One-minute quantitative separation of complex carbohydrates for food analysis using direct infusion Trapped Ion Mobility Spectrometry (TIMS)

Ultra-fast chromatography-free analysis of carbohydrates for food

Carbohydrate analysis is of great importance within the entire food industry for various reasons.
First, the determination and monitoring of disaccharides is of considerable interest in nutritional and biochemical studies since their excessive consumption is known to increase the risk of various diseases including dental cavities, weight gain, obesity, type 2 diabetes, and other chronic conditions. In this same context, the analysis of disaccharides is also mandatory for nutritional labeling of packaged food as well as quality monitoring for food development purposes. This type of analysis is required to make or verify food-labeling claims in calorie-reduced foods, to prove food authenticity, and for the detection of adulteration. Additionally, lactose estimation is required in low lactose or lactose-free foods. Monitoring of fermentation processes and products in food and ingredient production includes sugar and oligosaccharide analyses.

Fructo-oligosaccharides and other oligosaccharides are often used as food ingredients and are usually incorporated as dietary fibers in many food products.
Also, several oligosaccharides are considered as prebiotics, so their analysis is required to measure their contribution to dietary fiber and other health benefits attributed to these types of substances.
In this study, selected di-, tri-, and oligosaccharides in food were investigated using an ultra-fast one-minute loop-injection method on a Bruker timsTOF Pro instrument to generate reliable, quantitative high-throughput results. This work provided high-resolution, high-mass accuracy data at a rapid speed of one minute per sample and showed that trapped ion mobility spectrometry (TIMS) is a viable alternative for effective separation of isomers without the need for time consuming chromatography.

Keywords:
timsTOF Pro 2, food and beverage, food adulteration, oligosaccharides, DataAnalysis software, direct infusion

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Methods

Mixtures of nigerose, gentiobiose, isomaltose, and maltulose, at concentrations between 0.4 ppm to 62 ppm, were directly infused for the method evaluation. Similarly, disaccharide mixtures of sucrose, maltose, and lactose, each at 5 ppm, and trisaccharides raffinose and maltotriose, each at 6–7 ppm, as well as malto-oligosaccharides DP4 to DP10 and DP6 to DP16, were investigated. All samples were loop-injected without any chromatographic separation into a Bruker timsTOF Pro QTOF mass spectrometer for ion mobility separation and quantification with processing performed using DataAnalysis software.

Results

The high-accuracy collisional cross section (CCS) values for carbohydrates obtained with TIMS are shown in the far right column of the table in Figure 1. These values are in excellent agreement with reported literature-based CCS values obtained from ccsbase.net and can be used for identification in conjunction with other parameters including mass accuracy, isotope pattern, and MS/MS data.

Saccharide	Lit. value (Na+)	Number of Lit. Values	timsTOF Result (Na+)
Nigerose	-	-	173.9, 175.6, 178.5
Gentiobiose	180.5	1	178.4
Isomaltose	177.7 ± 0.3	2	178.0
Maltulose	181.3	1	175.6, 178.5, 180.0
Raffinose	209.6 ± 1.1	4	208.7
Maltotriose	212.2 ± 1.4	4	212.9
Sucrose	173.0 ± 2.96	4	173.9
Maltose	178.3 ± 0.9	5	179.2
Lactose	178.3 ± 0.9	5	176.3

Figure 1.
Literature-based collisional cross section values (CCS) in Å² versus experimental timsTOF CCS values.

Figure 2 (upper) illustrates the heat-maps obtained for the mixture of malto-oligosaccharides that were created across a wide mobility range with a high ion mobility resolution that successfully enables the identification of differently-branched compounds. The heat-map, showing *m/z* on the horizontal axis vs. ion mobility on the vertical axis, is color coded for relative intensities of the detected features. Various ion series are visible with different charge states and the outlined area represents a specific ion series that was isolated and deconvoluted to generate clean baseline-separated mass spectra with a mass difference of 81 Da despite the presence of mixtures or matrices and without chromatographic separation. This analysis was performed at a speed drastically faster than existing methods, thus reducing the run time per sample from hours to minutes.

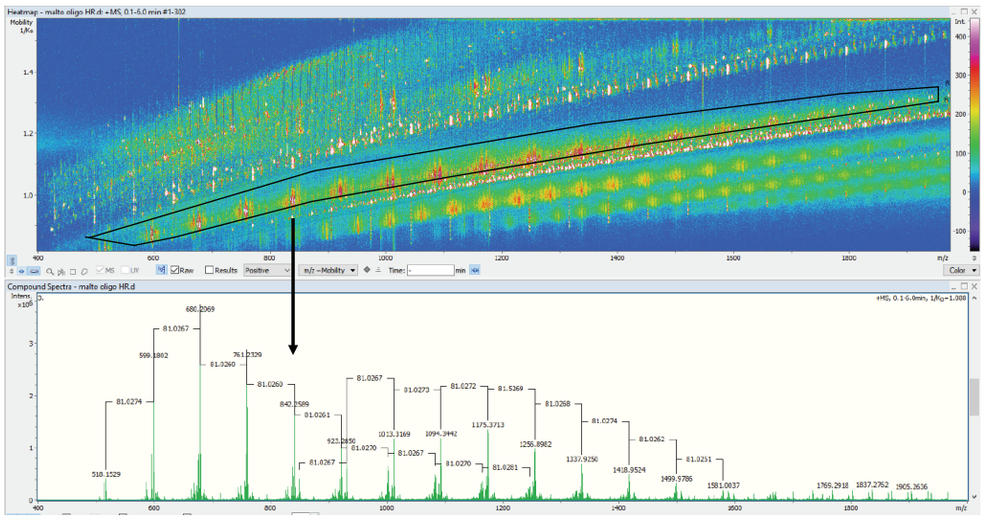

Figure 2.
(Upper) Heat map for infused malto-oligosaccharides and the outlined area showing the ion series extracted and displayed as discrete spectra (lower) separated by a mass difference of 81 Da.

