Analysis of Carbohydrates in Food Using a New Anion-Exchange Column SweetSep™ AEX200

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Introduction

High-Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) is a well-established method for the analysis of carbohydrates in food and life science. A novel anion-exchange stationary phase, SweetSep™ AEX200 (Antec Scientific), has been developed. This new resin based on monodisperse 5 µm particles is particularly suitable for high-resolution analysis of underivatized carbohydrates in complex food samples followed by sensitive direct quantification down to sub-picomole levels using PAD. To demonstrate the performance of the new SweetSepTM AEX200 column three applications will be presented for the analysis of carbohydrates, ranging from mono- to oligosaccharides, in (1) natural honey, (2) lactose-free products, and (3) dietary fiber products containing fructans. In all examples, the presented methods show fast, sensitive, and high resolution separation of carbohydrates in food.

Method & Instrumentation

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 sample tray cooling up to 4°C.
- CT2.1 Column Oven / Thermostat (5°C up to 85°C).
- SweetSep™ AEX200 4 x 200 mm column (pn 260.0010).
- DECADE Elite, electrochemical detector with dedicated flow cells (SenCell™ or FlexCell™) for carbohydrates.
- Data acquisition and instrument control via DataApex Clarity CDS or Thermo Scientific™ Chromeleon™ CDS.

Figure 1. ALEXYS Carbohydrate Analyzer.

Results

1. Analysis of sugars in natural honey

Profiling and quantification of sugars in honey can be used to assess floral origin, quality and adulteration of honey. Separation of 15 sugars commonly found in honey was achieved within 27 minutes with sufficient resolution (figure 2).

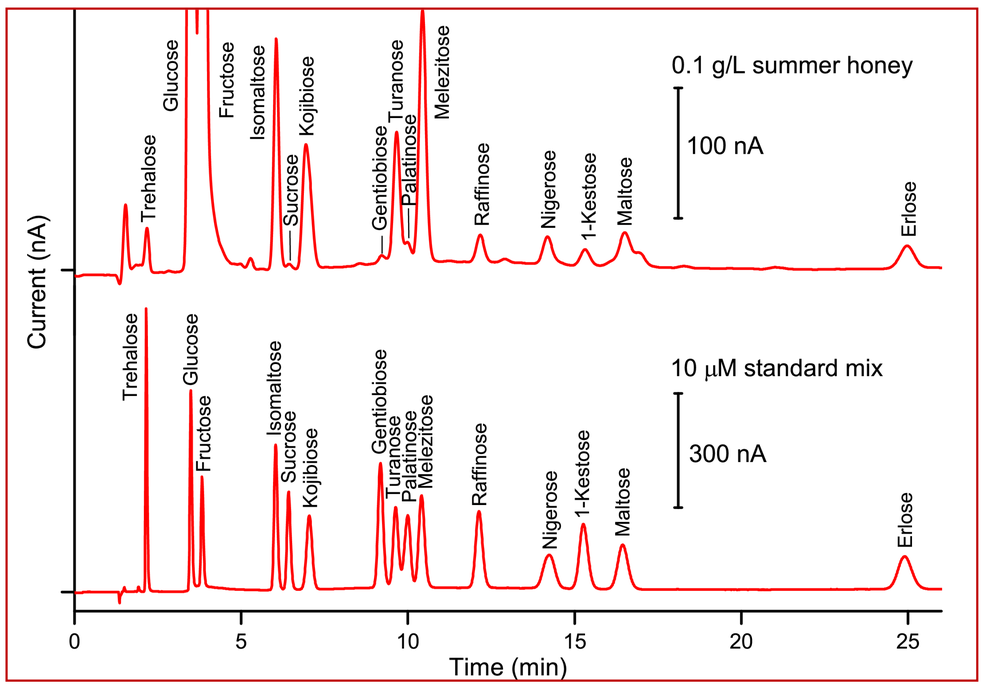

Figure 2. Analysis of honey on SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL injection of a 0.1 g/L Swiss summer honey sample. Bottom: 10 µL injection of a 10 µM standard of 15 sugars commonly found in honey. Isocratic elution: 68 mM NaOH, 0.7 mL/min, 20°C. Sample prep: simple dilution & filtering over a 0.2 µM PES filter.

