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Enhanced Retention of Polar Analytes Utilizing Novel 1.7 μm UPLC™ Particles for Hydrophilic Interaction Chromatography

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Hydrophilic interaction chromatography (HILIC) can improve HPLC assays for polar compounds that retain poorly using reversed-phase HPLC. Combining this chromatographic technique with highly efficient 1.7 μm UPLC™ BEH particles results in fast methods that exhibit improved polar retention, high sensitivity, superior chromatographic resolution, and long column lifetime.

HILIC is a chromatographic technique that has been used to improve retention of very polar analytes. This is achieved by utilizing a high organic-low aqueous mobile phase in combination with a polar stationary phase. These assays can be further enhanced by utilizing Ultra Performance Liquid Chromatography (UPLC™). This technique combines low dispersion, high speed instrumentation with 1.7 μm particle packed columns for improved resolution, sensitivity and speed.

Chromatographers can, therefore, meet the challenges of developing separations that completely characterize the constituents of samples.

The general application of HILIC for the analysis of polar basic analytes has been limited by available column chemistries. Maximum retention occurs at modestly elevated mobile phase pH, often leading to decreased column lifetime of silica-based materials. This has led to the development of a novel 1.7 μm UPLC™ BEH (bridged ethyl hybrid) HILIC particle that provides improved retention of polar species, and long column lifetimes at moderate pH values. The improvements are demonstrated here with three bases; uracil, 5-fluorocytosine and cytosine.

Experimental Conditions

Instrument: Waters ACQUITY UPLC™ with an ACQUITY UPLC™ TUV detector

Column: ACQUITY UPLC™ BEH HILIC

Column Dimensions: 2.1 \times 50 mm, 1.7 μm

Mobile Phase A: 95:5 acetonitrile: water with 10 mM ammonium acetate pH 5.5

Mobile Phase B: 50:50 acetonitrile: water with 10 mM ammonium acetate pH 5.5

Flow Rate: 0.5 mL/min

Gradient:

Time (min)	%A	Profile %B
0.0	99	1
2.0	1	99
2.1	99	1
2.5	99	1

Injection Volume: 2.0 μL

Sample: uracil, 5-fluorocytosine and cytosine

Sample concentration: 25 $\mu\text{g}/\text{mL}$

Sample diluent: 75:25 acetonitrile: methanol

Temperature: 30 $^{\circ}\text{C}$

UV detection: 254 nm

Sampling Rate: 40 Hz

Time Constant: 0.05

Results and Discussion

Figure 1 illustrates the retentive properties, good peak shape and reproducibility obtained with the ACQUITY UPLC™ BEH HILIC column over the course of these experiments.

Quantitative information is reported in Table I for 5-fluorocytosine and cytosine over the course of 2000 injections.

Table I: BEH HILIC

fluorocytosine				
Injection	V_o	TR	k	width @ 4.4%
10	0.33	0.77	1.32	0.028
500	0.33	0.77	1.32	0.028
1000	0.33	0.76	1.32	0.028
1500	0.33	0.76	1.30	0.027
2000	0.33	0.76	1.30	0.027
average		0.76	1.31	0.03
standard deviation		0.004	0.012	0.001
%RSD		0.495	0.929	1.985
cytosine				
Injection	V_o	TR	k	width @ 4.4%
10	0.33	0.92	1.79	0.029
500	0.33	0.92	1.78	0.028
1000	0.33	0.92	1.78	0.028
1500	0.33	0.91	1.75	0.028
2000	0.33	0.91	1.75	0.028
average		0.91	1.77	0.03
standard deviation		0.006	0.019	0.000
%RSD		0.640	1.090	1.586