Figure 3 shows the quantitative data for the fully separated saccharides that are observed at a run time of 1 min with a mobility resolving power of greater than 120. This ability to obtain high resolution at fast scan speeds facilitates the fast loop injection and effectively eliminates the need for time-consuming chromatography, where co-elution is common. The mobilograms are quantitative and reproducible for the analysis of a single sample in less than 1 min.

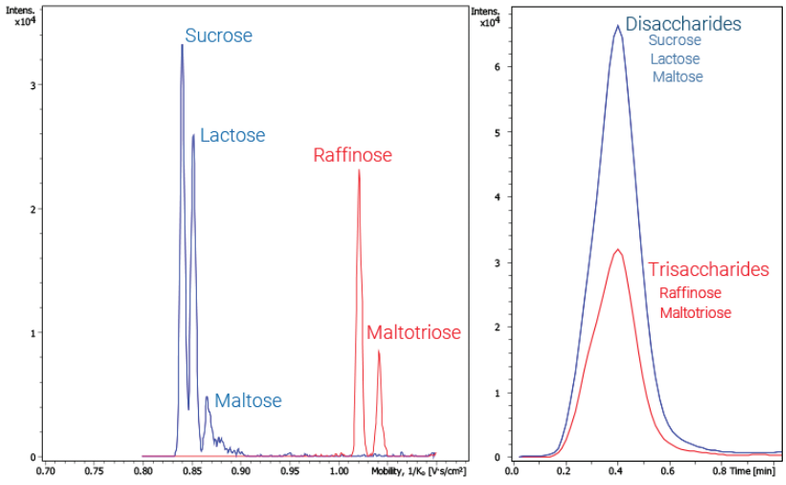

Figure 3.
Mobilogram showing fully separated disaccharides and trisaccharides (left) versus coelution by LC (right).

Finally, further demonstration of the potential for the benefit of using ion mobility separation for two isomeric trisaccharides is presented in Figure 4, where raffinose and maltotriose were fully baseline resolved as shown in the resulting direct injection mobilogram. The ability to clearly distinguish between isomers is a significant advantage of TIMS, with $1/K_0$ resolution values of 122.03 and 162.14, respectively.

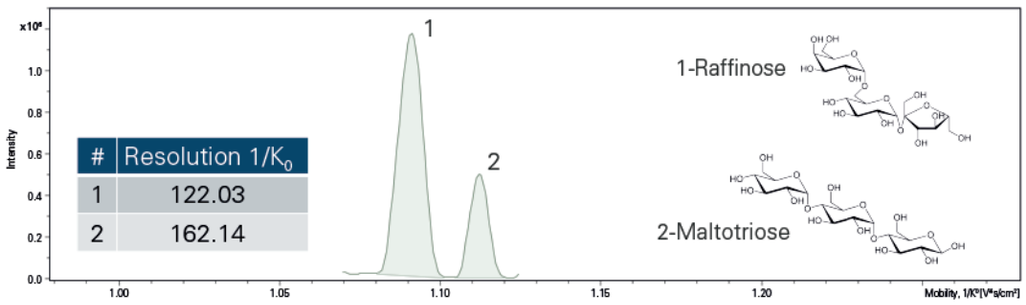

Figure 4.
Mobilogram showing baseline separation of isomeric trisaccharides Raffinose and maltotriose.

Conclusions

The current methods of sugar analysis are limited in their ability to accurately analyze sugars across different types of food matrices. Often, prior knowledge of expected sugar composition and presence in the investigated samples is required. Because of the complexity of matrices, most of the methods first require extensive sample cleanup and lengthy chromatographic separation, which can then often result in co-elution. Therefore, multiple analyses by different techniques often need to be performed for an accurate assessment of the sugars, significantly affecting the analysis time and possible sample throughput.

Trapped ion mobility spectrometry (TIMS) offers the accurate analysis of complex mixtures with fast MS/MS spectral rates higher than 100 Hz. TIMS provides accurate collisional cross section (CCS) values for the separation of generated ions, enhances the sensitivity of quadrupole time-of-flight (QTOF) mass spectrometry, and increases the confidence in identification or validation of compounds, with reproducible and quantitative mobilograms.

A quantitative method was developed for the direct infusion of samples for the analysis of di-, tri- and malto-saccharides (length of 4–10 monomers) that could be accomplished in one minute. A rapid and a simple method for the analysis of sugar which is applicable across diverse types of food matrices could be useful to the food industry in tackling the large number of the analysis required in various applications including nutritional labeling and also in reducing the extensive time currently required to complete this analysis.

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