

# Evaluation of Highly Stable Zwitterionic HILIC Columns Based on Hybrid Organic/Inorganic Particles

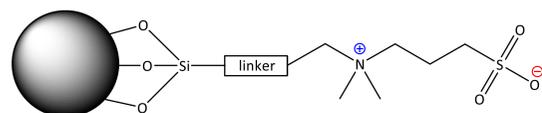
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## Introduction

- Hydrophilic Interaction Chromatography (HILIC) is widely used for separating polar analytes, such as those encountered in many metabolomics studies.
- Among HILIC stationary phases, zwitterionic materials employing sulfobetaine groups have proven to have the broadest utility.
- Many existing sulfobetaine columns suffer from poor stability in basic mobile phases, high batch-to-batch variability and/or low recoveries of metal-sensitive analytes.
- To address these challenges, we developed a new sulfobetaine stationary phase on ethylene-bridged hybrid (BEH™) organic/inorganic particles and packed it into column hardware modified using MaxPeak™ Premier Technology.
- Here, we developed a targeted LC/MS/MS method for 27 challenging metabolites using a pH 9 buffered mobile phase under gradient conditions



Structure of the zwitterionic sulfobetaine stationary phase for Atlantis™ BEH Z-HILIC

## Experimental

See Waters application notes 720007311, "Introducing Atlantis BEH Z-HILIC: a Zwitterionic Stationary Phase Based on Hybrid Organic/Inorganic Particles", July 2021 and 720000xxxxx "Separation of Pentose Phosphate Pathway, Glycolysis, and Energy Metabolites using an ACQUITY Premier System with an Atlantis Premier BEH Z-HILIC Column", Oct. 2021

## Results

### LC/MS/MS Chromatograms of Pentose Phosphate Pathway, Glycolysis, and Energy Metabolites

1	Pyruvic acid
2	Lactic acid
3	Deoxyribose 5-phosphate
4	Dihydroxyacetone phosphate
5	Ribulose 5-phosphate/Xylulose 5-phosphate
6	Ribose 5-phosphate
7	Glyceraldehyde 3-phosphate
8	Sedoheptulose 7-phosphate
9	Fructose 6-phosphate
10	2-Phosphoglyceric acid
11	3-Phosphoglyceric acid
12	Phosphoenolpyruvic acid
13	Glucose 6-phosphate
14	6-Phosphogluconic acid
15	Fructose 1,6-Bisphosphate
16	Flavin Adenine Dinucleotide
17	Acetyl Coenzyme A
18	Dihydrnicotinamide Adenine Dinucleotide
19	Adenosine Monophosphate
20	Nicotinamide Adenine Dinucleotide
21	Adenosine Diphosphate
22	Guanosine Monophosphate
23	Adenosine Triphosphate
24	Dihydrnicotinamide Adenine Dinucleotide Phosphate
25	Nicotinamide Adenine Dinucleotide Phosphate
26	Guanosine Diphosphate
27	Guanosine Triphosphate

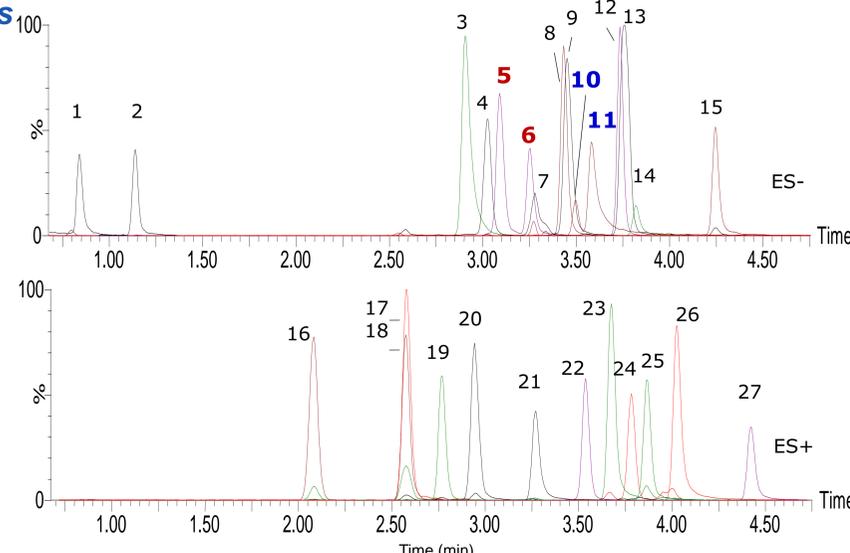


Figure 1. Representative chromatograms of 27 pentose phosphate pathway, glycolysis, and energy metabolites under pH 9 mobile phase conditions using an Atlantis Premier BEH Z-HILIC 1.7 μm 2.1 x 100 mm column  
**Note:** isobaric pairs ribulose 5-phosphate/ribose-5-phosphate and 2-phosphoglyceric acid/3-phosphoglyceric acid are well resolved

