

# Analysis of Carbohydrates in Microalgal Biomass Samples with HPAE-MS

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## INTRODUCTION

Efficient production of biodiesel from microalgae requires analysis of all cell products, including carbohydrates, lipids, and proteins. A complete characterization of the carbohydrate breakdown products is essential for nutrient recycling to determine which sugars are best absorbed by the algae. Carbohydrate content in microalgal biomass often contains complex mixtures of C5 and C6 sugars, requiring a separation technique with high resolution and high sensitivity. High-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) has proven to be an efficient tool for carbohydrate analysis.<sup>1</sup> The CarboPac® column family provides fast and efficient separations for a variety of carbohydrate samples, including monosaccharides, oligosaccharides, and linear polymer saccharides. To facilitate on-line coupling of HPAE with mass spectrometry, a carbohydrate membrane desalting device (CMD™) was developed for on-line desalting of the samples.<sup>2</sup> Here, the authors demonstrate that the complex carbohydrate mixture in microalgal biomass can be efficiently separated on a CarboPac MA1 column and analyzed with on-line electrospray ionization mass spectrometry (ESI-MS).

## EXPERIMENTAL CONDITIONS

### Chemicals and Materials

Mono- and disaccharide standards (Sigma)

Sodium hydroxide, 50% (w/w) (Fisher Chemicals, P/N SS254-500)

Lithium chloride (Sigma)

Deionized water, Type 1 Reagent Grade, 18.2 MΩ-cm resistivity or better

OnGuard® RP cartridge (Dionex, P/N 39595)

0.22 µm PVDF syringe filter (Fisher Chemicals)

1 mL and 10 mL plastic syringe (Fisher Chemicals)

### Columns

CarboPac MA1 Analytical column (4 × 250 mm, Dionex, P/N 044066)

CarboPac MA1 Guard column (4 × 50 mm, Dionex, P/N 044067)

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## Instrument Setup

All separations were performed on a Dionex ICS-3000 IC system, which includes an ICS-3000 DP gradient pump, AS autosampler, and ICS-3000 DC column compartment with electrochemical cell. Carbohydrates were detected using pulsed amperometry with a gold electrode and Ag/AgCl reference electrode using the standard quadruple waveform developed at Dionex. For MS detection, the column was connected to a CMD desalter (Dionex, P/N 059090), then to the MSQ Plus™ mass spectrometer. Pressurized water was pumped through the CMD as regenerating reagent. Postcolumn addition of 0.5 mM lithium chloride was mixed into the eluent at 0.1 mL/min using a mixing tee and AXP-MS pump (Dionex, P/N 060684). Detailed instrument setup is shown in Figure 1. The addition of lithium allowed mono- and disaccharides to form lithium adducts. The use of lithium allows detection of the sugars in the lithium form as opposed to mixed forms which may be caused by contamination of weaker adduct-forming cations, such as ammonium or sodium.

Chromatography and MS analysis was controlled by Chromeleon® Chromatography Data System (version 6.8). Reference spectra were collected using TUNE (Xcalibur, Thermo Fisher Scientific).

## Sample Preparation

Samples were obtained from General Atomics, San Diego, CA. To prepare samples for the HPAE-MS analysis, 2 mL of lysed microalgae sample was centrifuged at 12,000 rpm for 60 min. The supernatant was collected and centrifuged for an additional 30 min. The supernatant was collected and filtered through a 0.2 µm syringe filter to remove any remaining particles. The sample was passed through an OnGuard RP cartridge to remove hydrophobic components. The OnGuard RP cartridge was activated first with 5 mL methanol followed by 10 mL DI water using a 10 mL syringe. Next, 400 µL sample supernatant was mixed with 600 µL water and passed through the activated cartridge using a 1 mL syringe. The cartridge was then washed with 0.5 mL water and the eluted washing solution was combined with the initial eluent. The sample was then passed through another activated RP cartridge. This step was repeated until the sample was free of color. All the eluted solutions (~3 mL) were combined and concentrated to ~250 µL in a Speedvac (Thermo Fisher Scientific).

