

Gluten Intolerance Triggered by Fructans: A Sensitive Method for the Analysis of Fructans using HPAEC-PAD

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Introduction



- There is a need for a fast and accurate analysis method to detect and quantify fructans in food products.
- In this analysis High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) in combination with the new SweetSep™ AEX200 anion-exchange column is used.

Stationary Phase

The SweetSep™ AEX200 stationary phase is specifically developed for the separation of all classes of carbohydrates ranging from mono- up to polysaccharides using HPAEC-PAD/MS.

- Strong polymeric anion-exchange resin
- Highly monodisperse latex-coated particles (5 µm)
- Fast, high-resolution separation
- 2.1 mm ID and 4 mm ID version available

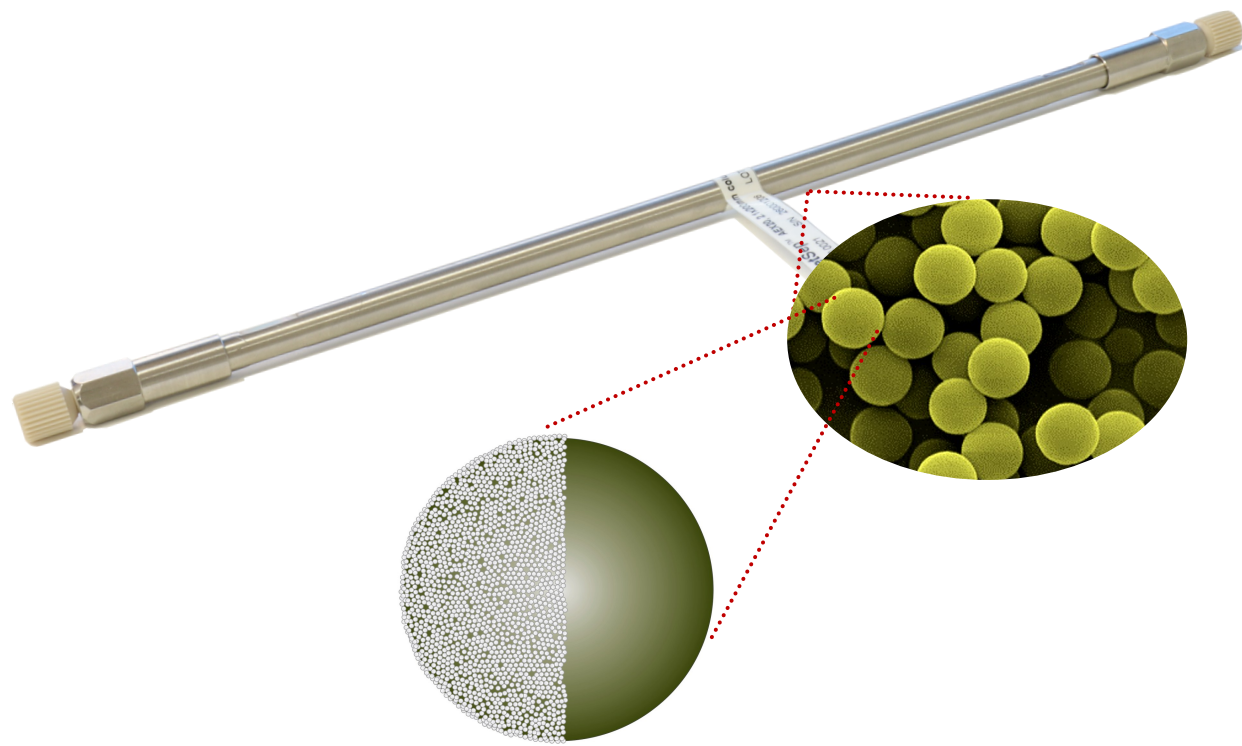


Figure 1. SweetSep™ AEX 200 column based on a polymeric stationary phase consisting of monodisperse 5 µm particles coated with latex nano beads, functionalized with quaternary amine groups (for clarity, only half of the nano-beads are shown).

Method & instrumentation

Dedicated metal-free HPAEC-PAD system consisting of:

- ET210 eluent tray, for sparging and blanketing of eluent with inert gas (N₂ 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).
- 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 2. ALEXYS™ Carbohydrate Analyzer (Antec Scientific)

Table 1: Adapted AOAC 2016.14/ISO 22579:2020 method

HPLC system	ALEXYS™ Carbohydrates Analyzer
Columns	SweetSep™ AEX200, 2.1 × 200 mm column, 5 µm SweetSep™ AEX200, 2.1 × 50 mm precolumn, 5 µm Borate ion trap, 2.1 × 50 mm column, 10 µm (Antec Scientific)
Mobile phase	A: DI Water B: 100 mM NaOH C: 100 mM NaOH + 100 mM NaOAc Eluents prepared & blanketed with Nitrogen 5.0
Flow rate	0.18 mL/min
Back pressure	about 200 bar, column net pressure about 130 bar
Injection	2.5 µL full loop injection
Temperature	35°C for separation and 45°C detection
Flow cell	SenCell with Au WE, stainless steel AE and HyREF palladium RE, AST 2
Potential waveform (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 µA
ADF	0.05 Hz
Range	10 µA/V

Results

1. Fructan analysis according to AOAC 2016.14/ISO 22579:2020

AOAC 2016.14/ISO 22579:2020 describes the method to determine the total fructan content in nutritional formula with HPAEC-PAD. This optimized method with the SweetSep™ AEX200 allows for direct analysis of fructan constituents without post-column addition and fast separation (figure 3).

