Optimizing UHPLC Resolution Using Fused-Core® Column Packings with Different Separation Selectivities

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Abstract

Fused-Core® particles, with an overall diameter of 2.7 µm and an inner diameter of 0.7 µm, demonstrate similar efficiency to sub-2-µm particles, but with one-half to one-third the back pressures. Although column efficiency (N) is an important parameter in the general resolution equation, resolution only increases by the square root of an increase in efficiency. Moreover, resolution has a direct linear relationship with the separation selectivity (α). Therefore, it is important to select packing materials which optimize symmetry to the lowest possible levels.

HALO Phase Descriptions and Characteristics for Column Used in this work

<table>
<thead>
<tr>
<th>Phase</th>
<th>Characteristics</th>
<th>Best Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8</td>
<td>Hydrophobic</td>
<td>Phenyl-Hexyl</td>
</tr>
<tr>
<td>RP-Amide</td>
<td>Amide derivatization</td>
<td>AcN/MeOH vs. 3 pHs</td>
</tr>
<tr>
<td>PFP</td>
<td>Ether derivatization</td>
<td>AcN/MeOH vs. 3 pHs</td>
</tr>
</tbody>
</table>

Column Phase Screening: Conditions

- Organic Modifier: AcN/MeOH
- Mobile Phase pH: 0.1% HCOOH
- Mobile Phase Gradient: 5–95% AcN/water, 0.1% HCOOH in 10 min.
- Mobile Phase Gradient Temp: 30°C
- Water Top Back Pressure: 80 psi
- Mobile Phase: 10 mL/min

Resolution Equation Shows that Selectivity is Most Effective Parameter to Change

Resolution is roughly proportional to selectivity, α, but only proportional to the square root of efficiency, N.

Most Effective Parameters to Change Selectivity

- Mobile phase B-solvent (acetonitrile, methanol, etc.)
- pH
- Buffer concentration

Experimental

- Instrument: Agilent 1200 series
- Column: 4.6 x 50 mm HALO RP-Amide
- Mobile phase: 5–95% AcN/water, 0.1% HCOOH
- Detection: UV @ 254 nm

Comparison of Selectivities: Benzoic Acids, Isocratic

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Log P</th>
<th>α</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-cyanobenzoic acid</td>
<td>3.6</td>
<td>1.48</td>
</tr>
<tr>
<td>benzaldehyde</td>
<td>4.2</td>
<td>1.86</td>
</tr>
<tr>
<td>acetaminophen</td>
<td>9.7</td>
<td>0.34</td>
</tr>
<tr>
<td>benzamide</td>
<td>15–16</td>
<td>0.74</td>
</tr>
<tr>
<td>benzyl alcohol</td>
<td>15</td>
<td>1.04</td>
</tr>
<tr>
<td>4-aminoacetophenone</td>
<td>4.6</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Summary and Conclusions

- HALO Fused-Core columns have comparable efficiency yet much lower backpressures than sub-2-µm columns
- Phases such as RP-Amide or Phenyl-Hexyl typically provide very different selectivities compared to C8 or C18 columns
- A variety of different phases are available for use in UHPLC systems
- Information regarding analyte-stationary phase interactions are provided to guide column selection for different sample types

Optimization Approach

- 5–10% ACN/0.1% formic acid, 12.5 mL/min
- Gradient times of 50 and 30 min
- Column temperature of 30 and 50°C
- Injection time for best column and multiple phase screening using all commercially available instrument protocols