Profiling Mono and Disaccharides in Juice, Wine, Beer, and Cider Using the ACQUITY UPLC H-Class System and the ACQUITY QDa Detector

Mark E. Benvenuti, Dimple Shah, and Jennifer A. Burgess
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS
When analyzing non-UV active sugars in beverages, coupling the ACQUITY UPLC® H-Class System with the ACQUITY® QDa® Detector provides a number of benefits such as:

■ Use of both retention time and mass-to-charge (m/z) to increase analytical specificity.
■ Obtain information-rich data from different sugars and sugar alcohols present in food products.
■ Provides a complementary detection method to Refractive Index (RI) and Evaporative Light Scattering (ELS) detectors that are commonly used for carbohydrate analysis.

INTRODUCTION
Sugars and sugar alcohols (or sugar polyols) are classes of carbohydrates that are important in human nutrition and natural constituents of foods. Some sugars are added to processed foods in order to enhance flavor, or to mimic fresh food products. With the increasing incidence of obesity and diabetes across the developed world, interest in monitoring sugar intake has vastly increased in recent years. Consequently there are now requirements to provide accurate information on product labeling in order to comply with the increasingly stringent regulatory demands.

The analysis of sugars and sugar alcohols remains a challenging application due to the lack of chromophores within their compound structure and the similarity between their molecules, many of which are simply isomers of one another. Because of its separation power, accuracy, and speed of analysis, HPLC has become the method of choice for the analysis of sugars.1,2 In order to detect these compounds, HPLC techniques have employed Refractive Index (RI) or Evaporative Light Scattering (ELS) detectors that are commonly used for carbohydrate analysis.

WATERS SOLUTIONS
ACQUITY UPLC BEH Amide Column
ACQUITY UPLC H-Class System
ACQUITY QDa Detector

KEY WORDS
Sugars, glucose, fructose, polyols, carbohydrate, maltose, inositol, cider, wine, beer, QDa
EXPERIMENTAL

UPLC conditions

LC system: ACQUITY UPLC H-Class
Runtime: 12.0 min with 15.0 min equilibration
Column: ACQUITY UPLC BEH Amide 1.7 µm, 2.1 x 100 mm
Column temp.: 85 °C
Mobile phase A: Water, 0.1% NH₄OH
Mobile phase B: Acetonitrile, 0.1% NH₄OH
Flow rate: 0.25 mL/min
Injection volume: 0.7 µL
Gradient table:

<table>
<thead>
<tr>
<th>Time</th>
<th>%A</th>
<th>%B</th>
<th>Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>90</td>
<td>6</td>
</tr>
<tr>
<td>12.0</td>
<td>40</td>
<td>60</td>
<td>6</td>
</tr>
<tr>
<td>12.01</td>
<td>10</td>
<td>90</td>
<td>6</td>
</tr>
</tbody>
</table>

MS conditions

MS system: ACQUITY QDa Detector
Ionization mode: ESI-
Capillary voltage: 0.8 V
Cone voltage: 4.0 V
Probe temp.: 600 °C
Acquisition rate: 1 Hz
SIR masses: see Table 2
Full scan range: 50 to 850 m/z

The ACQUITY QDa is the only mass detector that has been completely designed to be incorporated into a liquid chromatography system. It fits onto the LC stack, occupying the same space as a PDA detector. Extensive training is not required so users already familiar with HPLC can immediately make use of the selectivity and sensitivity that mass detection affords. In this application note we describe the profiling of sugars in orange juice, wine, beer, and cider using the ACQUITY QDa Detector coupled to ACQUITY UPLC H-Class System.

Table 1. SIR m/z used for the monosaccharides, disaccharides, and myo-inositol.

<table>
<thead>
<tr>
<th>Compound</th>
<th>SIR (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructose</td>
<td>215.1 [M+Cl⁻⁻]</td>
</tr>
<tr>
<td>Glucose</td>
<td>215.1 [M+Cl⁻⁻]</td>
</tr>
<tr>
<td>Myo-Inositol</td>
<td>179.2 [M-H⁺⁻]</td>
</tr>
<tr>
<td>Lactose</td>
<td>377.2 [M+Cl⁻⁻]</td>
</tr>
<tr>
<td>Maltose</td>
<td>377.2 [M+Cl⁻⁻]</td>
</tr>
<tr>
<td>Sucrose</td>
<td>341.3 [M-H⁺⁻]</td>
</tr>
</tbody>
</table>

Standard preparation

Individual 1000 mg/L stocks of the food sugars, fructose, glucose, sucrose, and maltose, along with myo-inositol were made in water. From these, a 50 mg/L mixed stock was prepared in 50:50 water:acetonitrile.

This stock was further diluted as necessary in 50:50 water:acetonitrile to determine retention times for the analytes.

