Novel Stationary Phase for High-Resolution Separation Of Carbohydrates in Biomass Hydrolyzates

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Introduction

High-Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) is the method of choice for the analysis of carbohydrates. It combines superior selectivity with sensitive detection down to femtomole levels without the need for derivatization or complicated sample preparation. A novel polymeric anion-exchange stationary phase SweetSep™ AEX20 has been developed based on monodisperse 5 µm particles. The size and exchange capacity of the latex nanoparticles was optimized to enable reproducible, high-resolution HPAEC-PAD analysis of mono– and disaccharides in a wide variety of carbohydrates samples. In this poster several methods are presented for the compositional analysis of neutral sugars, uronic acids and 5-hydroxymethyl furfural (HMF) in biomass hydrolyzates.

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

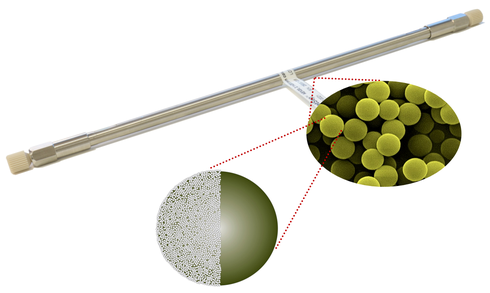

Figure 1. SweetSep™ AEX20 column based on a polymeric stationary phase consisting of monodisperse 5 µm particles coated with latex nano beads, functionalized with quaternary + tertiary amine groups.

Instrumentation

The **ALEXYS™ Carbohydrate Analyzer** is a dedicated metal-free HPAEC-PAD system consisting of:

- ET210 eluent tray, for sparging and blanketing of eluent with inert gas (N_2 or He).
- P6.1L quaternary LPG pump with 4 channel degasser.
- AS6.1L autosampler with cooling / heating (4°C up to 40°C)
- CT2.1 column oven / thermostat (5°C up to 85°C).
- SweetSep™ AEX20 2.1 x 200 mm column (pn 260.0021).
- DECADE Elite, electrochemical detector with dedicated flow cells (SenCell™ or FlexCell™) for carbohydrates.
- Data acquisition & instrument control via DataApex Clarity CDS Agilent OpenLab CDS or Thermo Scientific™ Chromeleon™ CDS.

Figure 2. ALEXYS™ Carbohydrate Analyzer (Antec Scientific).

Conditions

HPAEC-ECD conditions

HPAEC system	ALEXYS™ Carbohydrate Analyzer (Antec Scientific)
Columns	SweetSep™ AEX20, 2.1 x 200 mm analytical column, 5 µm (pn 260.0021) SweetSep™ AEX20, 2.1 x 50 mm precolumn, 5 µm (pn 260.0026) Borate ion trap, 2.1 × 50 mm trap column, 10 µm (pn 260.0031) All columns: Antec Scientific
Mobile phase (MP)	A: 10 mM NaOH, B: DI Water (resistivity > 18 MOhm.cm and TOC <5 ppb) C: 200 mM NaOH, D: 200 mM NaOAc. Eluents blanketed with Nitrogen 5.0
Flow rate	0.18 mL/min (back pressure 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 wave-form (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
I-cell	About 0.2—0.5 µA
Range, ADF	Range 10 µA/V, ADF 0.05 Hz

Results

Chromatogram of 12 neutral sugar and 5 uronic acid standards

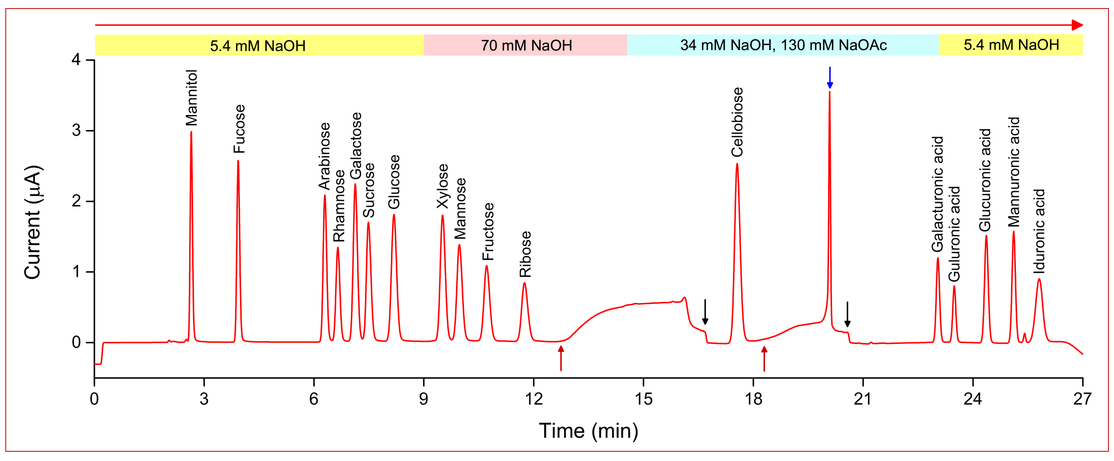

Figure 3. The chromatogram obtained from a 3 µL injection of the 100 µM sugar standard mix in DI water. The red ↑ arrows indicate the start of a baseline elevation. The black ↓ arrows indicate an autozero to remove the baseline current offset. The blue arrow indicates s a sharp OH^- peak due to displacement of a narrow zone of hydroxide ions from the column by the eluent containing acetate.

