Short application note

Food & beverage

Sugars in food according to GB5009.8-2023

Keywords

ALEXYS carbohydrate analyzer, HPAEC-PAD, DECADE Elite, SenCell, SweetSep™ AEX200, GB5009.8—2023 GB national standards for food safety China, galactose, glucose, fructose, sucrose, lactose, maltose, improved fast method

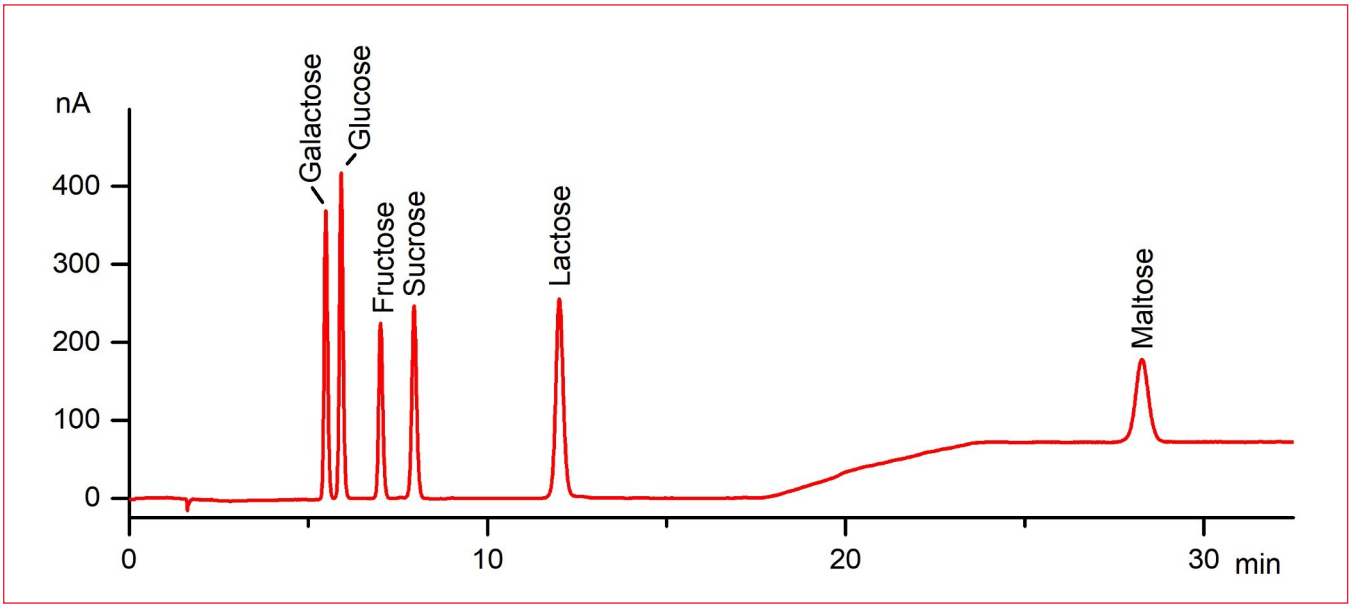

Fig. 1. Chromatogram obtained from an 10 µL injection of 10 µM galactose, glucose, fructose, sucrose, lactose and maltose in DI water. Separation was achieved using the LC-EC conditions and gradient program from standard GB5009.8—2023 shown in Table 1 and 2, respectively. Total run time 50 minutes including wash/regeneration and equilibration step.

Introduction

The monosaccharides glucose and fructose along with the disaccharides sucrose, maltose, and lactose are commonly added to flavor or preserve processed meat. To increase awareness of sugar intake and to comply with food labeling regulation, it is required to provide accurate information about added sugar content on food product labels.

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: DI water B: 200 mM NaOH Eluents blanketed with Nitrogen 5.0
Flow rate	0.7 mL/min
Backpressure	About 250—255 bar
Injection volume	10 µL
Temperature	4°C for sample cooling (AS6.1L), 25°C for separation (CT2.1), 35°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	2 µA/V
I-cell	About 0.1—0.3 µA
ADF	0.5 Hz

High-performance anion-exchange chromatography in combination with pulsed amperometric detection (HPAEC-PAD) is a powerful technique for sensitive analysis of sugars in food & beverages. In the Chinese national standard for food safety, GB5009.8-2023, a HPAEC-PAD method is described for the determination of fructose, glucose, sucrose, lactose and maltose in food products using a 4 x 250 mm anion-exchange column with a particle size 10 µm [1]. In this short application note we demonstrate fast high-resolution separation of these specific sugars based on the GB5009.8-2023 method, using the new Antec Scientific SweetSep™ AEX200 column. The AEX200 is an anion-exchange stationary phase based on a highly monodisperse 5 µm resin of ethylvinylbenzene-divinylbenzene copolymer coated with functionalized nanoparticles with quaternary amine exchange sites. The fast separation and high resolving power of the column is evident from figure 1, and enables the use of a faster simplified gradient program derived from GB5009.8-2023 to shorten both the run time and increase the sample throughput.

