Caramel coloring is a dark brown liquid or solid material used as a color additive. Caramel coloring results from the controlled heat treatment, often under pressure, of various food-grade carbohydrates, such as high-fructose corn syrup. There are four caramel colors: P-1, P-2, P-3, and P-4. Caramel color is used in various food products, such as cakes, candies, and beverages. In this study, a chromatographic and tandem mass spectrometry (LC-MS/MS) analytical methodology was evaluated for the identification and quantification of 4-methylimidazole (4-MEI), a compound that is classified as a potential byproduct, although 2-MEI is not in caramel color (2). A method that covers both 2- and 4-MEI allows for a single sample preparation method so that companies can demonstrate compliance with the new US regulation.

**Experimental**

**SPEC Sample Preparation**

An Agilent SPEC SPE disc is made of a monolith-like material, where the sorbent is covalently bonded to a highly porous, cross-linked polymer matrix. The SPEC SPE disc is designed to have high hydrophilic performance, large surface area, high volume capacity, and is robust to difficult samples.

**Results**

The mass spectrometer acquisition parameters, including transitions and precursor/product ions were optimized for both methods. (Table 1) The compounds were separated by single acquisition group, with retention times and a total run time less than 7 minutes.

**Conclusions**

The optimized sample preparation and analysis methods were used to analyze incurred samples of pumpernickel bread, which list caramel color as one of its ingredients. The results of this analysis indicated that 4-MEI was found in this bread at less than 18 ppb (calculated amount of 5.9 ppb).

**Figure 3. Incurred Sample: Pumpernickel Bread**

**Figure 4. Series of Liquid Caramel Colorings: 4-MEI Determination**

**Table 1. MS Acquisition Parameters**

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<thead>
<tr>
<th>Compound</th>
<th>m/z</th>
<th>Reaction (V)</th>
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<tbody>
<tr>
<td>2-Methylimidazole (2-MEI)</td>
<td>107</td>
<td>57</td>
<td>3</td>
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<tr>
<td>4-Methylimidazole (4-MEI)</td>
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<td>47</td>
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**Table 2. Relative Recoveries and RSDs for Pre-Spiked QC Samples**

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**Figure 2. Calibration Curves for 2-MEI and 4-MEI**

**Figure 1. Pre-Spiked Sample at 200 ppb**

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