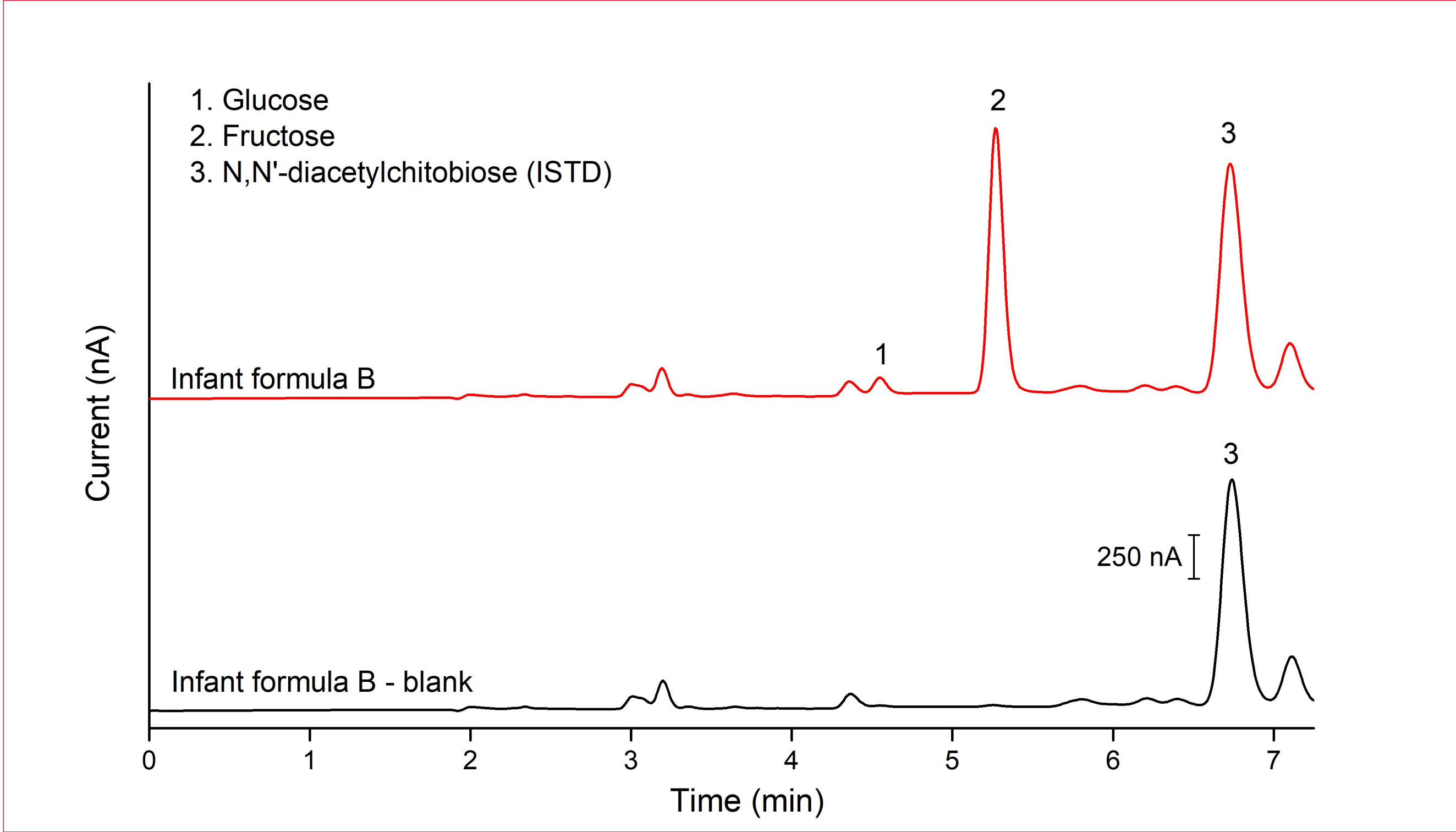


Figure 3. Analysis of infant formula on SweetSep™ AEX200 column, 2.1 mm ID × 200 mm. Top: 2.5 µL injection of a hydrolyzed infant formula. Bottom: 2.5 µL injection of a non-hydrolyzed test solution of infant formula. For gradient see application note 'Fructans in Infant Formula' on www.antecscientific.com

Table 2: Signal to noise ratio

Sample	S/N Glucose	S/N Fructose
Calibration standard (1.4 µg/mL fructose and 0.14 µg/mL glucose)	168	1264
Infant formula B containing fructan	245	4356

Table 3: Method performance requirements

Parameter	Value	Measured value	Within requirements?
LOQ (g/100g)	≤ 0.03	≤ 0.01	✓
Repeatability (RSD, %)	< 6	< 1	✓
Recovery (%)	90-110	98 - 100	✓

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 4).

