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Application Note

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

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Abstract

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, higher sensitivity, superior chromatographic resolution, and significantly long column lifetime.

Introduction

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 UltraPerformance 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 the 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

Experimental Conditions

Instrument:	Waters ACQUITY UPLC with an ACQUITY UPLC TUV detector
Columns:	ACQUITY UPLC BEH HILIC
Column dimensions:	2.1 x 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

Injection volume:	2.0 µL
Sample:	uracil, 5-fluorocytosine and cytosine
Sample concentration:	25 µg/mL
Sample diluent:	75:25 acetonitrile: methanol
Temperature:	30 °C
UV detection:	254 nm
Sampling rate:	40 Hz
Time constant:	0.05

Gradient

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

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 1 for 5-fluorocytosine and cytosine over the course of 2000 injections.

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]

Conclusion

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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