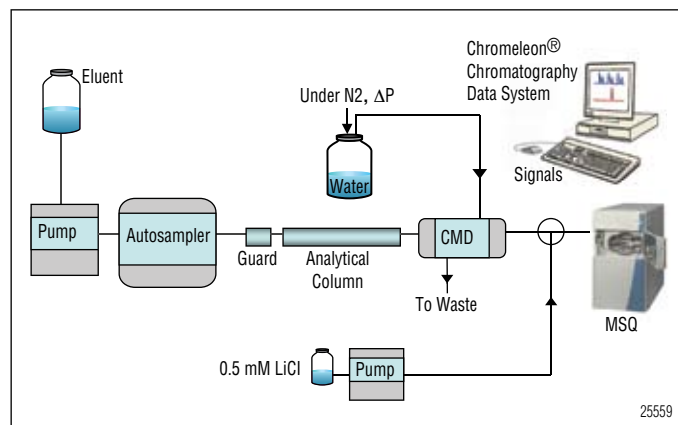


Figure 1. Instrument setup for on-line HPAE-MS analysis of carbohydrates.

## RESULTS

The separation profile of carbohydrates in microalgae samples on the CarboPac MA1 is shown in Figure 2. More than a dozen peaks were observed. On-line mass spectrometry analysis was used on the same samples to confirm the carbohydrate identity of the peaks. Note the similarities between the TIC and PAD profiles (Figure 3).

The full scan spectrum of each peak is shown in Figure 4. Because many mono- and disaccharides have identical mass-to-charge ratios, ( $m/z$ ) HPAE-PAD profiles of carbohydrate standards were compared with the sample profile. Comparison of their retention times helped to identify the peaks (Figure 5). Table 1 showed the identification results based on the peak's  $m/z$  and comparison with PAD profiles of the standards. These data indicated that monosaccharide alditols (fucitol, arabinol, inositol), monosaccharides (glucose, mannose, and others) and disaccharides (sucrose, maltose, and others) were present in the microalgal biomass, sucrose being the major sugar component.

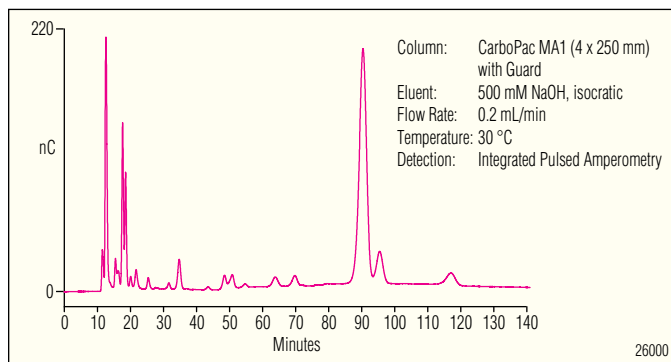


Figure 2. Separation profile of microalgae carbohydrates on the CarboPac MA1 column.

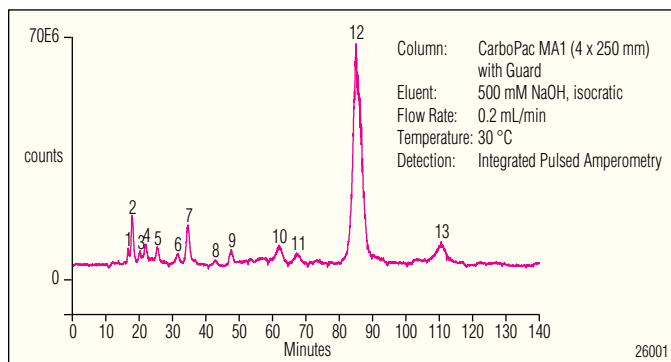


Figure 3. MS TIC profile microalgae carbohydrates.