Sample analysis — Acid hydrolysates of wood samples

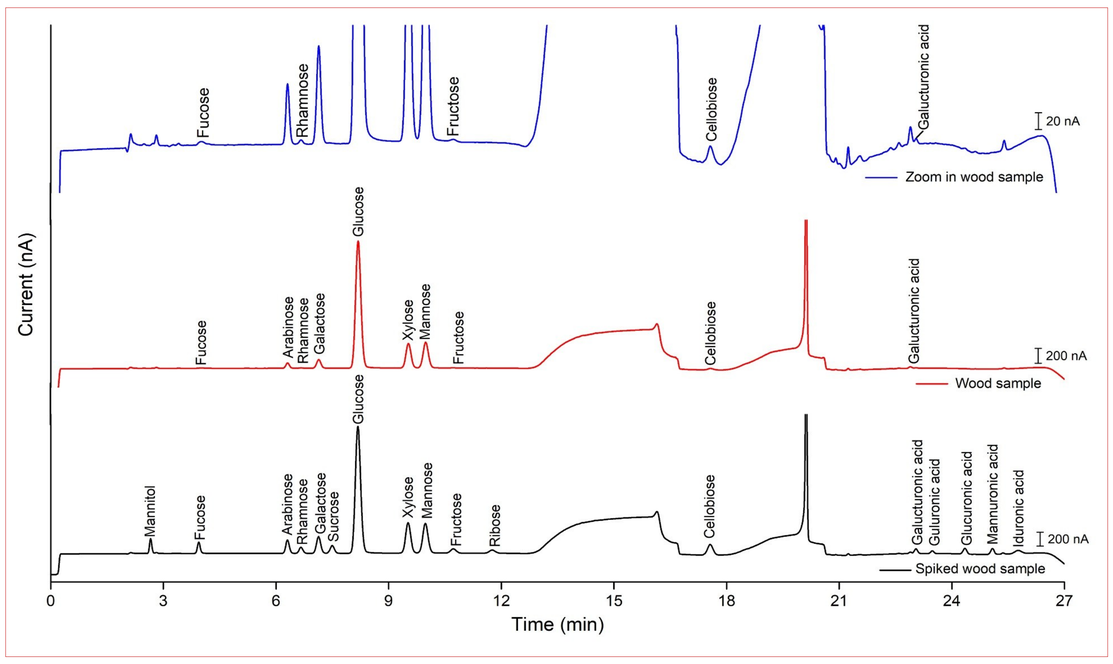

Figure 4. Chromatograms from 3 µL injections of the acid hydrolysates of wood sample spiked with 5 µM standards (bottom, black line), the acid hydrolysate of wood sample 100x dilution (middle, red line), and the zoom into the baseline of the acid hydrolysate of wood sample (top, blue line). LOD values are ranging from 3-41 ng/mL for the neutral sugars and 65-128 ng/mL for the uronic acids.

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.