Table 1. Quantification and adulteration criteria of honey samples

Parameters	*Pure honey criteria*	*Amount in sample*	*Within criteria?*
Sucrose	< 5 g/100 g	0.1 g/100 g	✓
Maltose	< 4 g/100 g	0.5 g/100 g	✓
Fructose + Glucose	> 45 g/100 g	54.5 g/100 g	✓
Fructose / Glucose	0.9 – 1.4	1.4	✓

2. Lactose in lactose-free dairy products

The presented method allows fast, high-resolution separation (within 8 minutes) of lactose and its isomers allolactose, lactulose and epilactose, including raffinose a trisaccharide which might be present in whole grain and cacao products containing dairy (figure 3).

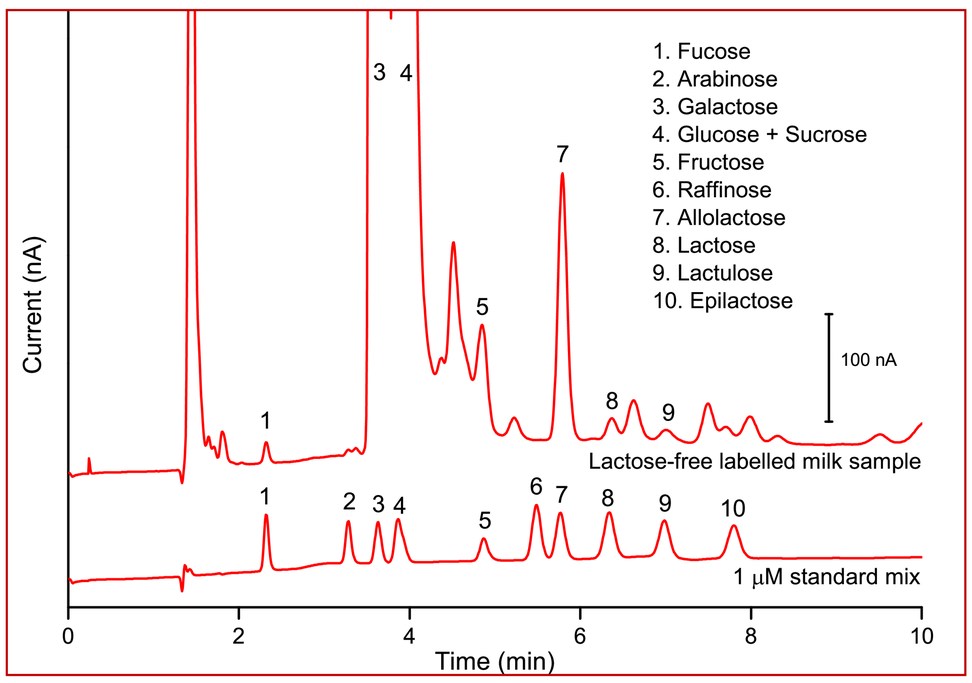

Figure 3. Analysis of milk on SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Top: 10 µL injection of a lactose-free labelled UHT milk sample. Bottom: 10 µL injection of a 1 µM standard of 11 sugars including lactose and its isomers. Isocratic elution: 12 mM NaOH + 3 mM NaOAc, 0.7 mL/min, 30°C. Sample prep: Carrez precipitation followed by centrifugation step and filtering of supernatant over a 0.2 µM PES filter.

Table 2. Lactose & allolactose, contents and sample recovery in lactose-free labelled products

Product	*Lactose*		*Allolactose*	
	*mg/100 g product**	*Recovery (%, n=3)*	*mg/100 g product*	*Recovery (%, n=3)*
Semi-skimmed milk UHT	1.2	99.5	26.9	119.0
Quark	1.2	78.9	7.5	91.4
Latte	3.8	97.1	24.3	115.6

*)upper limit of lactose concentration in lactose-free products is 10 mg / 100 g product

Table 3. Linearity, Limit of Detection (LOD) and Quantitation (LOQ) for Lactose and Isomers

Carbohydrate	*LOD*		*LOQ*		*Correlation coefficient r**
	(ppb)	*(nM)*	*(ppb)*	*(nM)*	
Allolactose	1.5	4.5	5.1	14.9	0.999
Lactose	1.5	4.5	5.1	15.1	0.999
Lactulose	1.1	3.3	3.7	10.9	0.999
Epilactose	2.3	6.8	7.7	22.6	1.000

*) concentration range of 0.1 - 40 µM (35 - 14×10^3 ppb).

