New Stationary Phase for the Superior Separation of all Classes of Carbohydrates in F&B

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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 quantification down to sub-picomole levels using PAD. To demonstrate the performance of the new SweetSep™ AEX200 column four applications are presented for the analysis of carbohydrates in honey (1), fructans (2), lactose in lactose free labelled products (3), and beer (4). In all examples, the presented methods show fast, sensitive, and high resolution separation of the carbohydrates of interest. Analysis conditions and performance data are described in detail in the respective application notes, downloadable on www.antecscientific.com.

Instrumentation

Dedicated metal-free HPAEC-PAD system
ALEXYS™ Carbohydrate Analyzer

- 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™ AEX200 4 x 200 mm column (pn 260.0010).
- DECADE Elite, electrochemical detector with dedicated flow cells (SenCell™ or FlexCell™) for carbohydrates.

ALEXYS™ Carbohydrate Analyzer.

Results

1. Honey fraud and adulteration

Selective separation of oligo- and polysaccharides in honey enables the detection of adulteration, helping to prevent food fraud and protect both producers and consumers (figure 1).

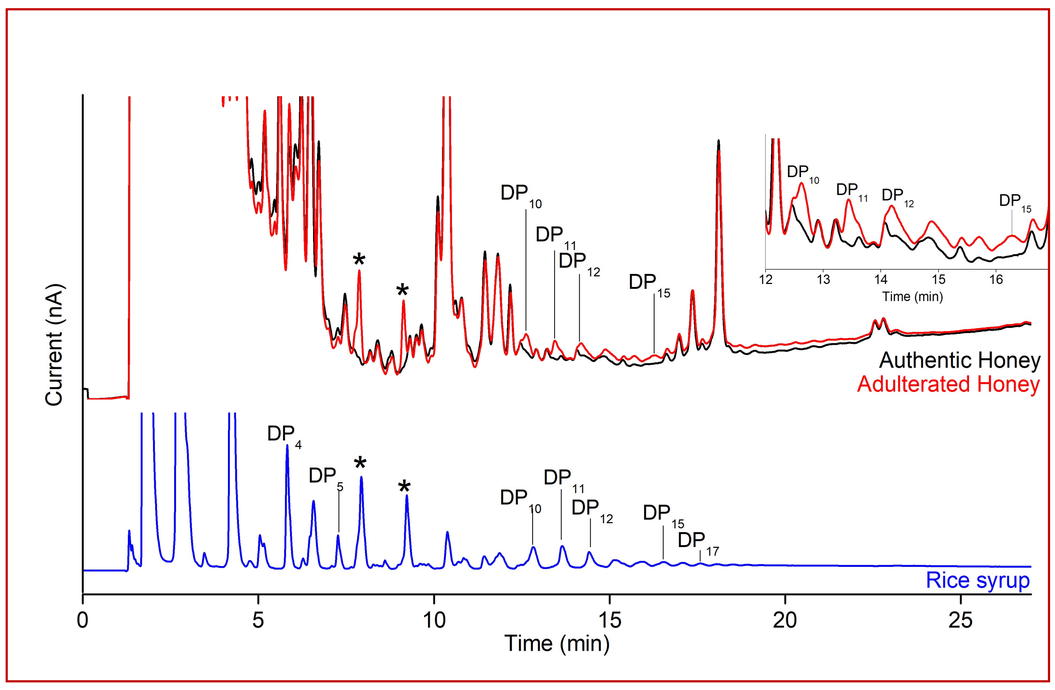

Figure 1. *Overlay of chromatograms of a 10 µL injection of 2.5 g/L rice syrup sample (blue trace), authentic avocado honey sample (black trace), and avocado honey sample adulterated with 1.1% rice syrup (red trace). Peaks marked with (*) are from the rice syrup. Linear gradient from 100 mM NaOH + 40 mM NaOAc to 100 mM NaOH + 450 mM NaOAc from t = 0 to t = 30 min.*

2. Inulin-type fructans

HPAEC-PAD analysis is a powerful tool for profiling the chain length distribution of inulin-type fructans. It enables fast, high-resolution separation of inulin, allowing detection of inulin with a degree of polymerization (DP) greater than 90 (figure 2).

