# Rapid and Sensitive HPAEC-PAD Analysis of Neutral Sugars and Uronic Acids in Biomass

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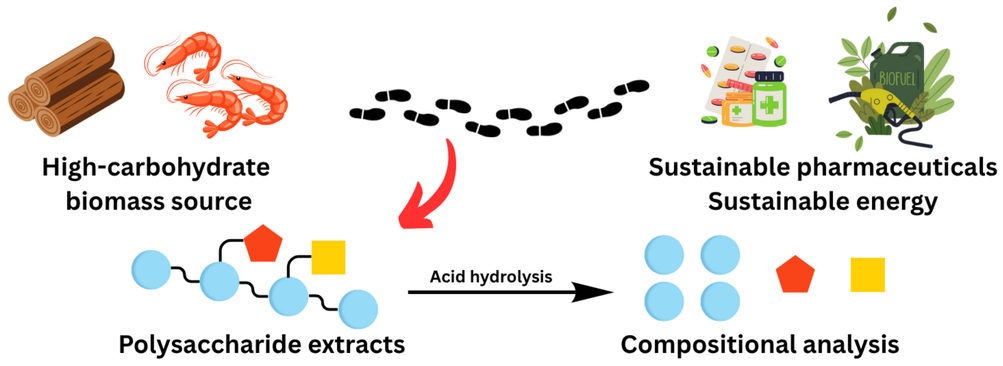
## Introduction




- Detailed understanding of biomass carbohydrate composition is an important step in developing its potential application
- The effectiveness of the extraction process directly influences the biomass's potential applications
- There is a need for a fast and accurate analysis method to evaluate composition of the extracted carbohydrates.
- Analysis using High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) in combination with the new SweetSep™ AEX20 anion-exchange column

## Stationary Phase

The SweetSep™ AEX20 stationary phase is developed for high-resolution separation of carbohydrates with HPAEC-PAD/MS.

- Rugged polymeric anion-exchange resin
- Highly monodisperse latex-coated particles (5 µm)
- Bifunctional anion exchange sites
- Fast, high-resolution separation
- Use of smaller ID column 2.1 mm × 200 mm

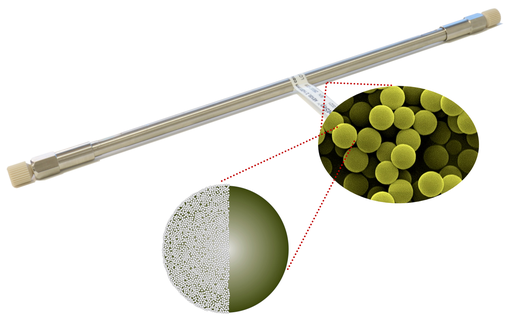


*Figure 1. SweetSep™ AEX 20 column based on a polymeric stationary phase consisting of monodisperse 5 µm particles coated with latex nano beads, functionalized with quaternary + tertiary amine groups.*

## Method and instrumentation

**HPAEC-ECD conditions**

| | |
|---|---|
| HPAEC system | ALEXYS™ Carbohydrate Analyzer |
| Columns | SweetSep™ AEX20, 2.1 mm ID × 200 mm analytical column, 5 µm<br>SweetSep™ AEX20, 2.1 mm ID × 50 mm precolumn, 5 µm<br>Borate ion trap, 2.1 mm ID × 50 mm column, 10 µm<br>All columns: Antec Scientific |
| Mobile phase (MP) | A: 10 mM NaOH<br>B: DI Water (resistivity > 18 MOhm.cm and TOC <5 ppb)<br>C: 200 mM NaOH<br>D: 200 mM NaOAc<br>Eluents blanketed with Nitrogen 5.0 |
| Flow rate | 0.18 mL/min |
| Backpressure | About 190 bar |
| Injection volume | 3 µL |
| Temperature | 27°C for separation, 45°C for detection |
| Flow cell | SenCell with Au WE, stainless steel AE, and HyREF Pd RE, AST 2 |
| PAD Potential waveform (4-step) | E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V<br>ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s |
| I-cell | About 0.2—0.5 µA |
| ADF | 0.05 Hz |
| Range | 10 µA/V |

