Carbohydrate Analysis with FID

Application Note

(U)HPLC: Carbohydrates/Sugars

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Abstract

Maltoheptaose and maltotriose were analyzed using HPLC with the Solvere™ flame ionization detector (FID). The FID response to both sugars is equivalent within error and increases linearly with concentration from 10 to 1000 ppm.

Introduction

Quantitative analysis of sugars, or carbohydrates, is important in the development of food, biofuels, and pharmaceuticals. HPLC is the most common technique for sugar analysis, however, there is no detector that can yield a uniform response to different sugars during solvent gradients. Previous data, shown in Figure 1, displays a consistent response of sucrose as the mobile phase is changed from aqueous to organic.

Here, we analyze two carbohydrates, maltoheptaose and maltotriose, from ca. 10 ppm to 1000 ppm using gradient HPLC and a Solvere™ detector. The detection scheme consists of a catalytic rotating disc that facilitates solvent removal and subsequent catalytic combustion for detection by catalytic flame ionization detection (FID). This technique allows for universal carbon response because all carbon atoms are converted to methane and counted by the FID.

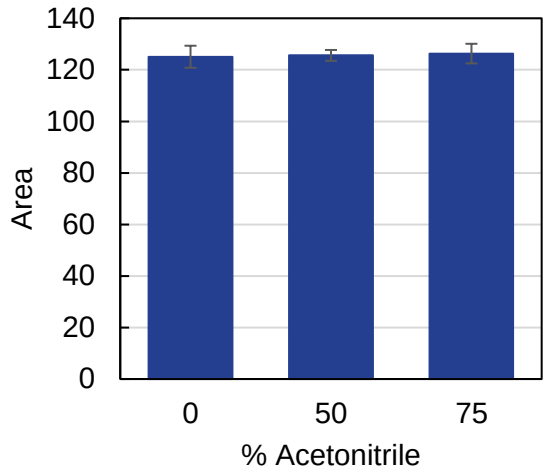

Figure 1. Consistent sucrose responses with varying mobile phase composition.

Experimental

Samples of maltoheptaose (M7753, Sigma Aldrich) and maltotriose hydrate (851493, Sigma Aldrich) were dissolved in HPLC grade water and diluted.

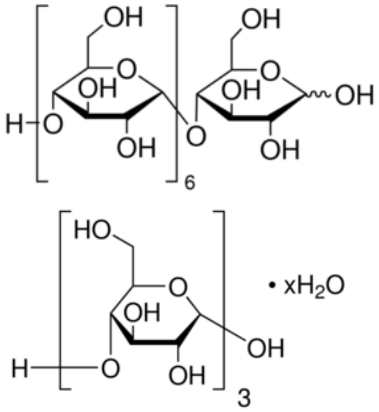

Figure 2. Diagrams of maltoheptaose (top) and maltotriose hydrate (bottom)

The samples were injected using an Agilent 1290 Infinity II LC System into a column (Zorbax SB-C18, Agilent), which was connected to the Solvere (v1, ARC) with a 300 mm x 0.12 mm ID 316SS tube and the following configuration:

HPLC conditions

Column	Zorbax SB-C18 (2.1 x 50mm, 3.5µm)
Column temp.	N/A
Test Standard	Various
Injection volume	20 µL
Pump flow rate	0.3 mL/min
Solvent A	Water, 0.1% formic acid
Solvent B	Acetonitrile

Gradient profile:

Time (min)	Percent B
0	10
1	10
3	95
13	95
13.1	10
20	10

Solvere™ conditions

Cell Temperature	120 °C
FID Temperature	400 °C
H_2	50 sccm
Air	350 sccm
Makeup	500 sccm (air)
Pressure	5 psig
Acquisition rate	3.125 Hz
Power	80%
Catalyst	S1-M

Results and Discussion

Figure 3 shows the detector response as a function of time for maltoheptaose and maltotriose injected at 497 and 506 ppm (µg/mL), respectively. Maltoheptaose displayed a smaller peak before the primary peak at higher concentrations. The area of this peak was included in the calculation of sugar response since these compounds were purchased as pure standards. It is assumed that this is an impurity in the sugar, but nonetheless included in the mass of the standard. Peak shapes are gaussian with tailing factors of 1.53 and 1.24 for maltoheptaose and maltotriose hydrate, respectively, and peak widths (FWHM) of 0.115 and 0.195 min, respectively.

