Short application note

Food & beverage

Sugars in sugar beets

Keywords

ALEXYS carbohydrate analyzer, HPAEC-PAD, DECADE Elite, SenCell, SweetSep™ AEX200, glucose, sucrose, fructose, quality control, EU regulation 1308/2013 on agricultural product market, ICUMSA method GS6-3, ICUMSA Method GS8-26.

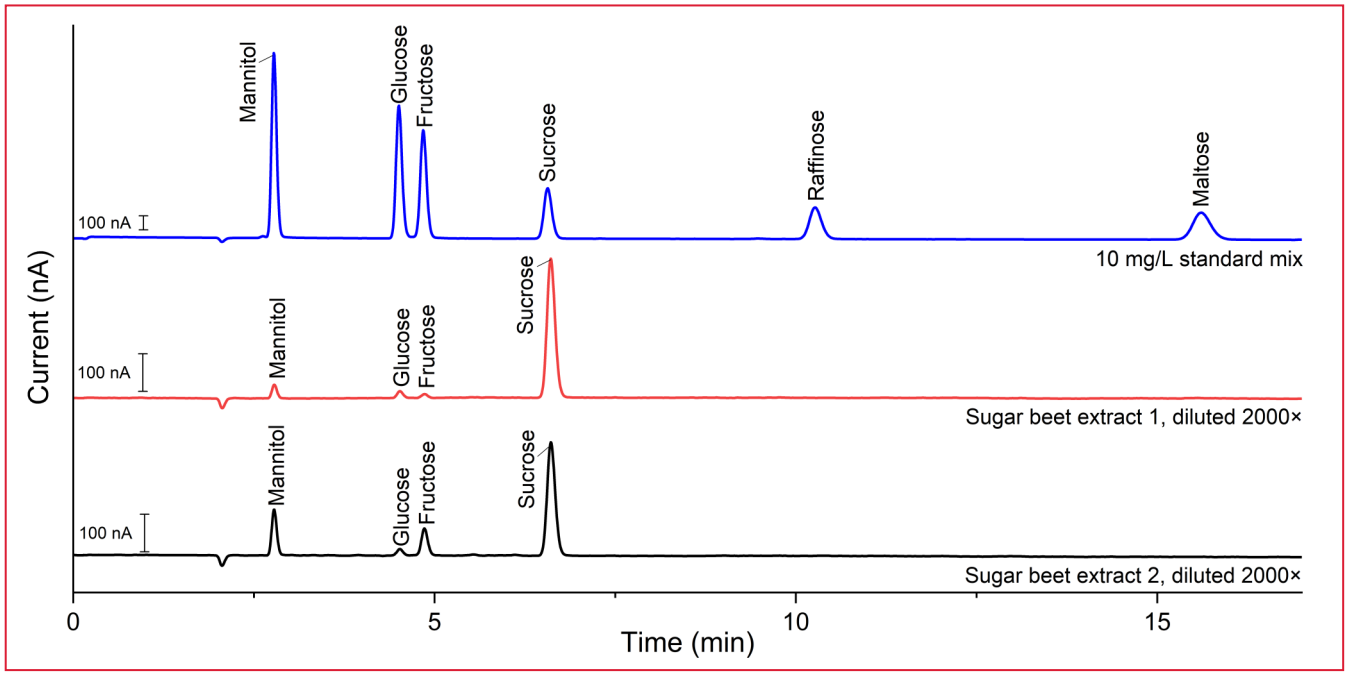

Fig. 1. Chromatogram obtained from an 2.5 µL injection of 10 mg/L sugar standards (top, blue trace), and two different sugar beet extracts diluted 2000× in DI water (middle and bottom, red and black traces), using the ALEXYS Carbohydrate Analyzer shown in Figure 2. Separation was achieved using the LC-EC conditions and gradient program shown in Table 1 and 2, respectively.

Introduction

Sugar beet trade and the common organisation of the EU sugar market are governed by Regulation (EU) No 1308/2013, which until 1 October 2017 set a minimum purchase price for quota beet based on the quantified sucrose content [1]. Although the quota and minimum-price regime were removed in the EU since then, the measurement of sucrose

Fig. 2. ALEXYS Carbohydrate Analyzer.

Table 1. HPAEC-PAD conditions

HPLC	ALEXYS™ Carbohydrate Analyzer (Antec Scientific)
Columns	SweetSep™ AEX 20, 2.1 × 50 mm precolumn, 5µm SweetSep™ AEX 20, 2.1 × 200 mm column, 5µm Borate ion trap, 2.1 × 50 mm column, 10 µm (all columns Antec Scientific)
Mobile phases	A: DI water, B: 200 mM NaOH and C: 100 mM NaOH + 500 mM NaOAc Eluents blanketed with Nitrogen 5.0
Flow rate	0.18 mL/min
Backpressure	About 220—230 bar
Injection volume	2.5 µL
Temperature	5°C for sample cooling (AS6.1L), 30°C for separation (CT2.1), 45°C for detection (DECADE Elite)
Flow cell	SenCell Au WE, HyREF Pd RE, AST setting 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
Range	20 µA/V
I-cell	About 0.1 µA
ADF	0.05 Hz

