Short application note

Food & beverage

Inulin from chicory

Keywords

ALEXYS carbohydrate analyzer, HPAEC-PAD, SweetSep™AEX200, Inulin, prebiotic, fructans, FOS, plant-derived oligo– and polysaccharides, sodium nitrate pushing agent, fast high-resolution separation > DP 90 within 25 min

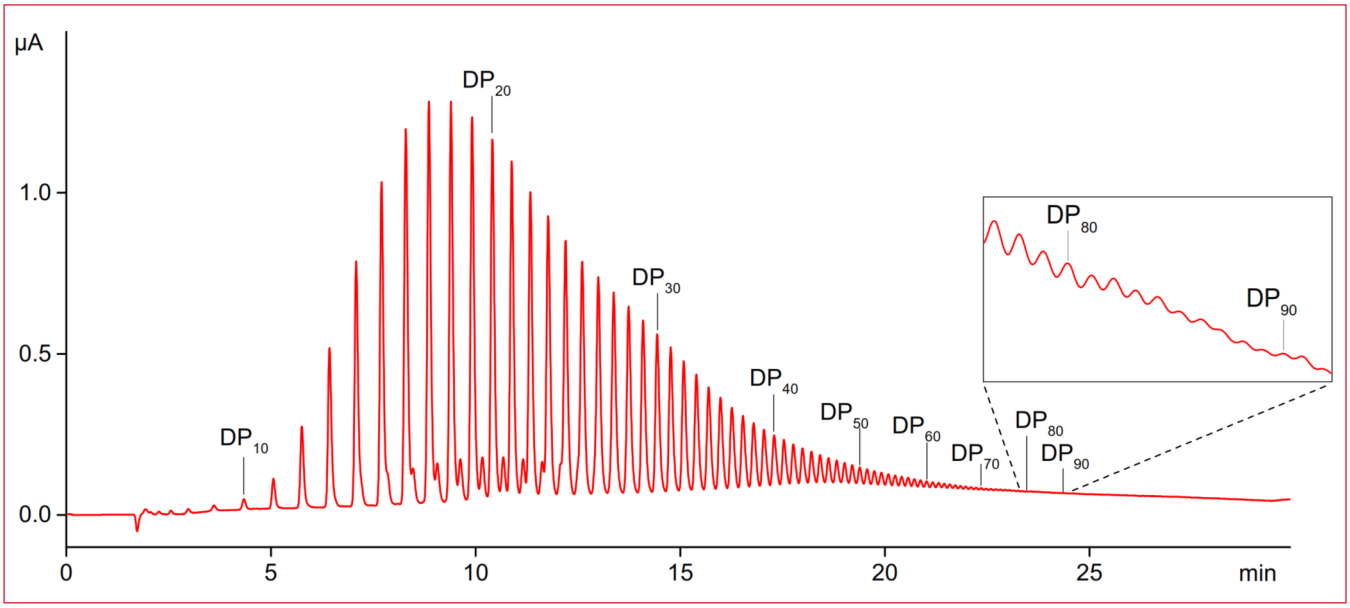

Fig. 1. Chromatogram obtained from an 10 µL injection of a 200 ppm solution of inulin from chicory in DI water. Separation was achieved using the LC-EC conditions and gradient program shown in Table 1 and 2, respectively. Inset(right): zoomed plot of area between DP80—DP90.

Introduction

Inulin-type fructans found in many types of plants are a group of naturally occurring polysaccharides consisting of fructose units joined by β(2→1) glycosidic bond, and typically have a terminal glucose unit [1,2]. High-performance anion-exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) is a powerful tool to profile the chain length distribution of inulin-type fructans [2].

Fig. 2. ALEXYS Carbohydrate Analyzer.

