

Separation of Polar and Hydrophilic Compounds Using a Zwitterionic Stationary Phase in Hydrophilic Interaction Liquid Chromatography

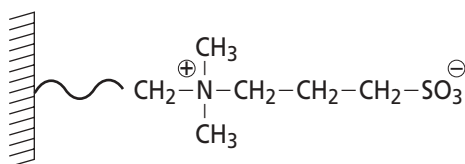
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Introduction

Hydrophilic interaction liquid chromatography (HILIC) is a separation technique suitable for polar and hydrophilic compounds, and is orthogonal to reversed-phase chromatography. Compounds that have little or no retention and that may be affected by wettability problems on a reversed-phase column generally have strong retention on a HILIC column. The separation mode uses an eluent containing a high content of water miscible organic solvent (e.g., acetonitrile) to promote hydrophilic interactions between the analyte and a water wetted hydrophilic stationary phase, and is thus a technique comparable, in principle, to traditional normal-phase chromatography. However, with respect to analyte solubility in the mobile phase and matrix compatibility, HILIC is superior, as the mobile phase compositions used are comparable to reversed-phase separations. At present there are only a few HILIC phases that are commercially available, but none is truly comparable with the ZIC®-HILIC phase. The highly polar zwitterionic column provides a unique environment particularly capable of solvating polar and charged compounds, which enables high performance HILIC separations. The zwitterionic stationary phase (see Figure 1) can interact with charged analytes via weak electrostatic interactions, as opposed to the strong electrostatic interactions obtained with plain silica or amino HILIC phases. In practice, this provides the chromatographer with a larger degree of freedom when choosing between buffer salts and ionic strength in method development.

This particular application note presents an example of an isocratic HILIC separation of purines and pyrimidines (i.e., RNA/DNA bases such as adenine, cytosine, guanine, thymine and uracil.) In addition to the separation, a comparison between two commonly used water miscible organic solvents, acetonitrile (weak) and methanol (strong) illustrates the solvent strength for ZIC®-HILIC separations. Finally, there is data illustrating the extreme flow-rate range that it is possible to use with the ZIC®-HILIC column, which demonstrates its suitability for high throughput separations.

Figure 1: The ZIC®-HILIC stationary phase.



ZIC®-HILIC Columns

The ZIC®-HILIC columns have a sulfobetaine type zwitterionic stationary phase covalently attached to 3.5, 5 and 10 µm particle size silica in conventional dimensions from capillaries to preparative scale.

The column can provide a selectivity benefiting from both hydrophilic- and weak electrostatic interactions, while maintaining a low eluent ionic strength, making the column an ideal choice for LC-MS analysis.

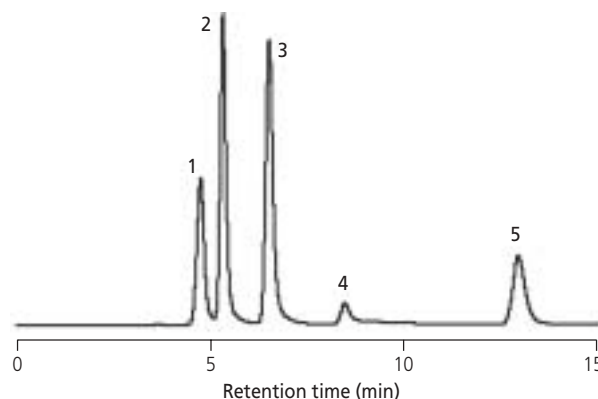
Experimental Conditions

Column: ZIC®-HILIC 150 × 2.1 mm, 5 µm
 Mobile phase: Acetonitrile/aqueous buffer containing ammonium formate (2.5 mM) and formic acid (25 mM); 80/20 (v/v)
 Flow-rate: 0.1 mL/min
 Detector: UV @ 254 nm
 Injection volume: 2 µL
 Sample: In elution order; thymine, uracil, adenine, guanine and cytosine all dissolved in mobile phase.

Results

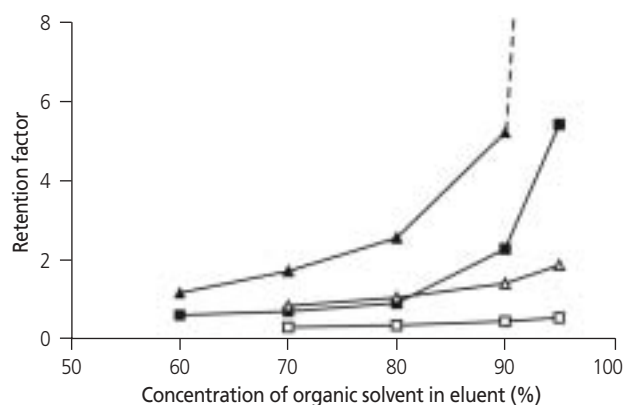
Satisfactory resolution for the five RNA/DNA bases can be achieved using a simple isocratic elution protocol, as seen in Figure 2. This

Figure 2: Separation of five nucleotides on a ZIC®-HILIC column.



