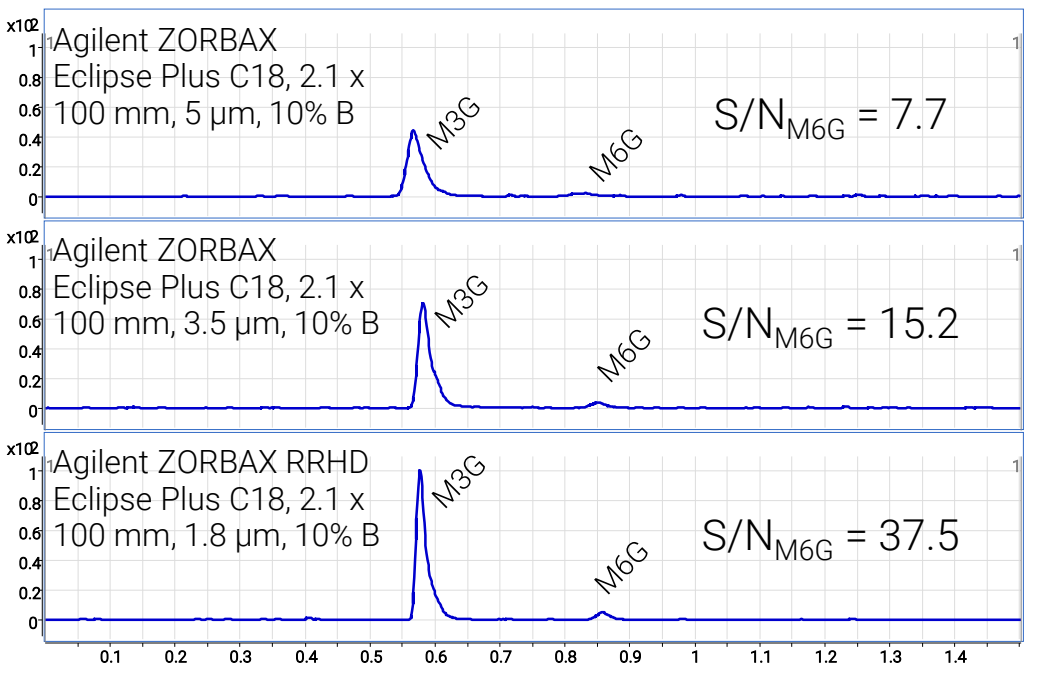
The availability of orthogonal HILIC phases, allows for varied selectivity and facilitates method development.



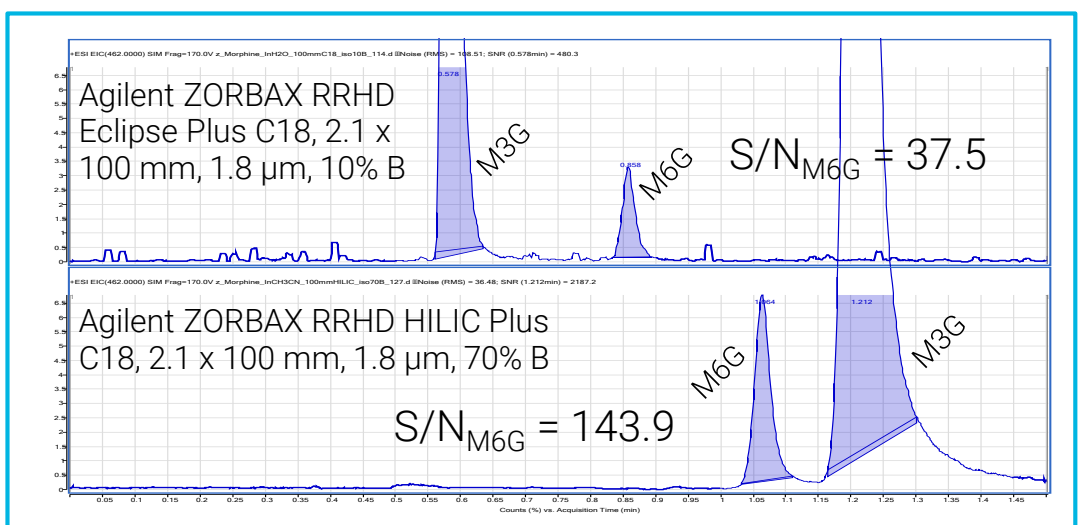
HILIC and UHPLC can Improve LCMS Sensitivity over RPLC

Chromatographic separation of morphine metabolites (morphine-3-β-D-glucuronide [M3G], and morphine-6-β-D-glucuronide [M6G]) is necessary because they are isobaric compounds. Signal-to-noise calculations for the least sensitive peak, M6G, are used as a metric to compare LC/ESI/MS sensitivity.