Table 1. HPAEC-PAD conditions

HPLC	ALEXYS™ Carbohydrate Analyzer (Antec Scientific)
Columns	SweetSep™AEX200, 4 x 50 mm precolumn, 5µm SweetSep™AEX200, 4 x 200 mm column, 5µm Borate ion trap, 4 x 50 mm column, 10 µm (all columns Antec Scientific)
Mobile phase	A: 100 mM NaOH - 500 mM $NaNO_3$ B: 100 mM NaOH
Flow rate	0.7 mL/min
Backpressure	185 - 195 bar
Injection volume	10 µL
Temperature	40°C for sample heating (AS 6.1L sample tray compartment), 35°C for separation, 45°C for detection
Flow cell	SenCell Au WE, HyREF Pd RE, AST setting 1
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
Range	5 µA/V
I-cell	About 0.2—0.5 µA
ADF	0.05 Hz

Larger molecules like oligo– and polysaccharides typically have a higher net negative charge under alkaline conditions and slower elution kinetics and are therefore strongly retained on AEX columns. To elute these macromolecules, sodium acetate is most frequently used in the mobile phase as 'pusher' anion due to its stronger elution power. As alternative sodium nitrate, can be used. Sodium nitrate is a more effective anion, requiring approximately 20% of the concentration of acetate to obtain the same retention time. In published studies it was claimed that the use of nitrate offered better reproducibility and lower limit of detection [3]. In this short application note fast high-resolution analysis of inulin is demonstrated based on separation on the new SweetSep™ AEX200 column using a sodium nitrate containing eluent. The high resolving power and detection sensitivity is evident from figure 1. Inulin with a degree of polymerization (DP) of more than 90 can be detected with this method.

Method

The setup & conditions of the method and gradient program are listed in table 1 and 2, respectively. The ALEXYS carbohydrate analyzer (fig. 2) is a dedicated HPAEC-PAD system with a metal-free flow path, optimized for the sensitive analysis of carbohydrates. The system consists of the ET210 eluent tray, a P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, and the DECADE Elite electrochemical detector. The ET210 eluent tray has an integrated gas distribution system to blanket the headspace of the eluent bottles with inert gas (Helium or Nitrogen) to avoid diffusion of CO_2 into the eluents and minimize the formation of carbonate ions.

Sample preparation: 20 mg/mL inulin in water was dissolved at 90°C in a water bath, subsequently diluted 100x and filtered (0.22 µm Polyethersulfone syringe filter). The inulin sample was kept to 40°C in the auto sampler prior to analysis, to prevent precipitation of the high molecular weight fraction.

Table 2—Gradient program

Time (min)	*%A*	*%B*	*Mobile phase*	*Description*
0	5	95	100mM NaOH + 25 mM $NaNO_3$	Gradient elution
30	40	60	100 mM NaOH + 200 mM $NaNO_3$	
30 - 45	40	60	100 mM NaOH + 200 mM $NaNO_3$	Column clean-up
45 - 60	5	95	100 mM NaOH + 25 mM $NaNO_3$	Equilibration

For research purpose only. The information shown in this short application note is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control and may be adjusted accordingly. Specifications mentioned are subject to change without further notice.

References

1. Wiki, "https://en.wikipedia.org/wiki/Inulin", https://en.wikipedia.org/wiki/Inulin
2. Antec Scientific Application note," Characterization of Inulin -type fructan mixtures", pn 220_021, 220_021
3. K. S. Wong et al., "Effects of pushing agents on the separation and detection of debranched amylopectin by HPAEC-PAD ", *J. of Liq. Chromatogr.*, 1995, 18(1), 63–80, https:/doi.org/10.1080/10826079508009221

Reagents and standards

NaOH (50% w/w/Certified)	Fisher Scientific, pn SS254-500
Sodium Nitrate, Suprapur 99.99%	Merck, pn 1.06546.0050
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
Inulin from Chicory > 95%	Carbosynth, pn YI01274

Ordering information

ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrate Analyzer - gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Columns	
260.0025	SweetSep™ AEX200, 4 x 50 mm precolumn, 5 µm
260.0020	SweetSep™ AEX200, 4 x 200 mm column, 5 µm
260.0030	Borate ion trap, 4 x 50 mm column, 10 µm
Software*	
195.0035	Clarity CDS single instr. incl. LC, AS module

*) The ALEXYS Carbohydrate Analyzer can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. Please Contact Antec for more details.

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