APPLICATION OF NOVEL ETHYLENE BRIDGED HYBRID PARTICLES FOR HYDROPHILIC INTERACTION CHROMATOGRAPHY

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INTRODUCTION

Hydrophilic interaction chromatography (HILIC) is a chromatographic technique that has been used to improve retention of very polar species that retain poorly in reversed-phase. This is achieved by utilizing a high organic, low aqueous mobile phase in combination with a polar stationary phase. Combining this chromatographic technique with highly efficient, 1.7 µm UPLC ethylene bridged hybrid (BEH) particles results in faster methods that exhibit improved polar retention, higher sensitivity, enhanced chromatographic resolution and significantly improved column lifetime. Chromatographers can, therefore, meet the challenges of developing separations that completely characterize the constituents of samples.

HILIC offers several benefits over reversed-phase chromatography with regards to MS response and simplification of sample preparation methods. Due to the highly organic (> 80%) mobile phase utilized in HILIC, sensitivity in electrospray MS is improved through efficient mobile phase desolvation and compound ionization.[1] Additionally, sample clean-up by protein precipitation or SPE can be directly analyzed without solvent evaporation and reconstitution, reducing sample handling steps and greatly improving the number of samples that can be analyzed.[2] In addition, 1.7 µm ACQUITY UPLC® BEH HILIC columns are built on the same BEH particle technology as XBridge™ HILIC HPLC columns. Therefore, scalability between HPLC and UPLC is easily achieved.

BEH HILIC PARTICLE TECHNOLOGY

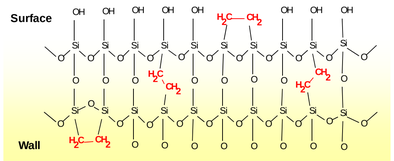

Figure 1. Ethylene Bridged Hybrid particle substrate used in both XBridge™ and ACQUITY UPLC® BEH HILIC columns. This substrate contains approximately one third the number of silanol groups found in a 100 % silica particle substrate.

RETENTION BEHAVIOR

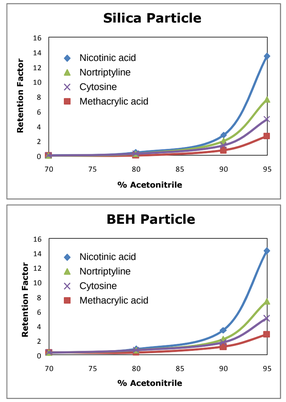

Figure 2. HILIC retention as a function of % ACN on silica and BEH particles. Mobile phase was 10 mM CH_3COONH_4 with 0.02% acetic acid (pH 5.0) and varying amounts of ACN. Flow rate was 0.5 mL/min. UV @ 210 nm. Silica column was Atlantis® HILIC, 2.1 x 50 mm, 3 µm. BEH column was ACQUITY UPLC® BEH HILIC, 2.1 x 50 mm, 1.7 µm. Sample diluent was 75:25 ACN:MeOH.

CHEMICAL STABILITY

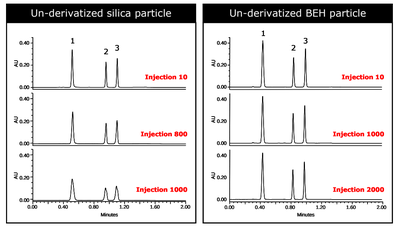

Figure 3. Chemical stability of silica and BEH HILIC columns at pH 5.0. Mobile phase A was 95:5 ACN:H_2O with 10 mM CH_3COONH_4 and 0.02% acetic acid (pH 5.5). Mobile phase B was 50:50 ACN:H_2O with 10 mM CH_3COONH_4 and 0.02% acetic acid. The gradient was from 1-99% B in 2 minutes. Flow rate was 0.5 mL/min. UV @ 254 nm. Peaks: 1 = uracil, 2 = 5-fluorocytosine, 3 = cytosine. Silica column was Atlantis® HILIC, 2.1 x 50 mm, 3 µm. BEH column was XBridge™ HILIC, 2.1 x 50 mm, 3.5 µm. Sample diluent was 75:25 ACN:MeOH.

1.7 µm BEH HILIC EFFICIENCY

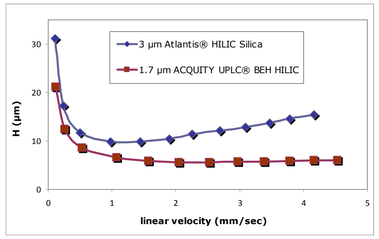

Figure 4. van Deemter curves for 3 µm HILIC silica and 1.7 µm BEH HILIC. Mobile phase was 90:10 ACN:H_2O with 10 mM NH_4COOH and 0.125% formic acid (pH 3.0). UV detection @ 254 nm. Test probe = cytosine. Sample diluent was 75:25 ACN:MeOH.

IMPACT OF ELUTION SOLVENT

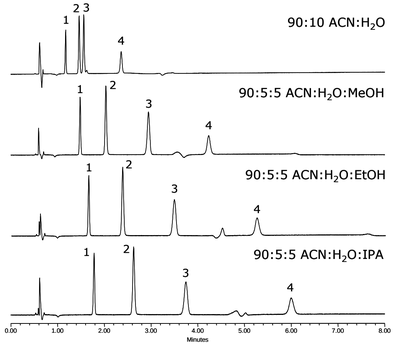

Figure 5. HILIC retention using different elution solvents. A concentration of 10 mM CH_3COONH_4 and 0.02% acetic acid (pH 5.0) was maintained in a 90% ACN mobile phase, and 5% of the aqueous content as substituted with MeOH, EtOH, or IPA. Flow rate was 0.5 mL/min. UV @ 210 nm. Peaks: 1 = methacrylic acid, 2 = cytosine, 3 = nortriptyline, 4 = nicotinic acid. Separation was performed on an ACQUITY UPLC® BEH HILIC column, 2.1 x 100 mm, 1.7 µm. Sample diluent was 75:25 ACN:MeOH.