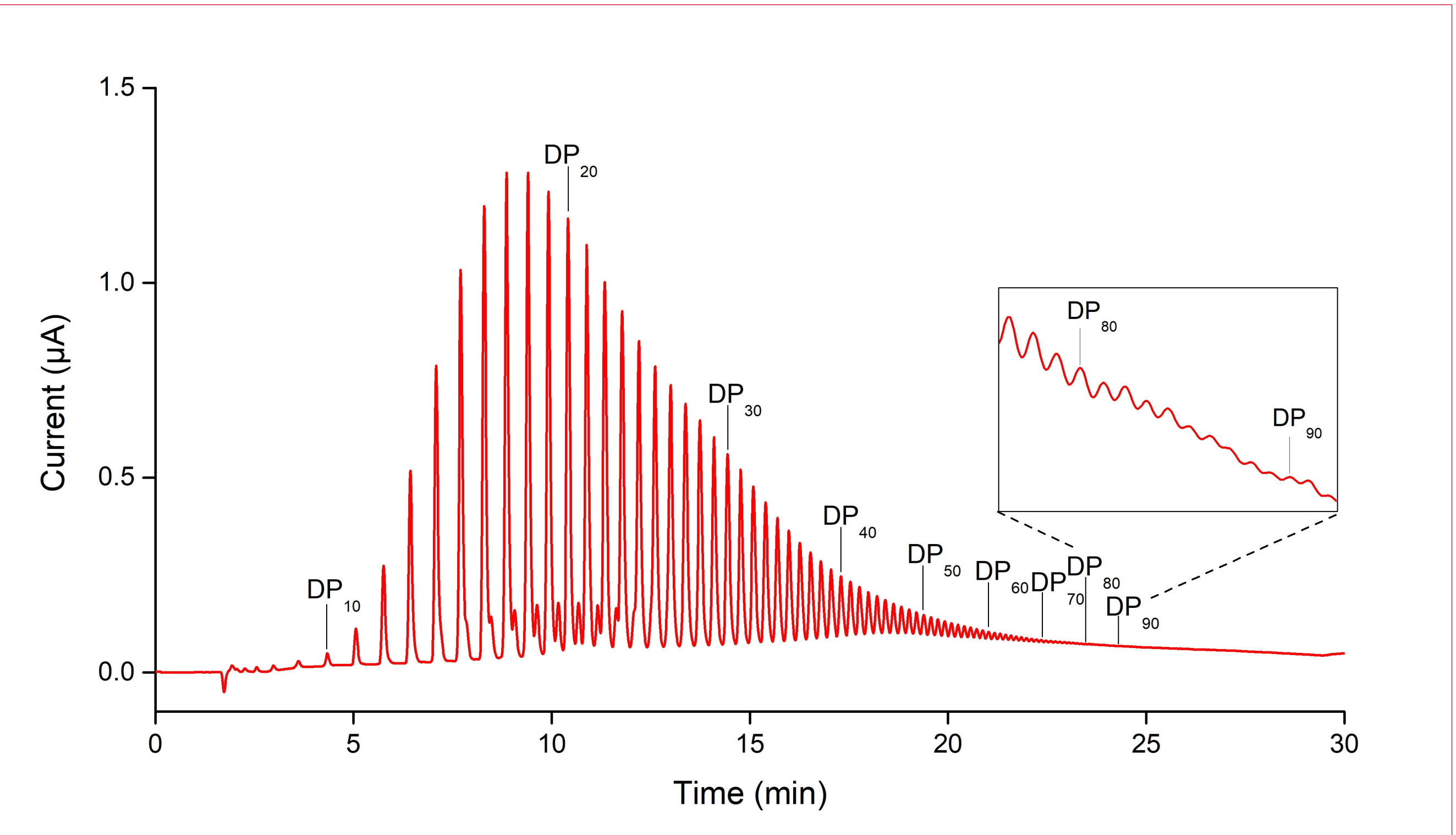


Figure 4. Chromatogram obtained from an 10 µL injection of a 200 ppm solution of inulin-type fructan in DI water. Inset (right): zoomed plot area between DP80 - DP90. Measured with the SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. See conditions and gradient in application note 'Inulin from chicory' on www.antecscientific.com.

Conclusions

A novel 4 × 200 mm and 2.1 × 200 mm anion-exchange column based on highly monodisperse 5 µm particles, SweetSep™ AEX200 (pn 260.0010 & pn 260.0011), was utilized for the analysis of fructans. The presented data obtained with the new SweetSep™ AEX200 column demonstrate:

- Fast, high-resolution separation of fructans in infant formula and inulin-type fructans.
- Optimized method for AOAC 2016.14/ISO 22579:2020 that meets all AOAC fructan analysis requirements.
- Sensitive quantification with S/N ratios at least 16 times above the required minimum.
- The SweetSep™ AEX200 in combination with the ALEXYS™ Carbohydrate Analyzer is the perfect choice for fructan analysis in foods and beverages.