Sample preparation

Samples of orange juice, hard cider, wine, and beer were purchased locally. The orange juice was centrifuged at an rcf of 2465 g for 30 mins. at room temperature to remove pulp. Two dilutions of the supernatant (1:100, 1:1000) were made in 50:50 water:acetonitrile. The beer samples were degassed by sonication, and filtered through a 0.2 micron PVDF filter, along with the hard cider and wine. Different dilutions were made in 50:50 water:acetonitrile. The hard cider and wine were diluted 1:100, and the beer was diluted 1:2.
RESULTS AND DISCUSSION

The separation of common food sugars including two monosaccharides (glucose and fructose), three disaccharides (sucrose, maltose, and lactose), and a sugar alcohol (myo-inositol) is shown in Figure 1. Multiple masses were monitored for each of the carbohydrates. Fructose, glucose, and myo-inositol all have a molecular mass of 180. Using ESI, myo-inositol forms a deprotonated molecular ion with \( m/z \) 179. The most abundant ion for fructose and glucose is the chloride adduct \([M+Cl^-]^-\) at \( m/z \) 215. Such chloride adducts have been previously reported to be used for MS analysis of some sugars as the intensities of the chloride adducts can exceed the \([M-H^+]^-\) of these analytes. The chloride adduct is also present for myo-inositol but at a lower response than \( m/z \) 179. Sucrose shows two abundant ions, the deprotonated molecular ion at \( m/z \) 341 and chloride adduct at \( m/z \) 377. Maltose has a dominant chloride adduct ion at \( m/z \) 377.

As can be observed in Figure 1, sucrose and myo-inositol show partial co-elution, but their different masses allow them to be easily distinguished using mass detection. Sucrose (\( m/z \) 341) elutes slightly earlier than myo-inositol (\( m/z \) 179 and \( m/z \) 215). For other, less selective detectors, further method development would be required in order to separate these analytes. With the ACQUITY QDa Detector, sucrose and myo-inositol can easily be distinguished without further method development.

![Figure 1. Chromatogram showing an overlay of multiple SIR channels (\( m/z \) 179, 215, 341, and 377) for the analysis of mono- and disaccharides and sugar alcohols using a gradient separation.](image-url)
This method was used to analyze the sugars present in a number of different beverage samples. The sugar profile of an orange juice sample is shown in Figure 2. Here we show that there are five major peaks that eluted with the SIR channels used to monitor the sugars and sugar alcohol. Peaks 1 and 2 correspond to fructose and glucose, respectively (apparent in SIR $m/z$ 215, with a small response at $m/z$ 179). Peak 4 ($m/z$ 341) corresponds to sucrose, and peak 5 corresponds to myo-inositol ($m/z$ 179). Peak 3 does not correspond to the retention time of any of the standards used in this application.

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Analyte</th>
<th>RT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fructose</td>
<td>3.2</td>
</tr>
<tr>
<td>2</td>
<td>Glucose</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>Unknown</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>Sucrose</td>
<td>6.7</td>
</tr>
<tr>
<td>5</td>
<td>Myo-Inositol</td>
<td>6.8</td>
</tr>
</tbody>
</table>

Figure 2. SIR chromatograms of: A. $m/z$ 179, B. $m/z$ 215, and C. $m/z$ 341 for the analysis of mono- and disaccharides and sugar alcohols in an orange juice sample. The vertical axis has been scaled to show responses relative to the largest peak (sucrose $m/z$ 341). Labeled peaks and their identity and retention time are displayed in the table inset.
Wine, beer, and cider samples were analyzed using the gradient method and the profiles of monosaccharides (using SIR channels of m/z 179 and 215), shown in Figure 3. The main sugars present in wine were glucose and fructose. Residual amounts were detected in the finished wines, with higher levels of fructose in many cases. Both fructose and glucose were detected in the two moscato wines (white and pink) and the cabernet sauvignon. Myo-Inositol was also detected in the wine samples. Sucrose and maltose were not detected in the wines (data not shown).

![Figure 3: Overlay of SIR chromatograms at m/z 215 (A-G) and m/z 179 (H-N) for the beverage samples and a mixed standard at 5 ppm.](image)

During the beer brewing process, soluble sugars such as maltose, glucose, and maltotriose are enzymatically produced from the malt starches. These sugars are subsequently converted to alcohol through the yeast fermentation process. Carbohydrates that are detected in finished beers either come from residual sugars that were not consumed in the fermentation process, or those that were added during a secondary fermentation process to produce additional CO₂. These residual sugars contribute to the sweetness of beer and they typically include combinations of fructose, glucose, sucrose, maltose, and maltotriose.

As shown in Figure 3, there were no detectable levels of glucose in the beer samples, and levels of fructose were extremely low. An example of a residual disaccharide detection is shown in Figure 4 for sucrose in the pale ale sample. The SIR chromatogram shows the sucrose peak (m/z 341) and spectrum from the simultaneous, full-spectrum analysis, which is shown with the spectrum from the sucrose standard. The black lager did not show an equivalent peak. Myo-Inositol was also detected in the beers and cider.
Figure 4. SIR chromatogram of m/z 341 in a mixed standard (A), and the pale ale beer sample (B). Spectra summed at the retention time of the major peak are shown in C and D for the standard and beer, respectively. The major ion in the spectrum (m/z 341) corresponds to the [M-H+]−.
CONCLUSIONS

The analysis of carbohydrates in food samples can be a challenging task given the mix of closely related UV-transparent compounds. With its high separation power, LC has become the method of choice, offering improvements in speed over other techniques. RI and ELS detection have been widely adopted in some laboratories. The combination of the ACQUITY UPLC H-Class System with the ACQUITY QDa Detector offers scientists a complementary detection system with the additional advantages of:

- Improved analytical selectivity by combining both retention time and mass analysis for compound identification.
- Detection of UV-transparent molecules using a sensitive and selective detector.
- The ability to discriminate between co-eluting components using their mass-to-charge ratios.
- Reduced burden for method development since baseline separation of all components is not required.
- The ability to deploy multiple methods on a single system that can rapidly change depending on method conditions.

References


