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INTRODUCTION

Hydrophilic interaction chromatography (HILIC) is a chromatographic technique that has been used to improve the retention of very polar species that are poorly retained in reversed-phase HPLC (RP-HPLC). HILIC offers several benefits over RP-HPLC with regards to MS response and simplification of sample preparation methods. Due to the highly organic (> 80%) mobile phase utilized in HILIC, signal intensity in electrospray MS is improved through efficient mobile phase desolvation and compound ionization. Additionally, sample clean-up by protein precipitation or SPE can be directly analyzed without solvent evaporation and reconstitution, reducing sample handling steps and increasing the number of samples that can be analyzed. In addition, 1.7 μm ACQUITY UPLC® BEH HILIC columns use the same BEH particle technology as XBridge™ HILIC HPLC columns. Therefore, scalability between HPLC and UPLC is easily achieved.

EXPERIMENTAL CONDITIONS

- System: Waters ACQUITY UPLC system with PDA detector and TQD mass spectrometer
- Columns: ACQUITY UPLC BEH HILIC column, 2.1 x 100 mm, 1.7 μm (Part Number: [186003461](#))
ACQUITY UPLC BEH C₁₈ column, 2.1 x 100 mm, 1.7 μm (Part Number: [186002352](#))
XBridge C₁₈, 2.1 x 100 mm, 3.5 μm (Part Number: [186003022](#))
XBridge HILIC, 2.1 x 100 mm, 3.5 μm (Part Number: [186004433](#))
Atlantis® HILIC, 2.1 x 100 mm, 3 μm (Part Number: [186002013](#))
- SPE: Oasis® MCX $\mu\text{Elution}$ 96-well Plate (Part Number: [186001830BA](#))

RESULTS & DISCUSSION

BEH particles exhibit chemical stability superior to that of silica particles. The performance of the silica-based column deteriorated rapidly after ~ 800 injections, whereas the BEH column showed no change in chromatographic performance in HILIC mode over the course of 2,000 injections.

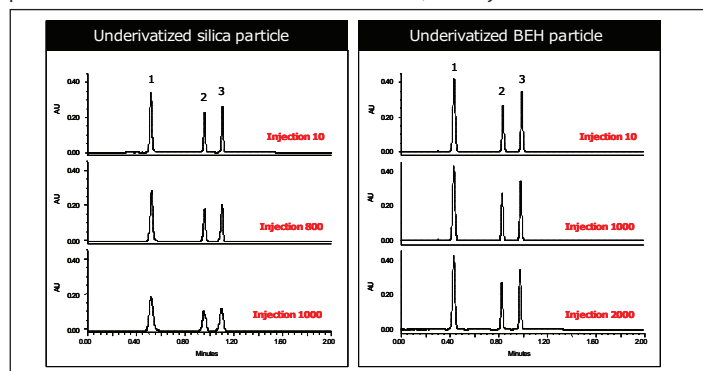


Figure 1. Chemical stability of silica and BEH HILIC columns at pH 5.0.

HILIC mobile phases contain a high amount of organic solvent, dramatically improving desolvation efficiency, resulting in higher MS signal intensity.

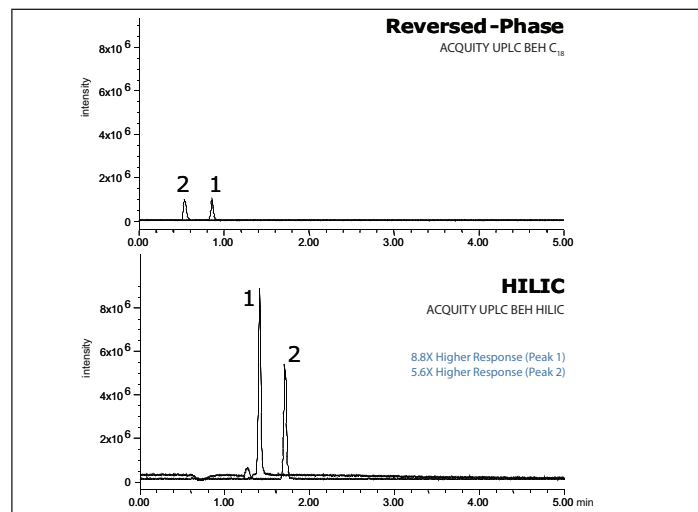


Figure 2. Comparison of ESI+ MS response in HILIC- and RP-UPLC for (1) acetylcholine and (2) choline.

CONCLUSIONS

Novel BEH particles were found to be useful for HILIC separations. They exhibited chemical resistance superior to that of silica particles at moderate pH over the course of 2,000 injections. Adding small amounts of alternative polar solvents enhanced retention of highly polar compounds. HILIC offers complementary selectivity and was found to yield up to 10-fold improvement in ESI-MS response when compared to RP-HPLC. Separations were found to be directly transferable between XBridge HILIC and ACQUITY UPLC BEH HILIC columns. Finally, direct analysis of SPE eluates is possible by HILIC without the need for dilution or time-consuming evaporation and reconstitution.

ORDERING INFORMATION

Products Used in this Application	Part Number
ACQUITY UPLC BEH HILIC column, 2.1 x 100 mm, 1.7 μm	186003461
ACQUITY UPLC BEH C ₁₈ column, 2.1 x 100 mm, 1.7 μm	186002352
XBridge C ₁₈ , 2.1 x 100 mm, 3.5 μm	186003022
XBridge HILIC, 2.1 x 100 mm, 3.5 μm	186004433
Atlantis HILIC, 2.1 x 100 mm, 3 μm	186002013
Oasis MCX $\mu\text{Elution}$ 96-well Plate	186001830BA

FOR COMPLETE UPLC APPLICATION NOTE, VISIT WWW.WATERS.COM: [720002711EN](#)