Affecting Selectivity and HILIC Retention on a FluoroPhenyl Stationary Phase

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Abstract & Introduction

In this study we have set out to explore the retention mechanisms of the FluoroPhenyl stationary phase. The FluoroPhenyl phase may be described as having Mixed-Mode and/or HILIC retention and selectivity. HILIC and Mixed-Mode offer retention mechanisms that vary, or are orthogonal to, typical reversed-phase columns like C18. These chemical interactions are generally not well understood or easily demonstrated, which may be frustrating and leave chromatographers not using the phase to the fullest potential.

The FluoroPhenyl phase offers unique selectivity by incorporating strongly electronegative fluorine atoms on a phenyl ring. In addition to traditional reversed-phase dispersive interactions, this phase may exhibit polar, cation-exchange, and HILIC retention. Our focus in this presentation is on method changes including mobile phase composition, acid strength, and acid concentration, and how these changes affect the selectivity and sensitivity of several target analytes. By demonstrating the influence of method conditions on this phase we aim to gather a better understanding of the interactions provided by the FluoroPhenyl phase and its use as a HILIC or Mixed-Mode phase.

Probes for FluoroPhenyl Retention Mechanism Experiments

Several common drug compounds were selected to demonstrate the varied retention behaviors of the FluoroPhenyl stationary phase (Figure 2). These chemical probes represent bases, weak acids, and neutral compounds to highlight differences in selectivity and the affinity of the FluoroPhenyl phase for hydrophilic and basic compounds.

Orthogonal Selectivity and Mixed-Mode Retention

One of the primary advantages of the FluoroPhenyl phase is that it offers alternate selectivity than traditional alkyl phases due to increased cation-exchange interactions. Under reversed-phase conditions, the Raptor FluoroPhenyl column provides more retention for positively charged bases (i.e. atenolol, nortriptyline, and amitriptyline) compared to the Raptor C18 column while maintaining equivalent retention for neutrals and weak acids (Figure 3). On the other hand, the FluoroPhenyl stationary phase is preferentially selective for bases in HILIC mode with neutrals and acids eluting near the column void. The ability of the FluoroPhenyl phase to operate with dual modes of retention is clearly demonstrated by a “U-shaped” retention profile (Figure 4). In addition, the use of electrospray ionization mass spectrometry (ESI-MS) in conjunction with the high percentage of organic solvent available in HILIC mode increases the efficiency of the desolvation process and results in added sensitivity. In table 1, intensity values from the retention profile are compared for atenolol, nortriptyline, and amitriptyline. Increased sensitivity is observed for both nortriptyline and amitriptyline. Sensitivity for atenolol is unchanged as it switches from a non-retained compound in reversed-phase mode to a retained compound in HILIC mode.

Effects of Acid Concentration & Strength

The cation-exchange retention mechanism may be influenced by both the strength of the acid utilized, as well as the concentration of a specific acid. When utilizing the cation-exchange retention mechanism, the effect of acid concentration served to lessen the retention of bases, with minimal change in retention for neutrals and weak acids in reversed-phase and HILIC modes (Figure 5). Similarly, for mobile phases modified with 0.1% acid, as the strength of the acid increases (acetic → formic), the retention of bases decreases (Figure 6).

Conclusions

• The FluoroPhenyl phase allows retention based on hydrophobicity and cation-exchange which results in a “U-shaped” retention profile for bases.
• Acid strength and concentration can be used to alter selectivity based on the cation-exchange mechanism of the FluoroPhenyl phase.

Table 1: Increased Sensitivity in HILIC Mode with ESI-MS

<table>
<thead>
<tr>
<th>Analyte</th>
<th>RP Intensity (40% Isocratic)</th>
<th>HILIC Intensity (90% Isocratic)</th>
<th>Increase in Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>5.0e4</td>
<td>5.0e4</td>
<td>-</td>
</tr>
<tr>
<td>Nortriptyline</td>
<td>7.5e4</td>
<td>1.3e5</td>
<td>73%</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>8.5e4</td>
<td>1.7e5</td>
<td>100%</td>
</tr>
</tbody>
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