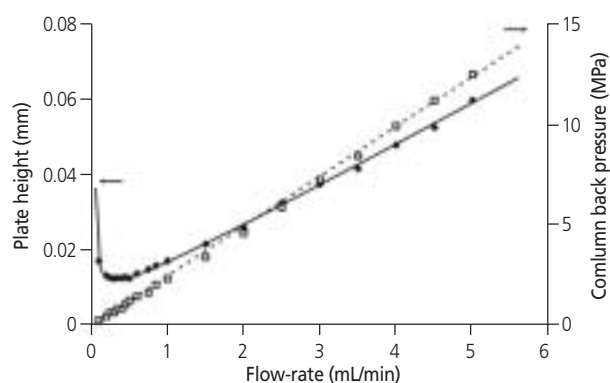
Peaks: 1 = thymine, 2 = uracil, 3 = adenine, 4 = guanine, 5 = cytosine.

Figure 3: Retention factor (k') as a function of the concentration of acetonitrile or methanol as organic modifier in the eluent.



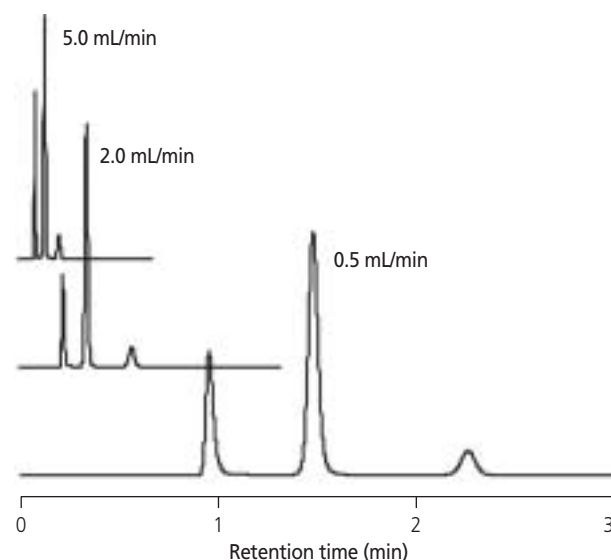
Formic acid (10 mM overall) in eluent. Triangles: cytosine; squares: adenine; filled symbols: acetonitrile; open symbols: methanol.

Figure 4: Separation efficiency and column back pressure as a function of flow-rate.



Cytosine diluted in mobile phase and separated on a 50 × 4.6 mm I.D. ZIC®-HILIC column with 5 µm particles.

Figure 5: Overlaid chromatograms at different flow-rates.



Separation of toluene (void volume marker), uracil and cytosine, all diluted in mobile phase and separated on a 50 × 4.6 mm I.D. ZIC®-HILIC column with 5 µm particles.

The interesting flow characteristics associated with the ZIC®-HILIC column distinguish it furthermore from other HILIC column brands. Figure 4, illustrates that optimal separation efficiency for cytosine is obtained at 0.5 mL/min on a 4.6 mm I.D. column, a value that is half of the normal flow-rate for other HILIC and common reversed-phase columns. In addition, the ZIC®-HILIC column has an extreme flow-rate working range. Figures 4 and 5 exemplify that it is feasible to perform a separation of toluene (void volume marker), uracil and cytosine using flow-rates between 0.1 and 5.0 mL/min, still within acceptable back pressure levels, which demonstrates its suitability for high throughput separations.

Conclusion

The ZIC®-HILIC column is a suitable tool for HILIC mode separation of purines and pyrimidines. By taking advantage of the weak electrostatic interactions between the analytes and the overall neutral zwitterionic stationary phase and performing proper tuning of the mobile phase, with respect to organic modifier, buffer salt, ionic strength and pH, the column exhibits a unique selectivity in the analysis of a wide range of compounds. Combined with the advantageous column flow characteristics, a wide range of applications, ranging from high throughput to preparative scale separations can be performed.

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illustrates the applicability for the ZIC®-HILIC column as it typically retains and resolves these compounds well. This application is an addition to the span of previous applications (separation of amino acids, amines, peptides etc.) where the ZIC®-HILIC column has proven to be successful. The five RNA/DNA bases represents compounds that elute with a retention factor (k') between 0.5 and 3 using the provisional optimal separation conditions. However, depending on the concentration and choice of mobile phase constituents, this is subject to changes. In Figure 3, a comparison between two commonly used water miscible organic solvents, acetonitrile and methanol illustrates the influence of solvent strength for HILIC separations. The weakest solvent for HILIC chromatography, acetonitrile provides higher increase in retention compared to methanol. The latter solvent is, although a viable alternative that may possibly provide a higher solubility for various compounds, nonetheless commonly associated with separations with lower efficiency.