## Results

### Chromatogram of standards

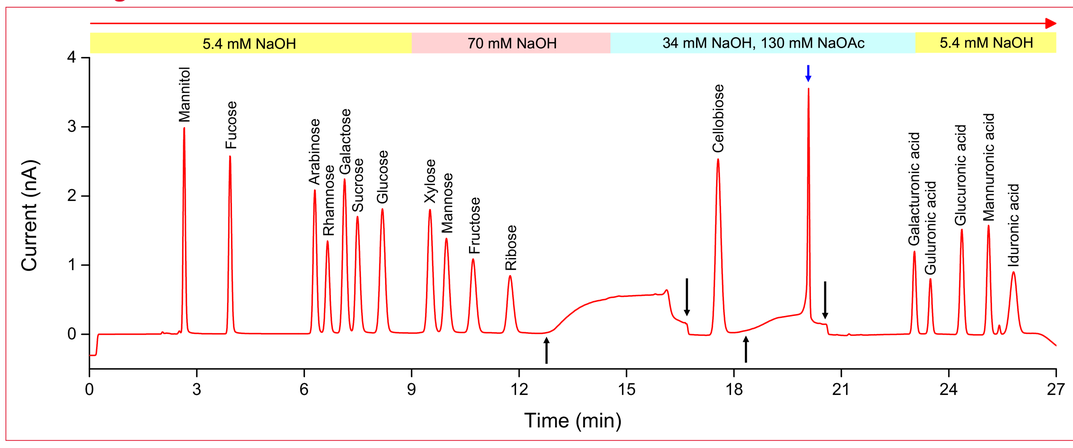



*Figure 2. The chromatogram obtained from a 3 µL injection of the 100 µM sugar standard mix in DI water. The black ↑ arrows indicate the start of a baseline elevation. The black ↓ arrows indicate an autozero to remove the baseline current offset. The blue arrow indicates a displacement of hydroxide ions from the column by the eluent containing acetate.*

### Sample analysis — Acid hydrolysates of wood sample

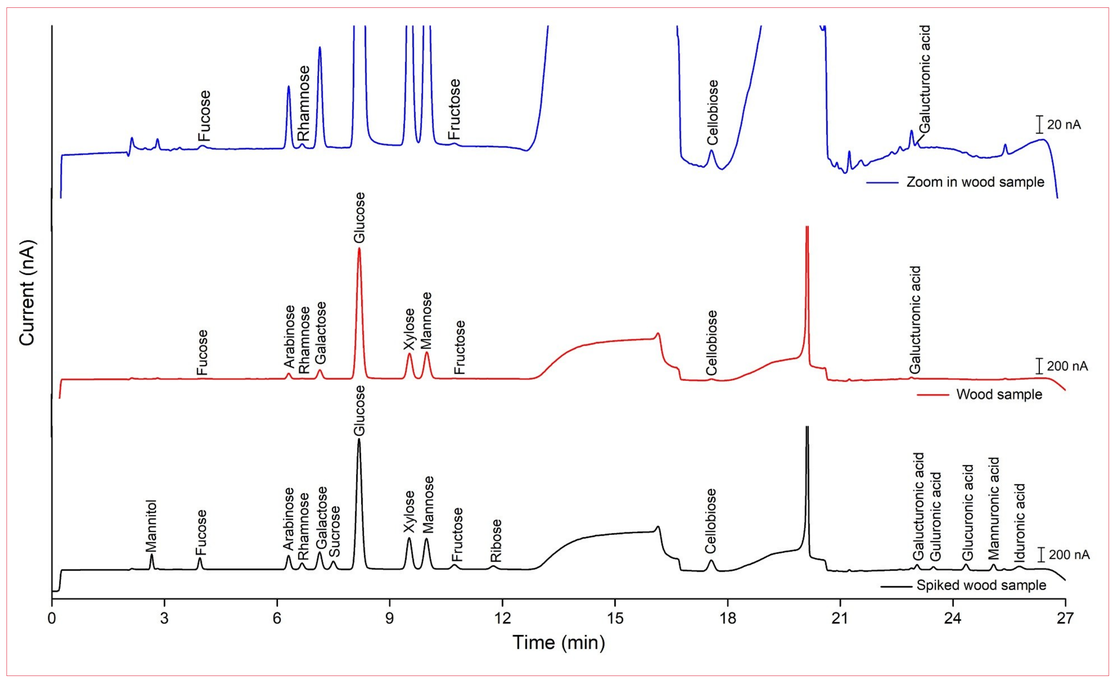



*Figure 3. Chromatograms from 3 µL injections of the acid hydrolysates of wood sample spiked with 5 µM standards (bottom, black line), the acid hydrolysate of wood sample (middle, red line), and the zoom into the baseline of the acid hydrolysate of wood sample (top, blue line).*