ENHANCED RETENTION OF POLAR COMPOUNDS

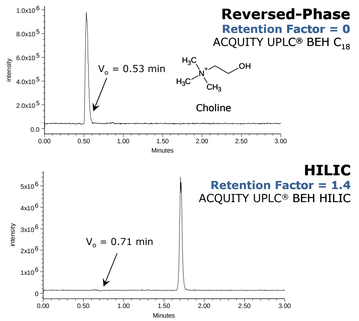

Figure 6. Retention comparison between HILIC and RP in UPLC. Isocratic mobile phase of 10% 10 mM NH_4COOH with 0.125% formic acid in H_2O (pH 3.0), 90% 10 mM NH_4COOH with 0.125% formic acid in 90:5:5 ACN:MeOH:H_2O. Flow rate was 0.5 mL/min. Detection performed using MS in SIR mode (m/z 103.9). Sample diluents were 75:25 ACN:MeOH with 0.2% formic acid for HILIC, and H_2O with 0.2% formic acid for RP-HPLC.

ENHANCED SENSITIVITY IN MASS SPECTROMETRY

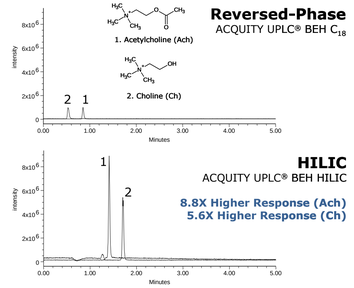

Figure 7. Comparison of ESI+ MS response in HILIC and RP UPLC. Isocratic mobile phase of 10% 10 mM NH_4COOH with 0.125% formic acid in 90:5:5 ACN:MeOH:H_2O. Flow rate was 0.5 mL/min. Detection performed using MS in SIR mode (m/z 146.2 for acetylcholine and m/z 103.9 for choline). Sample diluents were identical to Figure 6.

DIRECT SCALABILITY BETWEEN UPLC AND HPLC

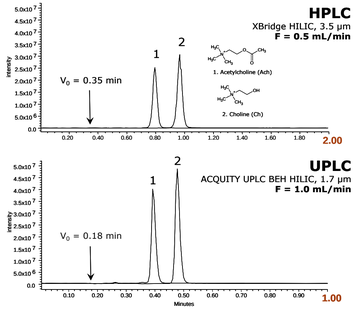

Figure 8. Scalability between HPLC and UPLC HILIC. Conditions identical to those in Figure 7.

ALTERNATE SELECTIVITY TO REVERSED-PHASE

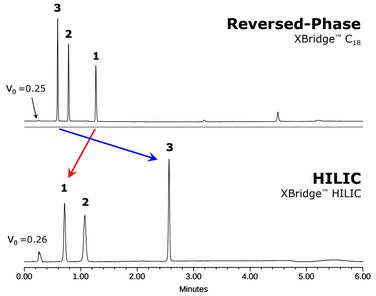

Figure 9. Selectivity differences between HILIC and RP-HPLC. For HILIC, mobile phase A was 10mM NH_4COOH in H_2O (pH 3.0), 0.125% formic acid in 50:50 ACN:H_2O. Mobile phase B was 10mM NH_4COOH in H_2O, 0.125% formic acid in 90:10 ACN:H_2O. Initial hold at 99.9% B for 1.05 min, followed by a linear gradient was from 99.9% to 0.1% B in 3.3 min. Flow rate was 0.6 mL/min, UV detection @ 280 nm, 30 °C. Each compound was present at 25 µg/mL. Peaks: 1 = 6-acetylmorphine, 2 = morphine, 3 = morphine-3β-D-glucuronide. Sample diluent was identical to Figure 6 for HILIC. Sample diluent for RP-HPLC was H_2O with 0.2% formic acid.

COMPETITIVE EVALUATION

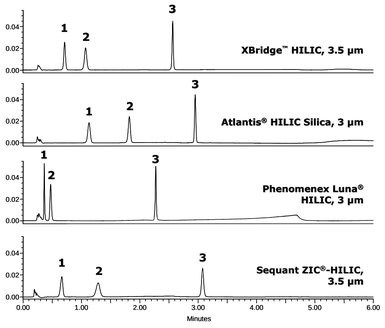

Figure 10. Comparison of different HILIC columns for the separation of 6-acetylmorphine (1), morphine (2), and morphine-3β-D-glucuronide (3). Conditions identical to those in Figure 9.

CONCLUSIONS

- **BEH particles exhibit similar retention to silica particles for HILIC separations.**
- **BEH particles exhibit superior chemical resistance over silica particles at moderate pH over the course of 2,000 injections.**
- **1.7 µm BEH HILIC columns give high resolution separations of polar compounds without compromising speed.**
- **Using alternative polar modifiers (MeOH, EtOH, etc.) enhances retention and changes selectivity in HILIC separations.**
- **HILIC offers complementary selectivity to RP-HPLC.**
- **Up to 10X improvement in ESI-MS response was observed when using HILIC instead of RP-HPLC.**
- **Separations were found to be directly transferable between XBridge™ HILIC and ACQUITY UPLC® BEH HILIC columns.**

References

1. E. S. Grumbach, D. M. Wagrowski-Diehl, J. R. Mazzeo, B. Alden and P. C. Iraneta, LCGC, Vol. 22, No. 10, 1010-1023 (October 2004)
2. W. Naidong, Rapid Commun. Mass Spectrom., 16, (2002) 1965-1975

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