Reliable HILIC LC-MS/MS Analysis of 4-Methylimidazole (4-MEI) on Raptor FluoroPhenyl Columns

- Increased retention for 4-MEI compared to a C18.
- Flexible Raptor FluoroPhenyl columns can be used in both HILIC and reversed-phase modes.
- Exceptional reproducibility assures accurate results analysis after analysis.

Caramel colorings are used in the food industry to impart a more desirable color to a wide range of foods and beverages. The many classes of colorings are produced in a variety of different ways, and those classes made using ammonia create 4-methylimidazole (4-MEI) as a byproduct. 4-MEI (CAS# 822-36-6) is classified by the International Agency for Research on Cancer (IARC) in Group 2B as possibly carcinogenic to humans [1]. This designation has generated interest in analytical methods that can be used to determine 4-MEI levels in both the colorings themselves to monitor formulations and also in foods and beverages to assess potential exposure. LC analysis of 4-MEI is a common approach, but as a small, polar compound, 4-MEI is difficult to retain using typical reversed-phase conditions and C18 columns.

As shown below, LC analysis of 4-MEI using HILIC mode and a Raptor FluoroPhenyl column is a much better strategy that yields strong retention and good peak shape. The highly reproducible performance and fast 2-minute analysis time (3.5 minute total cycle time) make this an excellent approach for labs needing high-throughput methods. Raptor FluoroPhenyl columns are recommended for LC analysis of 4-MEI in HILIC mode, but they can also be used in reversed-phase mode for other analyses, giving method developers the flexibility to use the column in both modes and then select the mode that performs best for their specific compounds of interest.

### LC Chromatogram

- **Column**: Raptor FluoroPhenyl (cat.# 9319A52)
- **Dimensions**: 50 mm x 2.1 mm ID
- **Particle Size**: 2.7 µm
- **Temp.**: 35 °C
- **Diluent**: Acetonitrile
- **Conc.**: 100 ng/mL
- **Inj. Vol.**: 5 µL
- **Mobile Phase**:
  - A: 0.1% Formic acid in water
  - B: 0.1% Formic acid in acetonitrile

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (mL/min)</th>
<th>%A</th>
<th>%B</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.6</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>2.00</td>
<td>0.6</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>2.01</td>
<td>0.6</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td>3.50</td>
<td>0.6</td>
<td>5</td>
<td>95</td>
</tr>
</tbody>
</table>

**Detector**: MS/MS
**Ion Mode**: ESI+
**Mode**: MRM
**Instrument**: UHPLC

### Peaks

<table>
<thead>
<tr>
<th>Peaks</th>
<th>tR (min)</th>
<th>Precursor Ion</th>
<th>Product Ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. 4-Methylimidazole</td>
<td>1.19</td>
<td>83</td>
<td>56</td>
</tr>
</tbody>
</table>

**Sample**
- **Diluent**: Acetonitrile
- **Conc.**: 100 ng/mL
- **Inj. Vol.**: 5 µL

**Inj. Vol.**: 5 µL

**Mobile Phase**
- A: 0.1% Formic acid in water
- B: 0.1% Formic acid in acetonitrile

**Time (min)**
- 0.00
- 2.00
- 2.01
- 3.50

**Flow (mL/min)**
- 0.6
- 0.6
- 0.6

**%A**
- 5
- 70
- 5
- 5

**%B**
- 95
- 30
- 95
- 95