Analysis of Polyphenolic Compounds and Vanillin in Cocoa Using Ascentis Express RP-Amide and MS Detection

By: Carmen T. Santasania, Reporter US Volume 28.3
carmen.santasania@sial.com

Cocoa, the principal component of chocolate, is reported to contain more than 800 different compounds (1). This complexity makes HPLC with mass spectrometric detection (LC-MS) an important analytical tool. In this short study, we looked at a few compounds (polyphenolics and vanillin) found in cocoa by LC-MS analysis. We chose Ascentis Express HPLC columns for their speed, efficiency, and ruggedness. The unique RP-Amide chemistry is ideal for these polar compounds.

Figure 1 shows the compounds that were examined in this study: the polyphenolic, anti-oxidant compounds catechin and epicatechin, which have reported health benefits. The extraction method was that described by Risner (2). A milk chocolate and an extra dark chocolate bar were tested. A 1 gram sample of the chocolate was added to 25 mL of warm (60 °C) water and allowed to melt with moderate stirring. A 2 mL aliquot of this solution was filtered through a 0.5 µm PVDF filter and cooled to ambient temperature prior to LC-MS analysis. Figure 2 shows the extracted ion chromatograms of a dark and milk chocolate sample by monitoring m/z 289.07. The analysis was run in negative ion mode on a single quadrupole mass spectrometer. The two samples are shown on the same scale to show the different amounts of catechins in the two types of chocolates analyzed. The observed ratio confirms literature reports that the darker the chocolate, the higher the levels of polyphenolic compounds (3).

Figure 1. Compounds Examined in the Study
Figure 2. Polyphenolic Compounds: LC-MS Extracted Ion Chromatogram of Dark and Milk Chocolate Samples (53929-U)

Figure 3 shows a comparison of vanillin content. Vanillin is a flavor enhancer that is added to many foods, including chocolate. Our results show higher levels of vanillin in the milk chocolate sample; not surprising since vanillin is known to reduce the bitterness of chocolate.

Figure 3. Vanillin: LC-MS Extracted Ion Chromatogram of Dark and Milk Chocolate Samples
In this brief report, we showed a simple extraction procedure followed by LC-MS analysis using Ascentis Express RP-Amide columns. Differences in catechin, epicatechin, and vanillin levels were observed in two types of chocolate. Additional applications using Ascentis Express and LC-MS to characterize chocolate samples are currently underway.

Materials

<table>
<thead>
<tr>
<th>Product #</th>
<th>Image</th>
<th>Description</th>
<th>Molecular Formula</th>
<th>Add to Cart</th>
</tr>
</thead>
<tbody>
<tr>
<td>53929-U</td>
<td></td>
<td>Ascentis® Express RP-Amide HPLC Column 2.7 µm particle size, L × I.D. 10 cm × 4.6 mm</td>
<td>wycena</td>
<td></td>
</tr>
</tbody>
</table>

References