SweetSep™ AEX200 a New Stationary Phase for Fast, High-Resolution Separation of Carbohydrates using HPAEC-PAD

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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™ AEX200 has been developed. The stationary phase is based on a monodisperse 5 µm resin coated with quaternary amine functionalized nanoparticles. The high uniformity and monodispersity of the resin allow for rapid, high-resolution separations of carbohydrates. The size and exchange capacity of the latex nanoparticles was optimized to enable the analysis of a wide variety of carbohydrates samples ranging from monosaccharides present in food, plants and glycoproteins up to oligosaccharides such as FOS (fructo-oligosaccharides) and N-linked glycans. In this posters several methods for the analysis of carbohydrates are presented demonstrating the performance of a new column based on AEX200 resin.

Table 1. Specifications SweetSep™ AEX200 column (pn 260.0010)

Type	Latex-coated pellicular resin
Particle diameter	5 µm
Material	ethylvinylbenzene-divinylbenzene copolymer
Crosslinking (%)	80%
Functionality	Quarternary amine groups
Column dimensions	200 x 4 mm ID
Ion Exchange Capacity	86 µeq per 4 x 200 mm column
Organic solvent limit	0 - 100% of any common solvent (cleaning)
Temperature limit	5 - 60°C (recommended 10 - 40°C)
pH range	0 - 14
Pressure rating	Max. 300 bar / 4500 psi
Typical back pressure	140 bar (0.7 mL/min, 12 mM NaOH, 30°C)

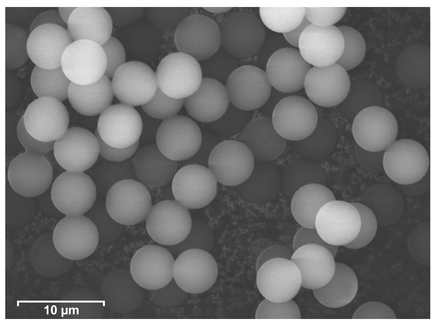

Figure 1. Scanning Electron Microscope (SEM) image of AEX200 resin. Magnification 1400 x, acceleration voltage 25 kV.

Instrumentation

The ALEXYS Carbohydrate Analyzer (Antec Scientific) 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 integrated 4-ch degasser
- AS6.1L autosampler with sample cooling (down to 4°C)
- CT2.1 column thermostat (temperature range 5°C - 85°C)
- DECADE Elite electrochemical detector with dedicated flow cells (SenCell™ or FlexCell™) for carbohydrates
- Instrument control & acquisition by DataApex Clarity CDS or Thermo Scientific™ Chromeleon™ CDS

Figure 2. ALEXYS Carbohydrate Analyzer.

Results

Column performance

Table 2. Column performance test results 4 x 200 mm AEX200 column #28

Compound	*Tr (min)*	*k*	*N*	*Rs*	*Symmetry /tailing*	*HETP (µm)*	*h*
Fucose	2.75	1.08	9427		1.34	21.2	4.2
Arabinose	4.39	2.33	12716	12.24	1.28	15.7	3.1
Galactose	5.19	2.93	10971	4.53	1.28	18.2	3.6
Glucose	5.69	3.31	8942	2.28	1.23	22.4	4.5
Sucrose	6.63	4.03	15601	4.17	1.16	12.8	2.6
Fructose	7.24	4.49	11589	2.534	1.50	17.3	3.5
Allolactose	10.84	7.21	16280	11.86	1.16	12.3	2.5
Lactose	11.67	7.84	13850	2.25	1.15	14.4	2.9
Lactulose	12.77	8.67	13530	2.64	1.28	14.8	3.0
Epilactose	14.58	10.05	16569	4.08	1.14	12.1	2.4