Original 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, DECADE Elite electrochemical detector and SenCell flow cell. 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, ensuring reproducible analysis. A 4 x 200 mm AEX200 analytical column in combination with a 4 x 50 mm AEX200 precolumn was used for the analysis.

Table 2. Gradient program GB5009.8-2023

Time (min)	*Mobile phase*	*%A*	*%B*	*Description*
0	20 mM NaOH	90	10	Elution & detection
15.0	20 mM NaOH	90	10	
21.0	40 mM NaOH	80	20	
32.0	40 mM NaOH	80	20	
32.1	200 mM NaOH	0	100	column clean-up/regeneration
42.0	200 mM NaOH	0	100	
42.1	20 mM NaOH	90	10	Equilibration to starting conditions
50.0	20 mM NaOH	90	10	

As a precaution a 4 x 50 mm borate ion trap was installed in the solvent line between pump and injector to eliminate the presence of borate contaminants in the mobile phase. Borate ions can form complexes with some carbohydrates causing peak tailing and thus loss of peak symmetry.

The temperature for separation is not specified in the GB5009.8-2023. In this case 25°C was selected as optimal separation temperature. A slightly lower flow rate of 0.7 mL/min was applied than specified in the GB5009.8-2023 method (1 mL/min). The optimum flow rate of 0.7 mL/min was determined based on measurements of the height equivalent to theoretical plate (HETP) as function of flow rate.

In figure 1 an example chromatogram is shown obtained with the GB5009.8-2023 method. A selection of the corresponding LC performance parameters together with the Limit of Detection (LOD) for all analytes of interest are listed in table 3.

Table 3. LC performance parameters & LOD

Compound	*Retention Time (min)*	*Efficiency (th.pl.)*	*Symmetry /Tailing*	*Resolution*	*LOD*	
					nmol/L	*µg/L*
Galactose	5.48	15,689	1.16	-	18	3
Glucose	5.92	16,573	1.10	2.42	16	3
Fructose	7.02	19,139	1.11	5.7	30	10
Sucrose	7.95	17,647	1.10	4.23	28	5
Lactose	12.01	16,279	1.07	13.21	27	9
Maltose	28.28	28,889	1.02	31.31	64	22

The peak efficiencies are about 15,000 - 19,000 theoretical plates, except for the late eluting maltose peak (29,000). This higher plate number is a result of the gradient elution. No significant peak tailing was observed for all analytes (tailing factor between 1.0 - 1.2). All peaks in the chromatogram are baseline separated ($r > 2$), including the epimers glucose and galactose. Good separation of glucose and galactose is important, because lactose containing samples might contain a small amount of galactose (and glucose) due to degradation of lactose. The disaccharide maltose is eluted at 28.3 min during the 40 mM NaOH gradient step. The 40 mM NaOH gradient step appears as a plateau in the chromatogram due to the higher ionic strength and pH, which resulted in a higher background current.

The LODs were calculated as the analyte response corresponding to 3× the ASTM noise (average peak-to-peak baseline noise of 10 segments of 0.5 min). The noise was determined using a 5-minute section of the baseline between t = 5 min to 10 min. The peak height responses obtained from a

10 µL injection of a 1 µM standards was used to calculate the LODs. Detection limits in the range of 16 - 64 nmol/L were obtained, demonstrating the high sensitivity of the HPAEC-PAD method. The repeatability of the method was evaluate by 6 repetitive injections of the 10 µM standards in DI water. Excellent repeatability was achieved with RSD values for peak area and height of < 0.3% and < 0.4%, respectively.

These results clearly demonstrate that with the SweetSep AEX200 fast high-resolution separation can be achieved superior to conventional anion-exchange columns with 10 µm particles.

Optimized fast method

It is evident from figure 3 that all sugars up to lactose elute within 12 minutes using the AEX200 column. Therefore, a faster simplified gradient program can be applied to shorten the elution time of maltose by almost a factor 2. The step gradient program is shown in table 4 and consist of an elution step using 20 mM NaOH for 10.5 minutes, followed by elution at 200 mM NaOH (3.5 minutes) and subsequent equilibration to the starting conditions (8 minutes). The LC performance parameters and LODs of the method are listed in table 5. It is clear that using the optimized fast method results in an almost 30 minute shorter run time, increasing the sample throughput with a factor 2.3, without compromising on performance. Moreover, the fast method resulted in a 4 fold improvement of the LOD for maltose, due to significant peak sharpening caused by the step-gradient elution of maltose at 200 mM NaOH.