**Sample quantification results (mg/mL) and Limit of Detection (ng/mL)**

| Compound | Wood | Chitin | LOD | Compound, cont'd | Wood | Chitin | LOD |
|---|---|---|---|---|---|---|---|
| Mannitol | - | - | 3 | Fructose | 0.003 | - | 10 |
| Fucose | 0.002 | - | 3 | Ribose | - | - | 10 |
| Arabinose | 0.046 | 0.026 | 4 | Cellobiose | 0.023 | - | 41 |
| Rhamnose | 0.003 | - | 6 | Galacturonic acid | 0.003 | - | 83 |
| Galactose | 0.090 | - | 5 | Guluronic acid | - | - | 111 |
| Sucrose | - | - | 10 | Glucuronic acid | - | - | 65 |
| Glucose | 1.674 | - | 6 | Mannuronic acid | - | - | 58 |
| Xylose | 0.246 | - | 5 | Iduronic acid | - | - | 128 |
| Mannose | 0.412 | - | 7 | | | | |

### Targeted analysis of uronic acids

For applications focusing solely on uronic acids, a fast and targeted analysis using isocratic separation conditions can be applied. This method significantly increases the sample throughput.

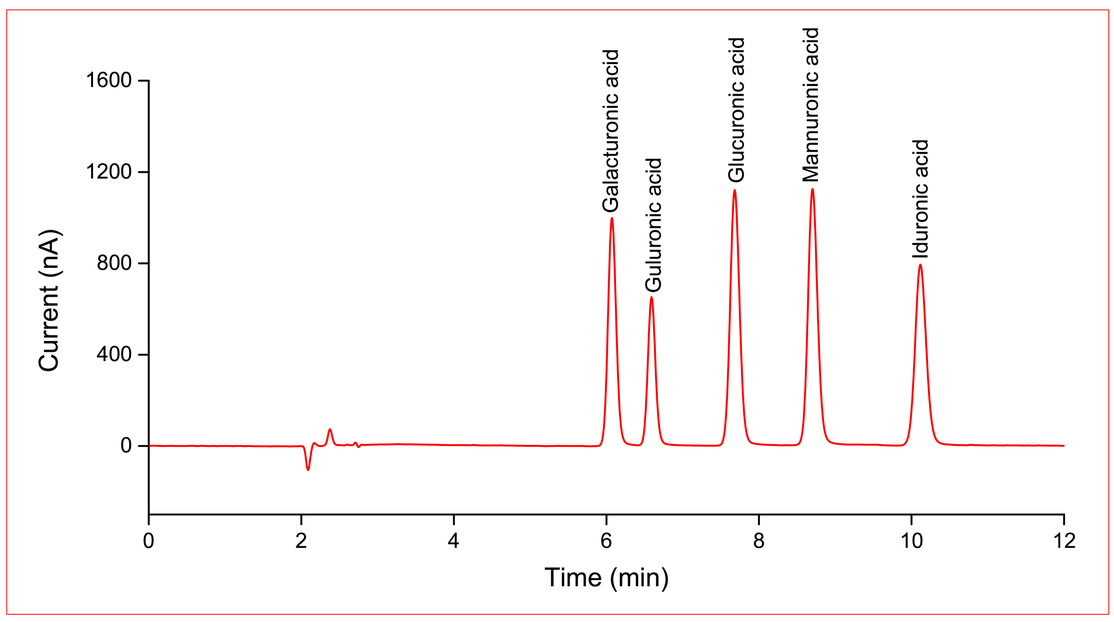



*Figure 4. Chromatogram obtained from 3 µL injections of the uronic acids standards. Isocratic elution using 34 mM NaOH + 130 mM NaOAc from t = 0 to t = 12 min. LOD values are 43, 62, 31, 37, and 34 ng/mL for galacturonic acid, guluronic acid, glucuronic acid, mannuronic acid, and iduronic acid, respectively.*

## Conclusion

High-resolution separation of wide range of neutral sugars and uronic acids was achieved within 26 minutes in one chromatographic run. The use of the 2.1 mm ID **SweetSep™ AEX20** columns allows for reduced mobile phase consumption and waste, aligning with green analytical chemistry principle. The presented data demonstrate:

- **Fast, high-resolution separation** of 12 neutral sugars and 5 uronic acids commonly present in biomass
- **High sensitivity** with limits of detection as low as 3 ng/mL—41 ng/mL (neutral sugars) and 65 ng/mL—128 ng/mL (uronic acids)
- Extraction efficiency can be optimized based on the sugar composition for further improving biomass utilization
- The use of ALEXYS Carbohydrate Analyzer in combination with SweetSep AEX20 is the ideal solution for the analysis of biomass hydrolysates