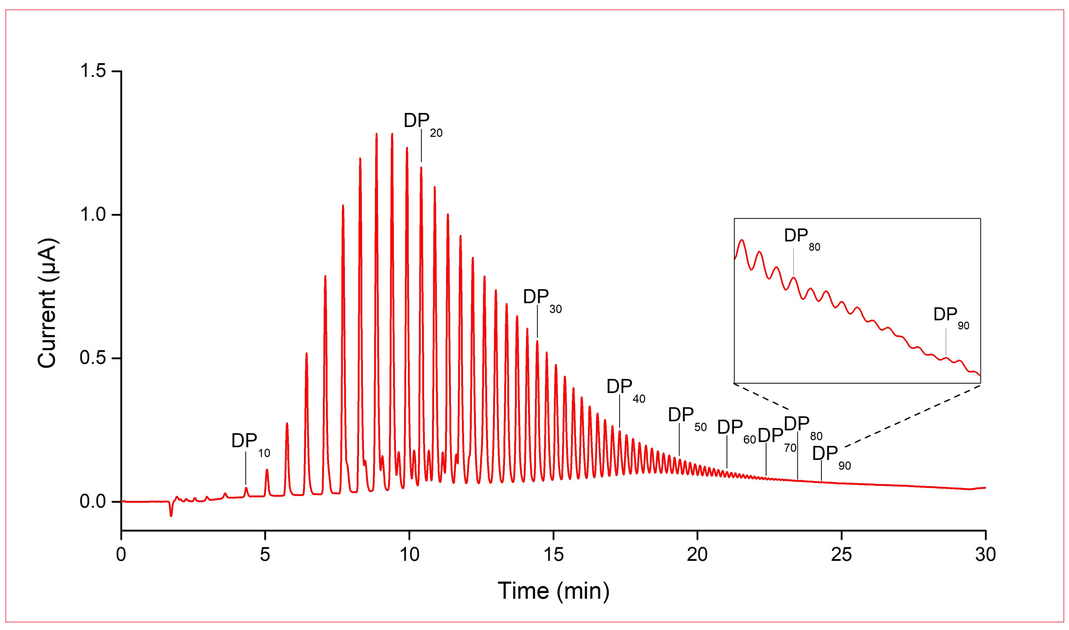

Figure 2. *Chromatogram obtained from an 10 µL injection of a 200 ppm solution of inulin-type fructan in DI water. Inset (right): zoomed plot area between DP80 - DP90. Linear gradient from 100 mM NaOH + 25 mM $NaNO_3$ to 100*

3. 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). Detection limits around 10 nmol/L are attainable for most of the sugars.