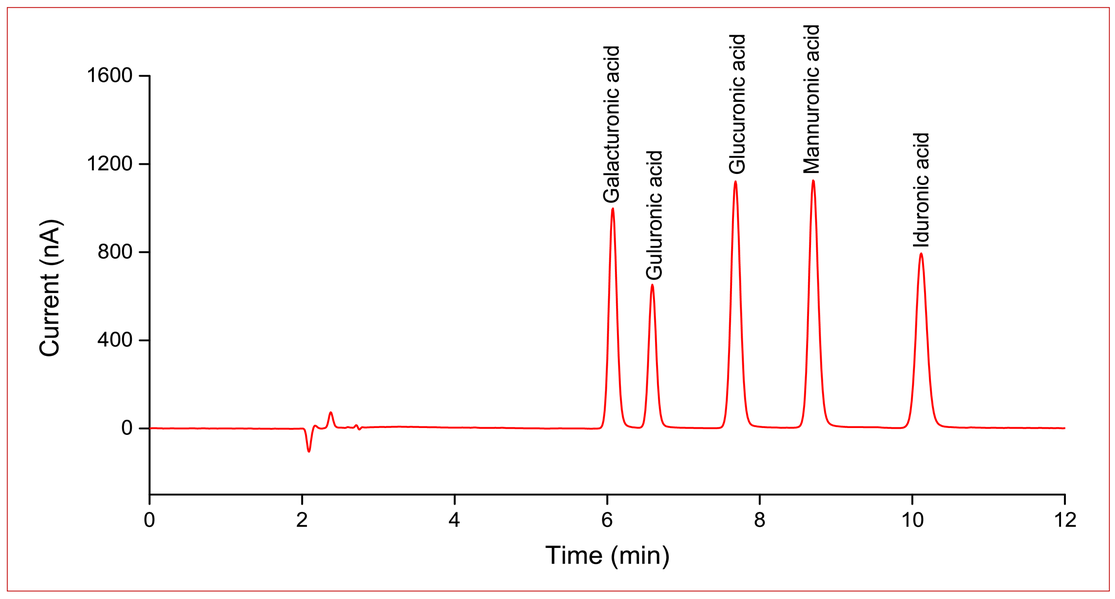

Figure 5. Chromatogram obtained from 3 µL injections of the uronic acids standards. Isocratic elution using 34 mM NaOH + 130 mM NaOAc from t = 0 to t = 12 min. LOD values are 43, 62, 31, 37, and 34 ng/mL for galacturonic acid, guluronic acid, glucuronic acid, mannuronic acid, and iduronic acid, respectively.

5-hydroxymethylfurfural (HMF) in dissolved pulp and paper grade pulp

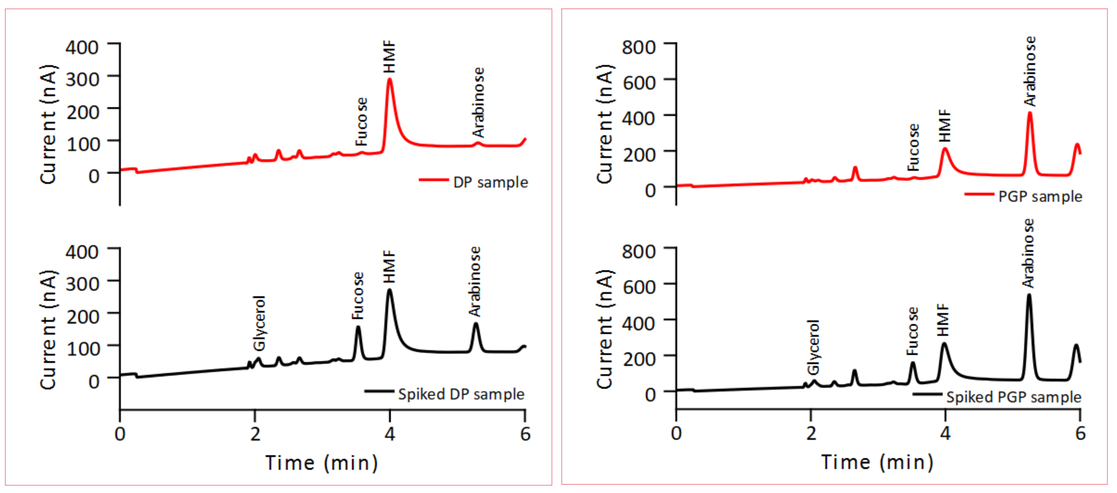

Figure 6. Chromatograms from 3 µL injections of an acid hydrolysate of a dissolved pulp (DP) and paper grade pulp (PGP) sample spiked with a 0.5 µg/mL standard mix (black line) and the acid hydrolysate of the DP and PGP sample 10x dilution (red line). Gradient elution using 10 mM NaOH to 15 mM NaOH from t = 0 to t = 2 min with a flowrate of 0.18 mL/min. The separation temperature was 25°C and detection temperature was 45°C. The LOD value for HMF is 10 ng/mL.

Conclusion

The novel SweetSep™ AEX20 anion-exchange microbore column was successfully utilized for the analysis of neutral sugars (mono– and disaccharides), uronic acids and HMF in various biomass hydrolyzates. The presented data demonstrate:

- **Fast, high-resolution separation (r> 1.5)** of HMF, 12 neutral sugars and 5 uronic acids commonly present in biomass hydrolyzates.
- **Sensitive quantification** with limits of detection as low as 3 ng/mL—41 ng/mL for the neutral sugars, 65 ng/mL—128 ng/mL for the uronic acids and 10 ng/mL for HMF.
- **Green analytical method:** 2.1 mm ID column minimizes solvent consumption and waste.
- The **ALEXYS™ Carbohydrate Analyzer** in combination with **SweetSep™ AEX20** offers a tailored solution for the selective & sensitive analysis of sugars in biomass hydrolysates.