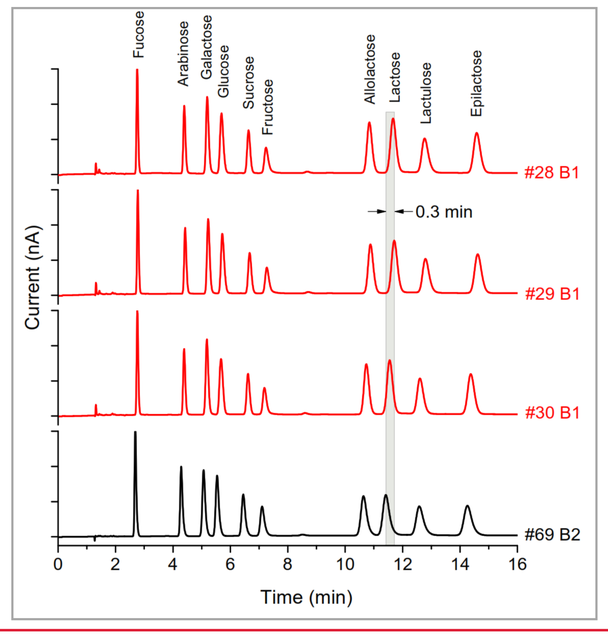

*Figure 3. Column reproducibility data. **Red curves:** comparison of three 4 x 200 mm columns #28, #29 and #30 packed with the same batch of AEX200 resin (B1) and run under identical LC conditions. **Black curve** (bottom): a 4 x 200 mm column (#69) packed with a different batch of AEX200 resin (B2). The maximum difference in retention time for Lactose between the columns was 0.3 minutes in this case. LC conditions (QAR): 10 µL injection of a 10 µM standard of 10 sugars in DI water, flow rate 0.7 mL/min, isocratic separation with 12 mM NaOH, T = 30°C.*

Analysis of mono-, di - and trisaccharides

Lactose in lactose-free products

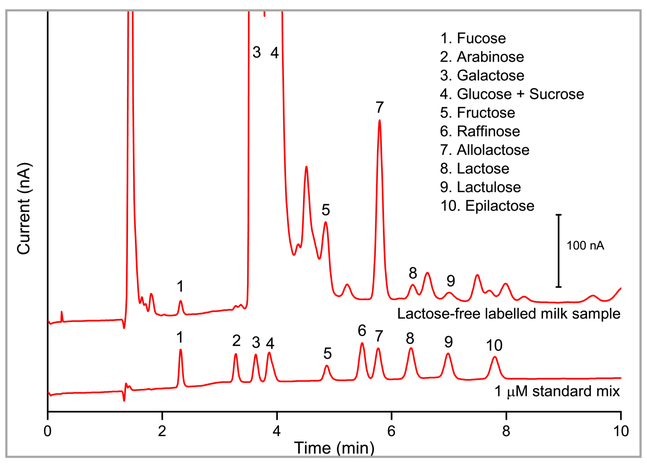

Figure 4. Analysis of milk on a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL injection of a lactose-free labelled milk sample. Bottom: 10 µL injection of a 1 µM standard of 11 sugars commonly found in milk. Isocratic elution: 12 mM NaOH + 3 mM NaOAc, 0.7 mL/min, 30°C.

Glycoprotein monosaccharides

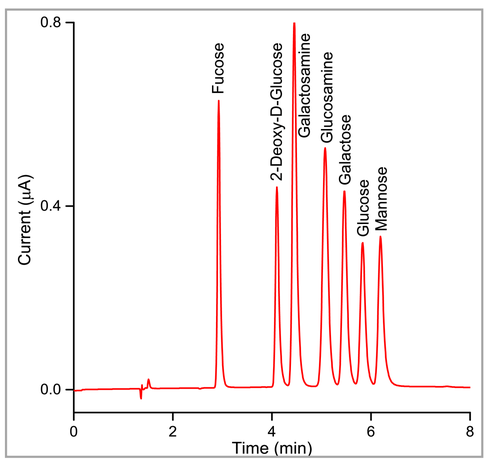

Figure 5. Separation of 10 µL injection of a 10 µM mixtures of glycoprotein monosaccharides on SweetSep™ AEX20 column, 4.0 mm ID × 200 mm. Isocratic elution: 17.5 mM NaOH, 0.7 mL/min, 30°C.

[18F]FDG - Fluorodeoxyglucose

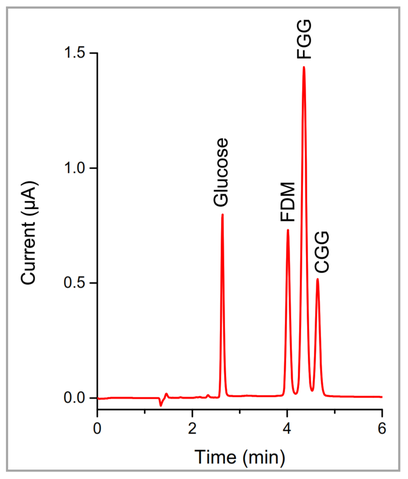

Figure 6. Impurity analysis of FDG and its side products FDM and CDG. 10 µL injection of a 25 µg/mL standard mix. Isocratic elution: 100 mM NaOH, 0.7 mL/min, 35°C. Resolution 2.0 and 1.7 between FDM-FDG and FDG-CDG, respectively (required Rs > 1.5).