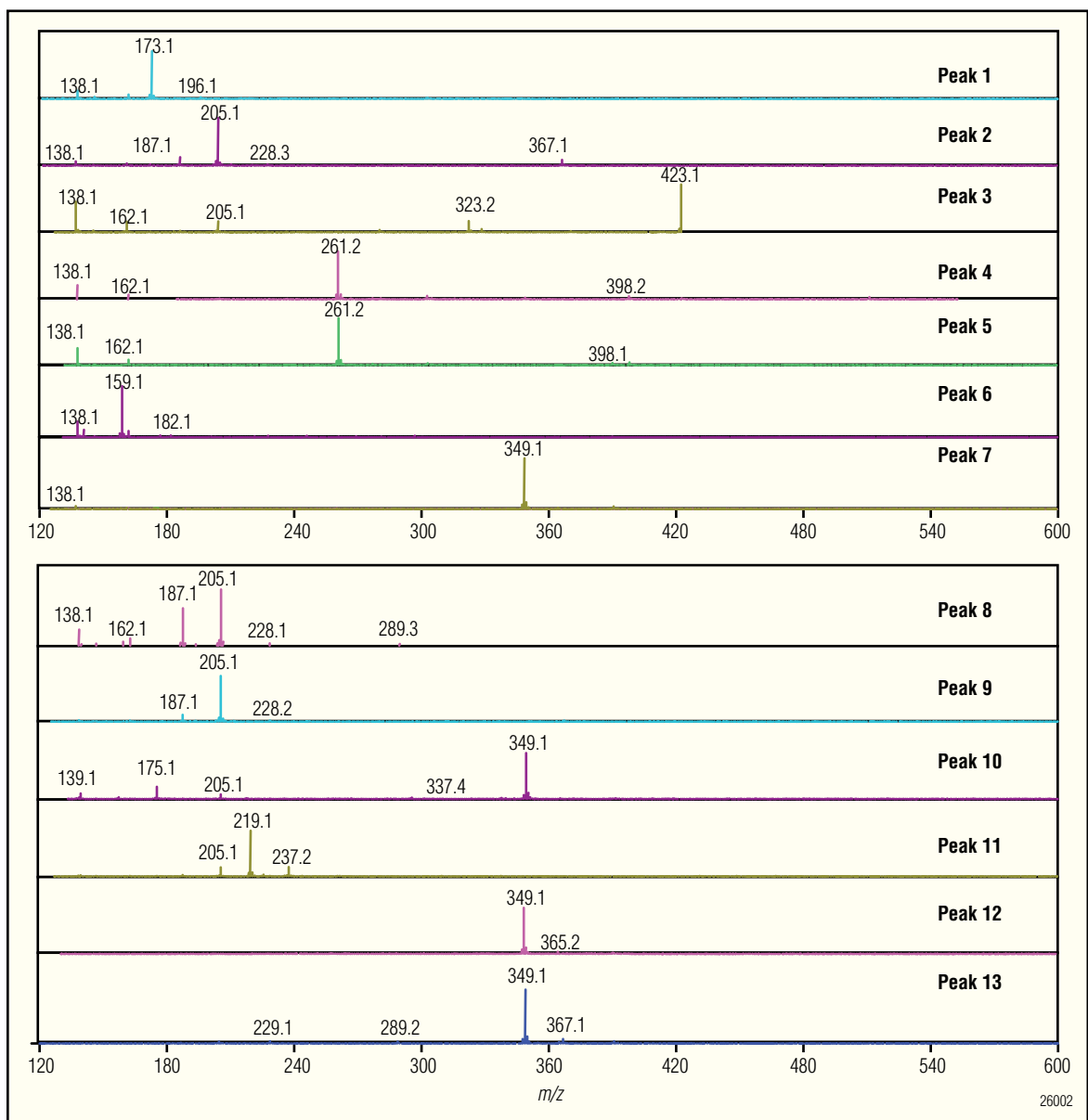
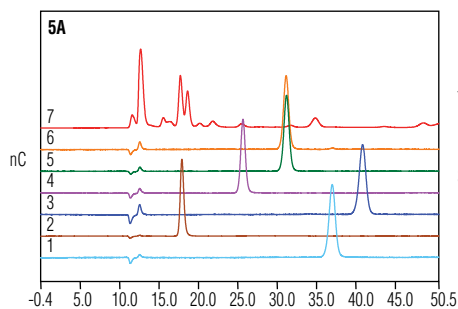
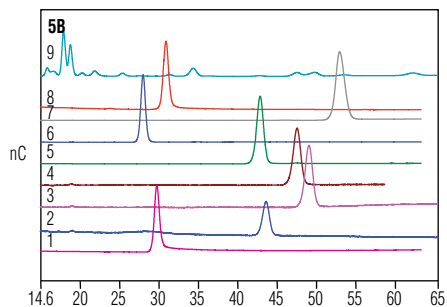


Figure 4. Background-subtracted ESI mass spectra of peaks shown in Figure 3.