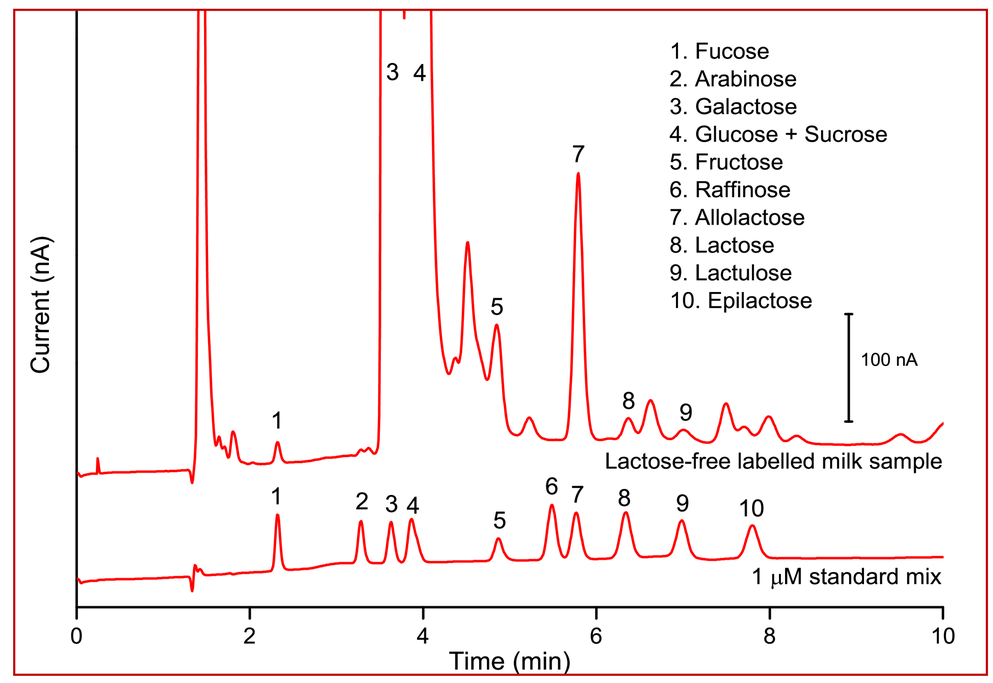

Figure 3. *Top: 10 µL injection of a lactose-free labelled UHT milk sample. Bottom: 10 µL injection of a 1 µM standard of 11 sugars including lactose and its isomers. Isocratic elution: 12 mM NaOH + 3 mM NaOAc, 0.7 mL/min, 30°C. Sample prep: Carrez precipitation followed by centrifugation step and filtering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

4. Sugars in beer

A HPAEC-PAD method is presented for the analysis of fermentable sugars and two additional non -fermentable sugars in beer samples. All sugars of interest are baseline separated and elute within 35 minutes (figure 4).

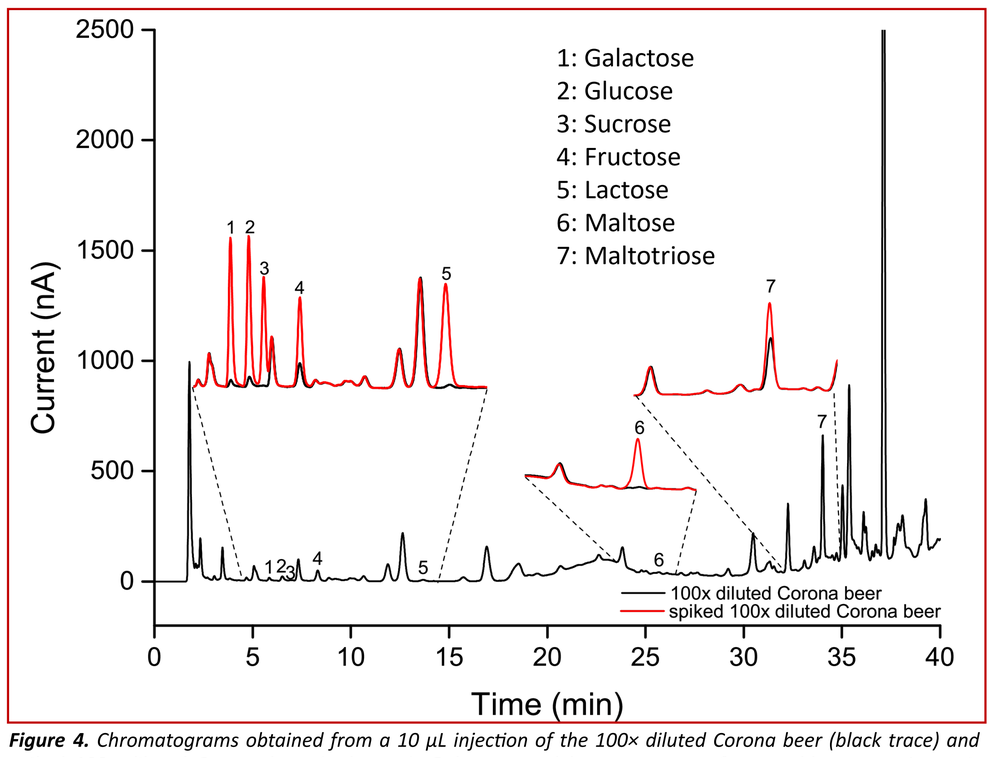

Figure 4. *Chromatograms obtained from a 10 µL injection of the 100× diluted Corona beer (black trace) and spiked 100x diluted Corona beer (red trace). Galactose and lactose are non-fermentable sugars. Isocratic elution at 9 mM NaOH from t = 0 to t = 15 min followed by a linear gradient to 100 mM NaOH within 10 minutes followed by another linear gradient to 100 mM NaOH + 100 mM NaOAc from t=25 to t=40 min*

Conclusions

A novel anion-exchange column based on highly monodisperse 5 µm particles, SweetSep™ AEX200, 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.
- **Sensitive quantification** of carbohydrates down to femtomole levels using the new column in combination with the ALEXYS™ Carbohydrate Analyzer.

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