### Reproducibility

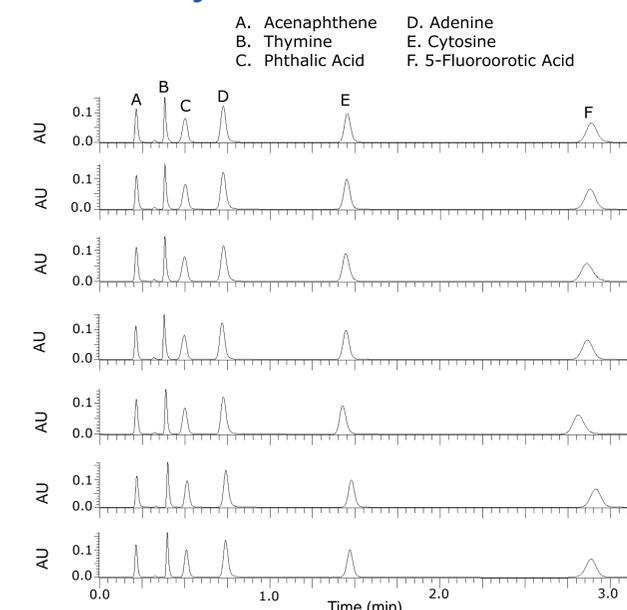


Figure 2: Chromatograms comparing the separation of a mixture of acids, bases and neutrals for seven different batches of Atlantis Premier BEH Z-HILIC 1.7 μm material

### High pH Chemical Stability

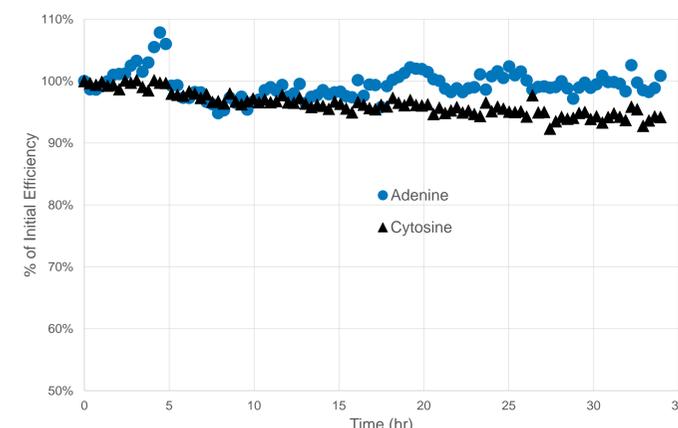


Figure 3: Stability at pH 11/70°C for an Atlantis Premier BEH Z-HILIC 1.7 μm 2.1 x 50 mm column

### Improved Peak Shape for Metal Sensitive Analytes

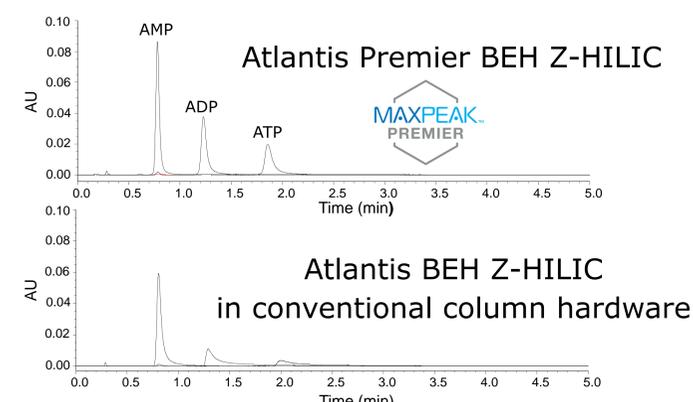


Figure 4: Performance for metal-sensitive analytes for 1.7 μm Atlantis BEH Z-HILIC in MaxPeak Premier vs conventional column hardware

## Conclusions

- Atlantis Premier BEH Z-HILIC columns combine:
  - Strong retention of polar compounds
  - Stability from pH 2 – 10
  - High efficiency
  - Excellent batch-to-batch reproducibility
  - Excellent peak shape for metal-sensitive analytes
- This combination of attributes makes Atlantis Premier BEH Z-HILIC columns particularly well-suited for polar metabolomics assays.