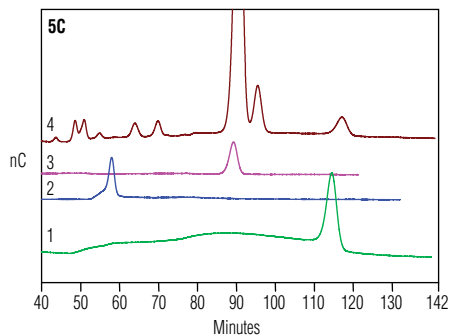
Column: CarboPac MA1 (4 x 250 mm) with Guard  
 Eluent: 500 mM NaOH, isocratic  
 Flow rate: 0.2 mL/min  
 Temperature: 30 °C  
 Detection: Integrated Pulsed Amperometry

Samples: 1. Mannitol 5. L-arabitol  
 2. Inositol 6. Dulcitol  
 3. Xylitol 7. Algae sample  
 4. D-arabitol



Column: CarboPac MA1 (4 x 250 mm) with Guard  
 Eluent: 500 mM NaOH, isocratic  
 Flow rate: 0.2 mL/min  
 Temperature: 30 °C  
 Detection: Integrated Pulsed Amperometry

Samples: 1. Galactosamine 6. Fucose  
 2. Arabinose 7. Galactose  
 3. Xylose 8. Glucosamine  
 4. Glucose 9. Algae sample  
 5. Mannose



Column: CarboPac MA1 (4 x 250 mm) with Guard  
 Eluent: 500 mM NaOH, isocratic  
 Flow rate: 0.2 mL/min  
 Temperature: 30 °C  
 Detection: Integrated Pulsed Amperometry

Samples: 1. Maltose  
 2. Lactose  
 3. Sucrose  
 4. Algae sample

26003

**Table 1. Identification of Sample Peaks Based on Mass-to-Charge Ratios and Retention Times**

| Peak | <i>m/z</i>  | Adduct                | Possible Identification  |
|------|-------------|-----------------------|--|
| 1    | 173.1       | 166 + 7               | (Fucitol + Li) <sup>+</sup>  |
| 2    | 205.1/187.1 | 180 + 7, 180 + 7 + 18 | (Inositol + Li) <sup>+</sup> , (Inositol + Li + H <sub>2</sub> O) <sup>+</sup> |
| 3    | 423.1       | Unknown               | Unknown  |
| 4    | 261.2       | Unknown               | Unknown  |
| 5    | 261.2       | Unknown               | Unknown  |
| 6    | 159.1       | 152 + 7               | (Monoalditol + Li) <sup>+</sup> (L-Arabitol)                                   |
| 7    | 349.1       | 342 + 7               | (Disaccharide + Li) <sup>+</sup>   |
| 8    | 205.1/187.1 | 180 + 7               | (Monosaccharide + Li) <sup>+</sup> (Mannose)                                   |
| 9    | 201.1/187.1 | 180 + 7               | (Monosaccharide + Li) <sup>+</sup> (Glucose)                                   |
| 10   | 349.1       | 342 + 7               | (Disaccharide + Li) <sup>+</sup>   |
| 11   | 219.1       | Unknown               | Unknown  |
| 12   | 349.1       | 342 + 7               | (Disaccharide + Li) <sup>+</sup> (Sucrose)                                     |
| 13   | 349.1       | 342 + 7               | (Disaccharide + Li) <sup>+</sup> (Maltose)                                     |

## CONCLUSIONS

Using simple sample preparation procedures, the carbohydrate content in lysed, microalgal biomass was separated on a CarboPac MA1 column with HPAE-PAD and analyzed using on-line mass spectrometry after treatment with the Carbohydrate Membrane Desalter. Peaks were identified based on their molecular weights and retention times.

## REFERENCES

1. Rocklin, R.; Pohl, C. Determination of Carbohydrates by Anion-Exchange Chromatography with Pulsed Amperometric Detection. *J. Liq. Chromatogr.* **1983**, *6*, 1577–1590.
2. Thayer, J.; Rohrer, J.; Avdalovic, N.; Gearing, P. Improvements to In-Line Desalting of Oligosaccharides Separated by High-pH Anion-Exchange Chromatography with Pulsed Amperometric Detection. *Analytical Biochem.* **1998**, *256*, 207–216

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