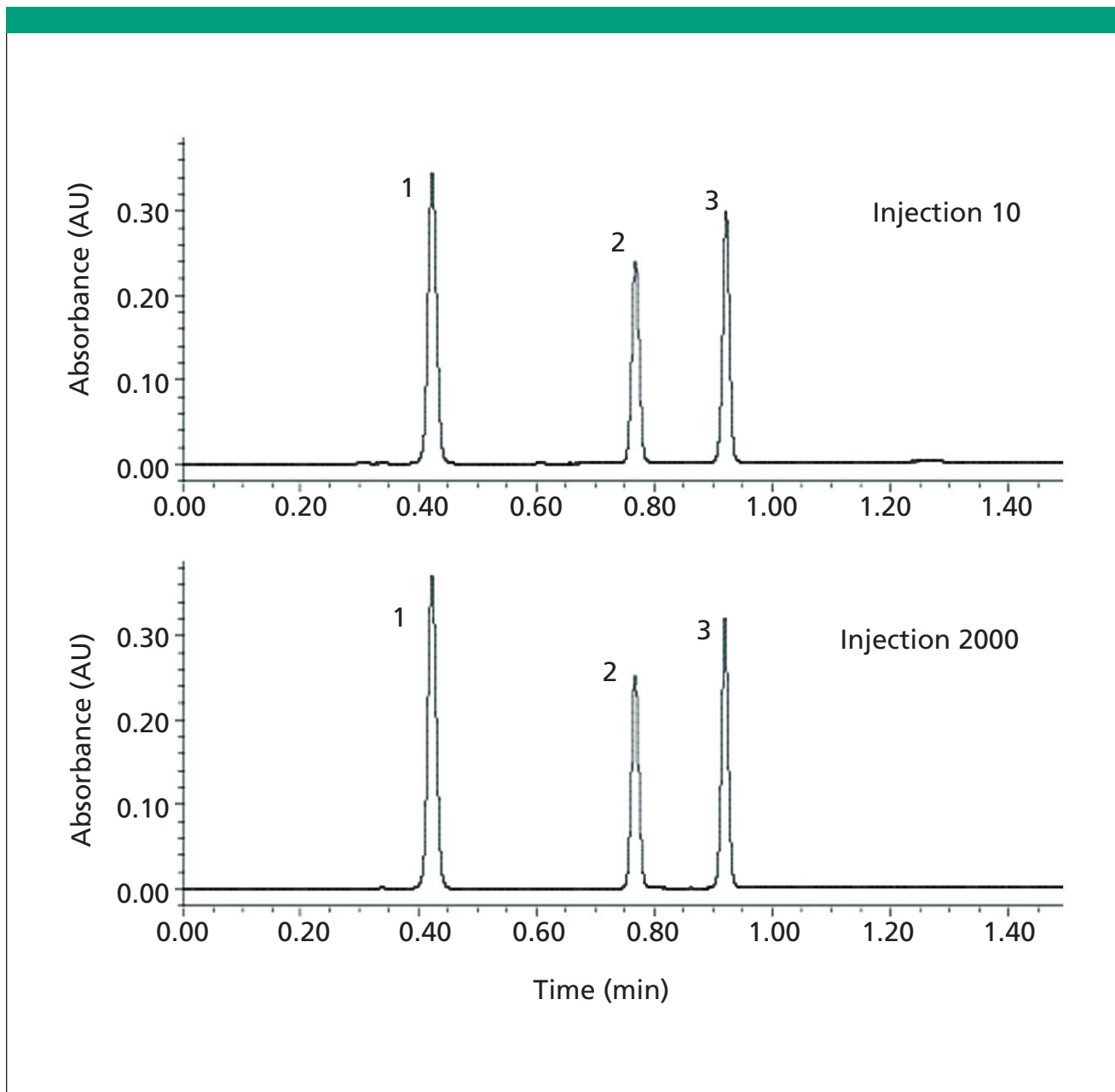


Figure 1: Overlay of injections 10 and 2000 at pH 5.5. Peaks are (1) uracil, (2) 5-fluorocytosine, and (3) cytosine.

Retention factors of 1.31 and 1.77 are observed for these very polar compounds. There is no loss of retention or change in peak width for either analyte over the course of 2000 analyses. In addition, as demonstrated, this highly volatile mobile phase improves sensitivity in electrospray MS through efficient mobile phase desolvation and compound ionization (1).

Conclusions

The 1.7 μm ACQUITY UPLC™ BEH HILIC column offers a unique selectivity that retains and resolves polar analytes. Retention times, peak shapes, and peak areas are highly reproducible over the length of the study demonstrating long column

lifetimes at moderate pH values.

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)

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