Method sensitivity can be increased five-fold by exchanging a 5 µm column for a 1.8 µm column.



Method sensitivity can be increased four-fold by exchanging a C18 for a HILIC column. The HILIC column produces a taller peak, while also reducing baseline noise.

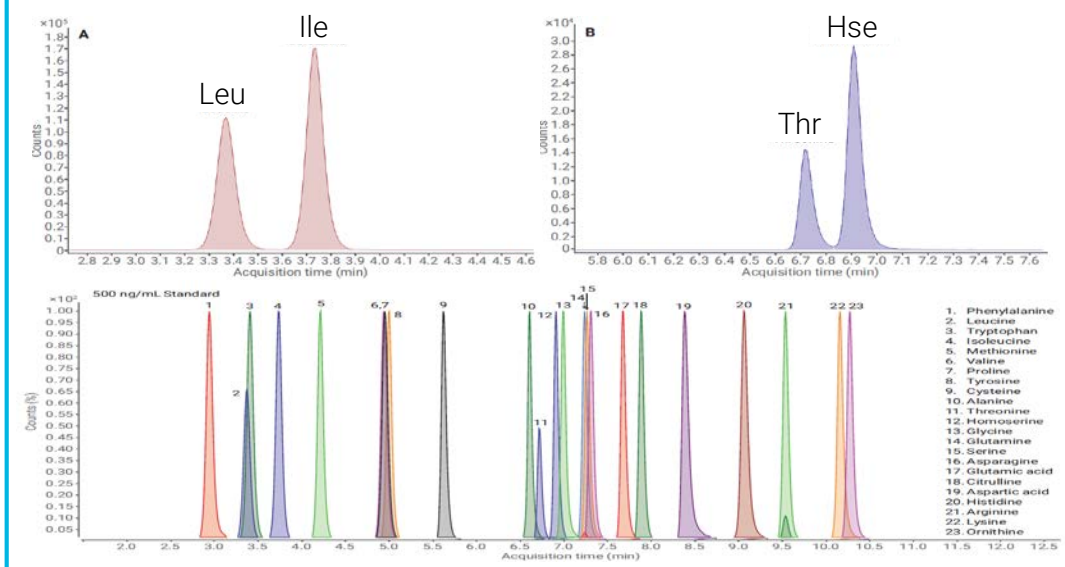


Agilent 1290 Infinity LC System, Agilent 6410A LC/MS, A: 10 mM ammonium formate pH 3.2, B: acetonitrile / 100 mM ammonium formate pH 3.2 (9:1), 0.4 mL/min, Isocratic elution, Injection Volume: 2 µL, Column: 25 °C, MS: ESI+, SIM, 250 °C, 11 L/min, 30 psi, 4000 V, 200 V delta EMV, 20 ms dwell time, Sample: Normorphine, m/z 272, Morphine, m/z 286, Morphine-6-β-D-glucuronide (M6G), m/z 462, Morphine-3-β-D-glucuronide (M3G), m/z 462; Agilent App Note 5991-0245EN

Transferring this morphine metabolite analysis from a traditional 5 µm RPLC column to a UHPLC 1.8 µm HILIC column improves LC/MS sensitivity by a factor of 20.

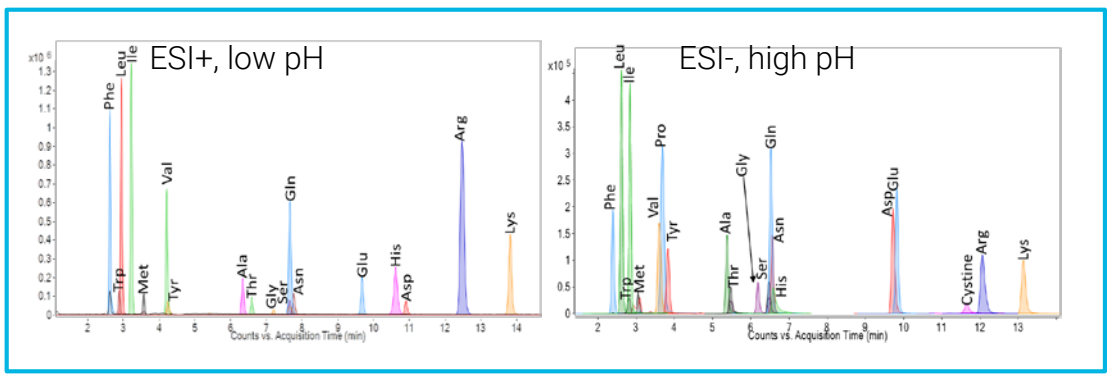
Chromatographic Resolution of Isobars

The utility of HILIC columns to improve LC/MS method performance is further shown by chromatographically resolving several pairs of amino acid isobars.