Table 2. Gradient program

Time (min)	*Mobile phase*	*%A*	*%B*	*%C*	*Description*
0—15	70 mM NaOH	65	35	0	Elution & detection
15—20	100 mM NaOH, 100 mM NaOAc	40	40	20	Column clean-up and regeneration
20—25	200 mM NaOH	0	100	0	
25—40	70 mM NaOH	65	35	0	Equilibration, starting conditions

and other sugars remains essential because farmers are paid based on the extractable sugar per unit area. The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) Method GS6-3 describes an official standard for accurate sucrose quantification in sugar beet based on polarimetry after clarification with aluminum sulphate [2], and may serve as a basis for beet purchasing contracts. ICUMSA Method GS8-26 determines the contents of other sugars such as mannitol, glucose, fructose, and raffinose in beet brei extracts and juices by HPAEC-PAD [3, 4]. These sugars represent impurities under the EU regulatory definition of white sugar, which requires 99.5% or more sucrose by weight. Glucose and fructose in particular accumulate as beet deteriorates, making their accurate separation and quantification important indicators of beet quality. In this work, the ALEXYS Carbohydrate Analyzer (Figure 2) equipped with the SweetSep AEX20 column is used for selective separation and accurate quantification of these sugars in sugar beet extracts, enabling beet quality assessment and processing control in the sugar industry.

Analysis of sugars in sugar beets extracts

An example of a selective and sensitive HPAEC-PAD analysis of sugar beet extracts is shown in Figure 1. Under the conditions described in Table 1 and Table 2, six sugar standards are baseline separated. Four of these sugars (mannitol, glucose, fructose, and sucrose) were detected in two different sugar beet extracts, even after 2000× dilution. Sucrose is the major sugar found in these samples. The limit of detection (LOD) was calculated as the analyte response corresponding to 3× ASTM noise (ASTM noise was calculated from t = 11—15 min). The LODs for all sugars ranges from 0.02 to 0.11 mg/L, indicating a sensitive method for quantification of the relevant sugars in sugar beets extracts.

The method's excellent repeatability was achieved with RSD values (n = 10, consecutive injections of 10 mg/L standard mix) for peak area and retention time of < 0.7% and < 0.5%, respectively. The method linearity was investigated over a concentration range of 0.05—100 mg/L (8 calibration points), and fitted with a quadratic fitting ignoring the origin and weighted using 1/concentration^2. An average relative standard error of 5.6% was obtained for all compounds, demonstrating the high accuracy of the calibration curves.

Table 3. Sample quantification and recovery

Sample	*Compound*	*Amount in the undiluted sample (mg/L)*	*Recovery (%)*
Sample 1	Mannitol	558	108
	Glucose	400	97
	Fructose	216	100
	Sucrose	20784	102
	Raffinose	n.d.	91
	Maltose	n.d.	96
Sample 2	Mannitol	1848	103
	Glucose	398	104
	Fructose	1936	107
	Sucrose	16810	125
	Raffinose	n.d.	108
	Maltose	n.d.	108

*n.d. = not detected

The carbohydrates found in the samples were quantified, and the results listed in in Table 3. The recovery of the sugars was also calculated based on a standard addition (spiking) method, comparing the amount of the analytes in the sample, spiked sample, and the amount of standard mix (1 mg/L final spike concentration). The recovery values for all analytes ranged between 90—125%.

$$Recovery\ (\%) = 100 \times \frac{Amount_{spiked\ sample} - Amount_{sample}}{Amount_{standard}}$$

Conclusion

A highly selective and sensitive method for analyzing sugars in sugar beet extracts is demonstrated using the ALEXYS™ Carbohydrate Analyzer in combination with the SweetSep™ AEX20 column. Simultaneous quantification of sucrose alongside glucose, fructose, mannitol, and raffinose in a single run addresses the analytical scope defined by ICUMSA Methods GS6-3 and GS8-26, providing sugar beet industries with the sensitivity and selectivity needed to monitor juice purity, detect sucrose degradation, and protect final product compliance with the 99.5% sucrose specification required under Regulation (EU) No 1308/2013.

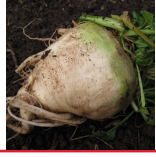

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Table 4. Ordering information

ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrate Analyzer - gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Columns	
260.0026	SweetSep™ AEX20, 2.1 × 50 mm precolumn, 5 µm
260.0021	SweetSep™ AEX20, 2.1 × 200 mm column, 5 µm
260.0031	Borate ion trap, 2.1 × 50 mm column, 10 µm
Software*	
195.0035	Clarity CDS single instr. incl. LC, AS module

*) The ALEXYS Carbohydrate Analyzer can also be controlled by Thermo Fisher Scientific Chromeleon™ CDS and Agilent OpenLab CDS. Please contact Antec Scientific for more details.

Table 5. Reagents, standards and sample prep accessories

NaOH (50% w/w/Certified)	Fisher Scientific, pn SS254-500
Sodium acetate trihydrate, HPLC grade	Fisher Scientific, pn 10122400
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
Fructose	Sigma Aldrich, pn F0127
Glucose	Sigma Aldrich, pn G8270
Sucrose	Sigma Aldrich, pn S9378
Maltose monohydrate	Sigma Aldrich, pn M5885
Mannitol	Sigma Aldrich, pn 3340-100G
Raffinose pentahydrate	Fisher, pn 11473867
Syringe filter	0.22 µm PES (Polyethersulfone) 25 mm Ø FFL/MLS
Nitrogen 5.0 (purity 99.999%)	Messer Netherlands, pn 100542102

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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.