STABILITY OF HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY (HILIC) STATIONARY PHASES UNDER LOW AND HIGH PH TESTING CONDITIONS

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INTRODUCTION

HILIC stationary phase stability has been studied sporadically and under specific assay conditions. While this tests the stability of a given column for a given assay, direct comparisons between HILIC stationary phases cannot be accurately drawn. However, by using accelerated pH stability tests with generic conditions across all columns, direct comparisons between columns can be investigated.

Accelerated low pH stability testing was performed on six stationary phases using 0.5% Trifluoroacetic acid (TFA) at 70°C. Additionally, an accelerated high pH stability test was performed on eight stationary phases in triplicate using a challenge mobile phase with ammonium bicarbonate at pH 11.3 at 70°C. Direct comparisons between similar stationary phases will be made.

METHODS

Low pH Stability Testing
System: ACQUITY™ UPLC™ H-Class
Columns: 2.1 x 50 mm
Column Temp: 70°C
Mobile Phase A: 0.5% TFA in Water
Mobile Phase B: 0.5% TFA in Acetonitrile
Flow Rate: 0.4 mL/min
Gradient: Start at 5% A, hold for 0.25 min. Linear gradient to 50% A in 2.75 minutes. Hold at 50% A for 0.5 minutes. Return to 5% A and hold for 2.5 minutes.
Sample: Trimethyl ammonium chloride (25µg/mL) (TMPA), Sodium p-toluenesulfonate (25µg/mL) (Tosylate), and Uridine (7µg/mL).
Detection: UV @ 254 nm

High pH Stability Testing
System: ACQUITY™ ARC™
Columns: 2.1 x 50 mm
Column Temp: 70°C
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Mobile Phase C: 100 mM ammonium formate pH 3.0
Mobile Phase D: 60:40 Acetonitrile:Water with 10mM ammonium bicarbonate pH 11.3
Flow Rate: 0.4 mL/min
Gradient: See Table 1.
Sample: Adenine (3.7 µg/mL) and Cytosine (7.7 µg/mL) (HILIC)
Detection: UV @ 260 nm

Table 1. Gradient Method for High pH stability Testing

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>%A</th>
<th>%B</th>
<th>%C</th>
<th>%D</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0</td>
<td>95</td>
<td>5</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>13.73</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>34.30</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>35.97</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>39.27</td>
<td>50</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>40.93</td>
<td>10</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>44.23</td>
<td>10</td>
<td>90</td>
<td>0</td>
<td>0</td>
<td>6</td>
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<td>5</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>68.13</td>
<td>0</td>
<td>95</td>
<td>5</td>
<td>0</td>
<td>11</td>
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</table>

RESULTS AND DISCUSSION

Low pH Stability Testing

For most of the phases, the changes in retention were <10%, except for the Ascentis Si column which showed a 19% increase in retention for the basic probe trimethyl ammonium chloride (TMPA). This would indicate that all tested phases are relatively stable at low pH, as for some phases, such as the BEH HILIC, CORTECS HILIC, Ascentis Si, and Accucore HILIC, there is no attached ligand, which would account for the stability of these phases. For the two phases which have attached ligands, the columns appear to be designed for low pH stability as only minor changes in retention are detected.

High pH Stability Testing

In reversed-phase liquid chromatography, the high pH stability of silica phases has been extensively studied.3,4 It has been established that in high pH mobile phases, the silica backbone of the particle is hydrolyzed leading to particle dissolution, and loss of efficiency. Theoretically, the same phenomenon should happen for HILIC columns, however no test has been performed to compare the stability of HILIC phases under such conditions.

For the hybrid columns (BEH Amide and BEH HILIC) are very stable during this testing showing <5% loss in efficiency. Interestingly, the unbounded BEH particle is slightly less stable than the bonded BEH Amide. The attached amide ligand appears to help further protect the hybrid particle from being dissolved. This is not the case for TSKGel Amide, which shows poor stability. For that column, the amide group is not able to protect the silica particle from being dissolved, leading to a significant loss in efficiency.

The silica particles, as expected, are less stable under these test conditions. However, not all silica particles have the same stability. The Atlantis HILIC column, which shows a loss of ~50% appears more stable than the Ascentis Si column, which showed ~65% loss. This could be due to the difference in surface areas of the silica particle. The CORTECS and Accucore HILIC solid-core silica particles show a smaller difference in stability, which is not statistically significant.

For the hybrid columns, the BEH HILIC and CORTECS HILIC column after testing. As the images show, the hybrid particles show no signs of bed loss while the CORTECS HILIC bed is obviously degraded and a void has formed.

Table 2. % Change in retention time for three probes on 6 columns subjected to accelerated low pH stability testing.

<table>
<thead>
<tr>
<th>Column</th>
<th>% Change in RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEH HILIC</td>
<td>0.00 - 4.55 - 0.23</td>
</tr>
<tr>
<td>BEH Amide</td>
<td>-7.03 0.00 - 1.62</td>
</tr>
<tr>
<td>CORTECS HILIC</td>
<td>0.00 - 6.35 - 0.01</td>
</tr>
<tr>
<td>Ascentis Si</td>
<td>4.41 10.95 - 0.18</td>
</tr>
<tr>
<td>Accucore HILIC</td>
<td>0.00 - 1.60 0.93</td>
</tr>
<tr>
<td>ZIC-HILIC</td>
<td>0.00 - 0.00 - 0.71</td>
</tr>
</tbody>
</table>

REFERENCES


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