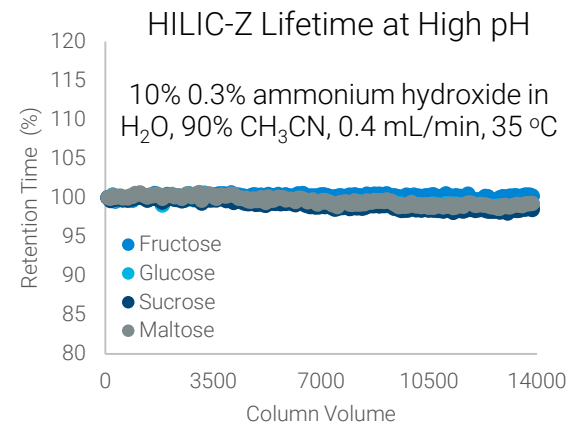
Column used was 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z; A: 20 mM Ammonium Formate pH 3.0 in water, B: 20 mM Ammonium Formate pH 3.0 in acetonitrile/water (9:1), 100-70% B in 11.5 minutes, 0.5 mL/min, 1 µL injection, 25C, MS Source: ESI+, 330C, 13 L/min, 35 psi, 390C, 12 L/min, 1500 V, dMRM; Agilent App Note 5991-8922EN

Amino acids can be analyzed at high or low pH. Generally positive ion mode (low pH) will be more sensitive, though which mode is used may also depend on what other analytes need to be monitored simultaneously.



High pH Stability with Agilent InfinityLab Poroshell 120 HILIC-Z

InfinityLab Poroshell 120 HILIC-Z is a novel zwitterionic phase. Its hybrid particle technology improves overall particle ruggedness at extended pH, enabling long lifetimes and fewer column changes.



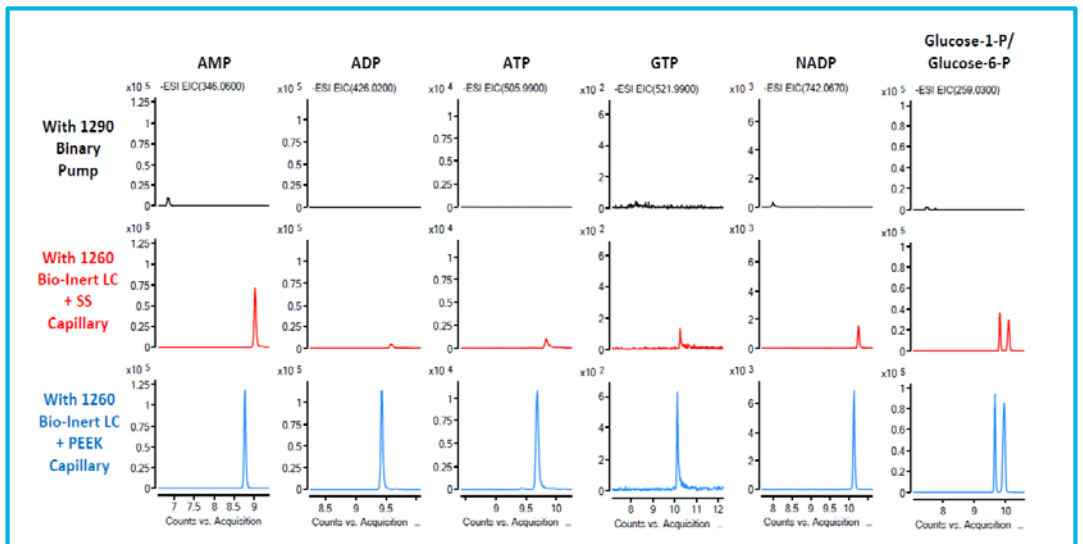
- High pH and temperature stable: Up to pH 12 and 80 °C
- Tolerates samples with high salt or buffer content
- High peak capacity and wide polarity range