3. Analysis of fructooligosaccharides from inulin

Profiling of fructo-oligosaccharides (FOS) in dietary fiber products is based on gradient elution with acetate modifier. Excellent separation of short-chain FOS and long-chain inulin standards was achieved up to degree of polymerization (DP) of 60 (figure 4).

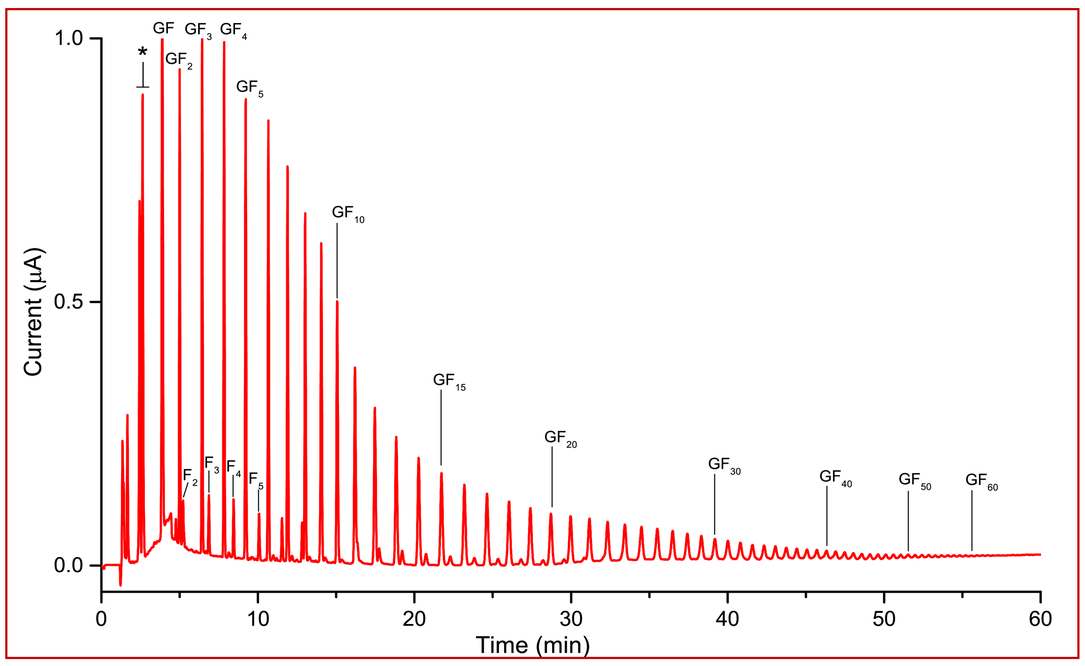

Figure 4. Chromatogram of 10 µL injection of a 200 ppm inulin standards on SweetSep™ AEX200 column. Elution: 0 min: 100 mM NaOH, 12 min: 100 mM NaOH + 180 mM NaOAc, 60 min: 100 mM NaOH + 450 mM NaOAc, 0.8 mL/min, 25°C.

Conclusion

A novel 4 x 200 mm anion-exchange column based on highly monodisperse 5 µm particles, SweetSep™ AEX200 (pn 260.0010), was utilized for the analysis of carbohydrates in various food samples. The presented data obtained with the new **SweetSep™ AEX200 column** demonstrate:

- **Fast, high-resolution separation** of mono-, di- and trisaccharides in various sample matrices such as honey and lactose-free dairy products.
- **Sensitive quantification** of carbohydrates up to femtomole levels using the new column in combination with the ALEXYS carbohydrates analyzer.
- High-resolution separation of high-molecular weight oligosaccharides. In case of fructo-oligosaccharides from inulin up to a degree of polymerization DP of 60.

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