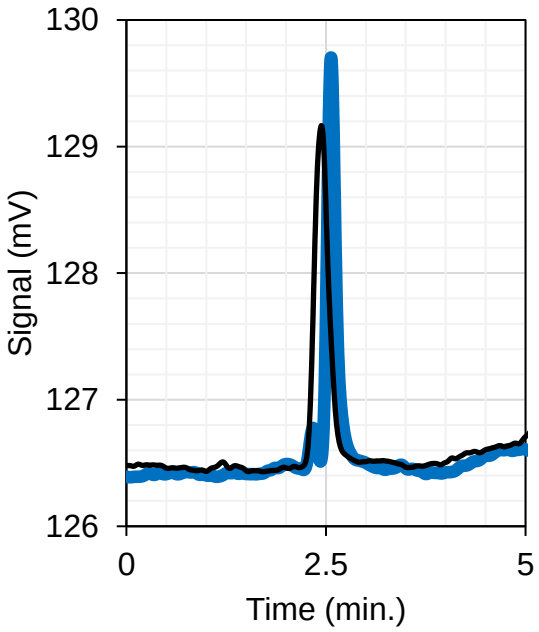

Figure 3. Overlay of chromatograms of maltoheptaose (blue, thick) and maltotriose (black, thin) at 500 ppm.

The integrated responses for each sugar are shown in Figure 4 from ca. 10 to 1000 ppm. The responses are both linear over the range with Pearson's correlation coefficients of 0.9992 and 0.99996. Both compounds give similar per carbon response indicated by the overlapping trend lines and similar slopes (1.62 and 1.58, respectively). The responses are likely equivalent within error. Minimum detection limits (MDL) were calculated based on the signal required to give a signal-to-noise ratio of 3. The MDLs were calculated as 15.55 ppm and 30.44 ppm for maltoheptaose and maltotriose, respectively.

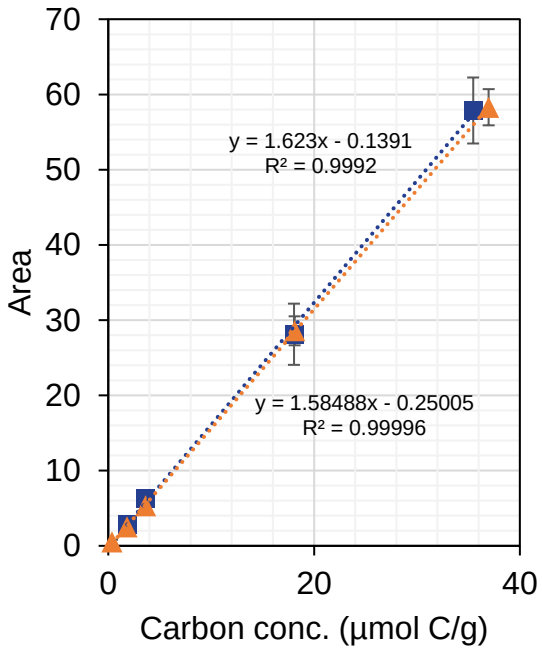

Figure 4. Integrated response of maltoheptaose (orange triangles) and maltotriose hydrate (blue squares) as a function of carbon concentration of the samples from 10-1000 ppm.

Conclusions

- Solvere™ CSD gives an equimolar carbon response for maltoheptaose and maltotriose
- Response is highly linear from 10-1000 ppm
- Minimum detection limits are 15-30 ppm in the current configuration

Contact Us

For more information or to purchase a Solvere™, please contact ARC at 612-787-2721, or by email at contact@activatedresearch.com.

Please visit ARC's website for details and additional technical literature, www.activatedresearch.com.

Printed in the USA
October 26, 2020
SA-APP-2034