Results and Discussion

Inert LC Systems and Columns

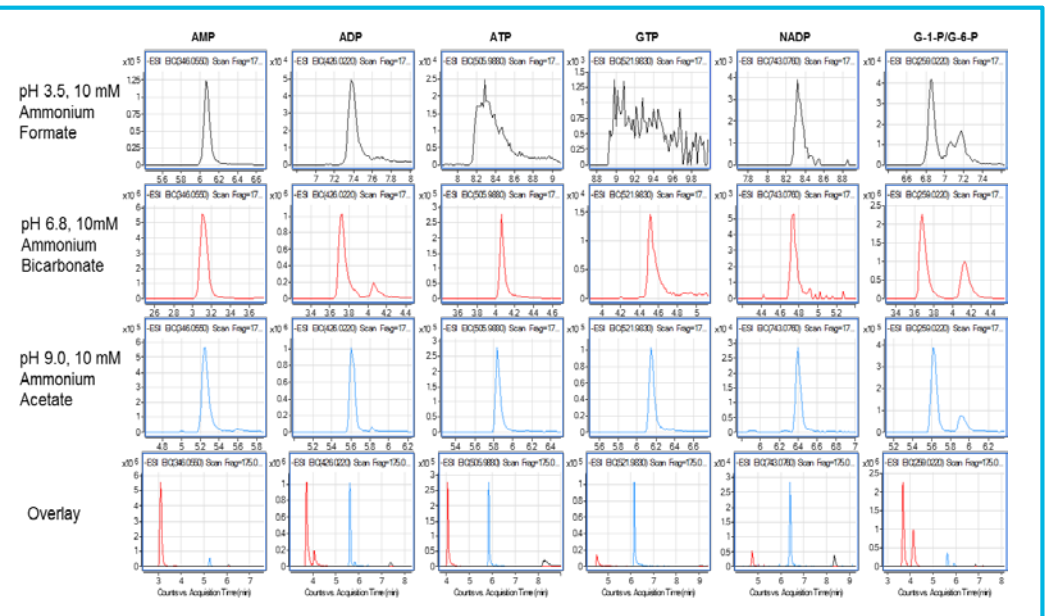
Additional gains in method sensitivity are possible with the use of an inert LC system and column. The stainless steel in the LC system flow path is replaced with PEEK and titanium, while the HILIC column is made with PEEK-lined stainless steel hardware. Reactive nucleotide phosphates show significant improvement in signal-to-noise with a fully inert LC setup.

Using an Agilent Bio-inert LC System with PEEK capillaries combined with a PEEK-lined Agilent InfinityLab Poroshell 120 HILIC-Z column can improve metabolite analysis, pH 6.8, 40 °C



Column used was 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (PEEK-lined stainless steel); A: 10 mM Ammonium Formate pH 6.8 in water, B: acetonitrile + 10 mM Ammonium Formate pH 6.8, 95-30% B in 10 minutes, 0.25 mL/min, 0.2 µL injection (5 ng each on column), MS Source: ESI-, m/z 191.02, 346.06, 426.02, 505.99, 521.99, 742.067, 743.067, 259.03

Additional improvements can be made to metabolite analytical sensitivity by choosing an appropriate mobile phase buffer pH, metabolites shown are at 5 ppm

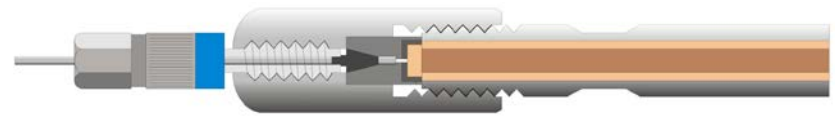


Column used was 2.1 x 100 mm, 2.7 µm Agilent InfinityLab Poroshell 120 HILIC-Z (PEEK-lined stainless steel); A: buffer varies, see figure above, B: acetonitrile + buffer varies, see figure above, 95-30% B in 10 minutes, 0.25 mL/min, 0.2 µL injection (5 ng each on column), MS Source: ESI-, m/z 191.02, 346.06, 426.02, 505.99, 521.99, 742.067, 743.067, 259.03

PEEK-Lined Stainless Steel Column Hardware

Agilent offers the InfinityLab Poroshell 120 HILIC-Z in PEEK-lined columns for added sensitivity with sticky compounds. The hardware is a PEEK-lined stainless steel with PEEK-coated titanium frits.

- The PEEK liner allows for a metal free flow path. The PEEK polymer's chemical inertness ensures the integrity of your samples by minimizing unwanted surface interactions, and also allowing operation under harsh solvent or pH conditions.
- The stainless steel provides strong walls for high pressure usage within the UHPLC realm.



For Best Results, Use the Full InfinityLab Bio-inert Solution:

- InfinityLab Bio-inert LC System
- Bio-inert quick connect heat exchanger, PN: G7116-60009
- All Agilent PEEK/SST Bio-inert capillaries with Quick Turn fitting (5067-5966) or UHP-FF fitting Bio-inert (5067-5695)
- Please note that the one piece PEEK finger tight fittings should not be used with the PEEK-lined columns. The combined compression and rotation may cause internal damage to the column. Only fittings with ferrules should be used.

Conclusions

- HILIC can improve the analysis of small polar metabolites compared to reversed-phase LC
  - HILIC gives better retention for polar molecules
  - HILIC mobile phases are more volatile, allowing for better MS sensitivity
- Orthogonal HILIC phases allow for easier method development and resolution of isobars
- Agilent InfinityLab Poroshell 120 HILIC-Z is high pH stable for more method and mobile phase flexibility
- PEEK-lined stainless steel hardware can provide additional gains in method sensitivity when combined with an inert LC system

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