Honey

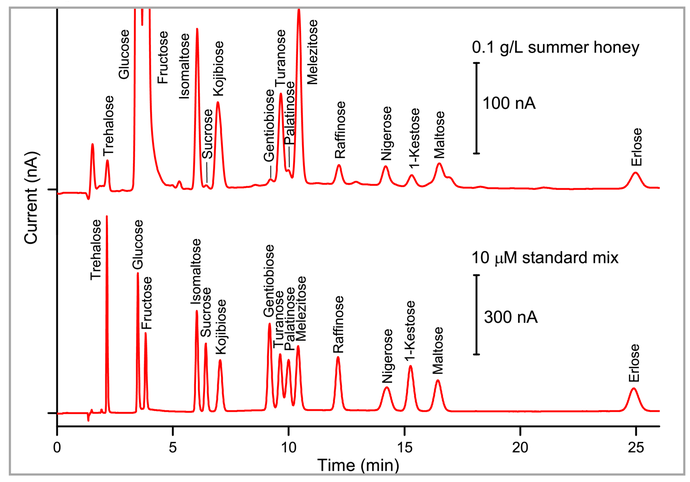

Figure 7. Analysis of honey on a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL injection of a 0.1 g/L Swiss summer honey sample. Bottom: 10 µL injection of a 10 µM standard of 15 sugars commonly found in honey. Isocratic elution: 68 mM NaOH, 0.7 mL/min, 20°C.

Analysis of oligosaccharides

Fructo-oligosaccharides (FOS)

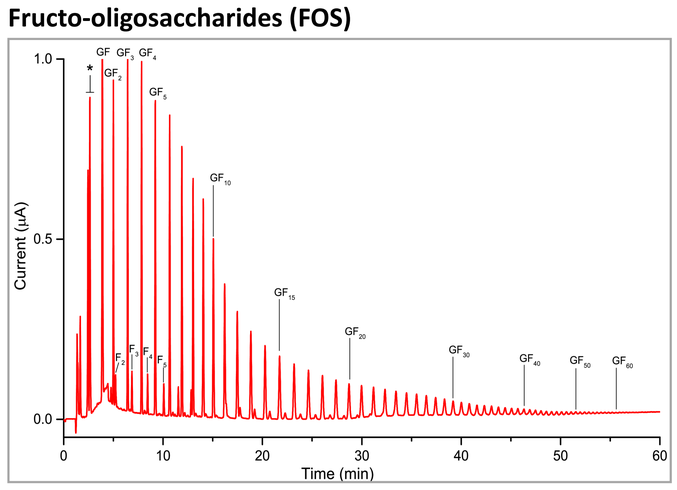

Figure 8. Gradient elution of 10 µL injection of a 200 ppm inulin from chicory on a SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Gradient elution: 0 min: 100 mM NaOH, 12 min: 100 mM NaOH + 180 mM NaOAc, 60 min: 100 mM NaOH + 450 mM NaOAc, 0.8 mL/min, 25°C.

Intact N-glycans

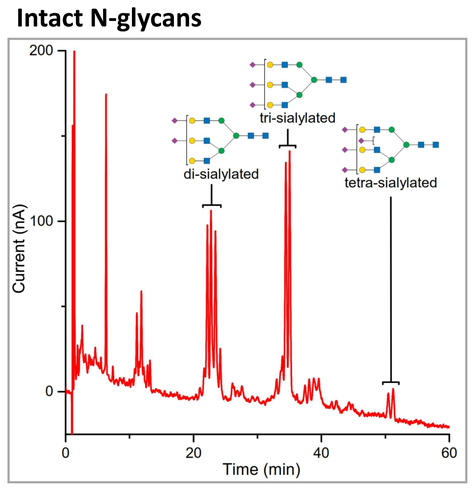

Figure 9. Separation of N-glycans standard containing di– tri– and tetra-sialylated oligosaccharides on a SweetSep™ AEX 200 column, 4.0 mm ID × 200 mm. Gradient elution: 0 min: 100 mM NaOH + 6 mM NaOAc, 70 min: 100 mM NaOH + 190 mM

Conclusion

A new stationary phase based on highly monodisperse 5 µm particles coated with quaternary amine functionalized nanoparticles, **SweetSep™ AEX200**, has been developed which enables:

- **Fast, high-resolution separation** of carbohydrates at moderate column back pressures
- Analysis of carbohydrates over a high molecular weight range: **from mono– to oligosaccharides** with a high degree of polymerization
- Analysis of complex mixtures of carbohydrates with both ECD and MS detection* (desalter required depending on the volatility of the buffer system used).

*) Data shown in poster P-OMIC-20, poster session 1

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