References

1. GB National standards for food safety, People's Republic of China, "Determination of fructose, glucose, sucrose, maltose and lactose in food using ion chromatography", GB 5009. 8—2023, implemented 2024-03-06

Table 4. Adapted fast gradient program

Time (min)	*Mobile phase*	*%A*	*%B*	*Description*
0	20 mM NaOH	90	10	Elution & detection
10.5	20 mM NaOH	90	10	
10.52	200 mM NaOH	0	100	Elution maltose & column clean-up/ regeneration
14	200 mM NaOH	0	100	
14.02	20 mM NaOH	90	10	Equilibration to starting conditions
22.0	20 mM NaOH	90	10	

Table 5. LC performance parameters & LOD

Compound	*Retention Time (min)*	*Efficiency (th.pl.)*	*Symmetry /Tailing*	*Resolution*	*LOD*	
					nmol/L	*µg/L*
Galactose	5.44	15,696	1.08	-	18	3
Glucose	5.87	16,474	1.10	2.39	16	3
Fructose	6.95	19,020	1.10	5.65	30	10
Sucrose	7.85	17,511	1.10	4.11	28	5
Lactose	11.85	16,082	1.05	13.13	27	9
Maltose	15.17	214,075	1.04	13.17	14	5

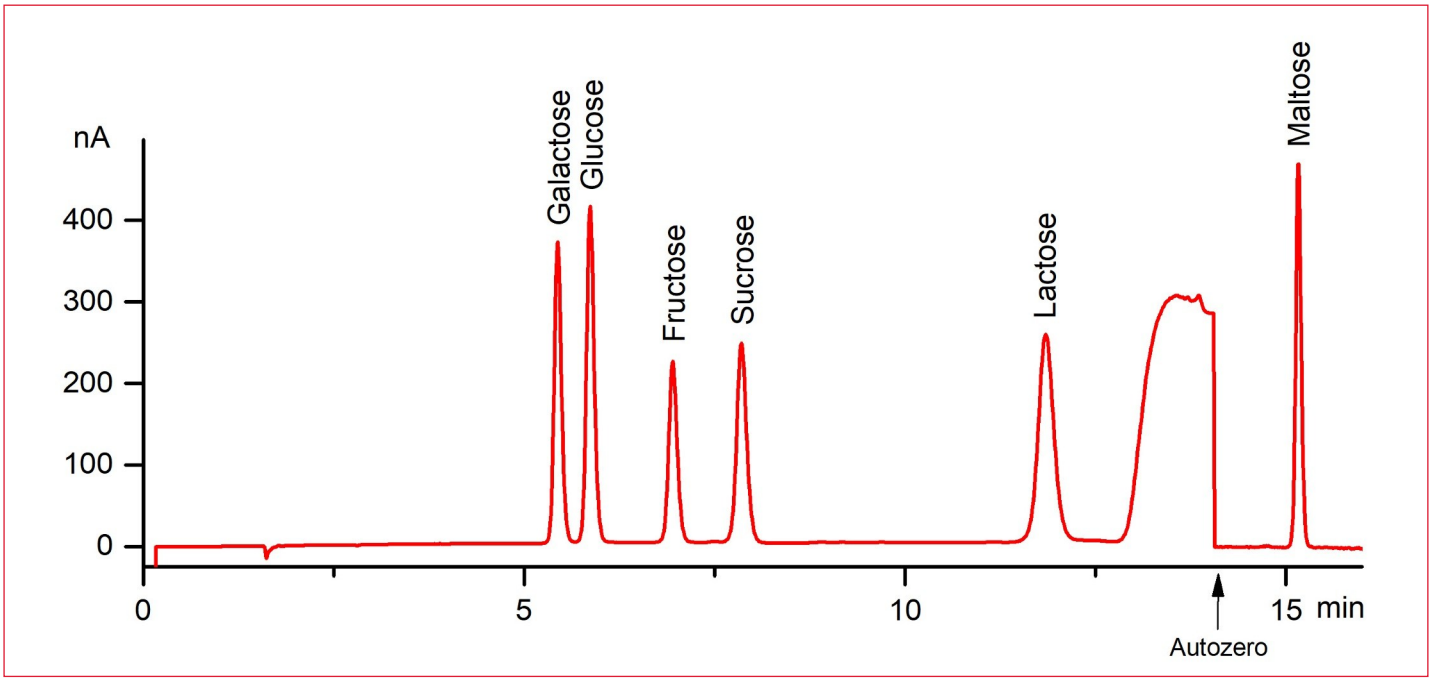

Fig. 3. Example chromatogram obtained from an 10 µL injection of 10 µM galactose, glucose, fructose, sucrose, lactose and maltose in DI water. Separation was achieved using the LC-EC conditions listed in table 1 and the adapted fast gradient program shown in table 2. At t=14.0 min an autozero was executed in the detector timed event table to zero the baseline prior to elution of maltose.

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 Scientific for more details.

Reagents, standards and sample prep accessories

NaOH (50% w/w/Certified)	Fisher Scientific, pn SS254-500
DI water 18.2 MΩ.cm, TOC < 5 ppb	YoungIn Chromass Aquapuri Essence+ 393
Galactose	Sigma Aldrich, pn G0750
Fructose	Sigma Aldrich, pn F0127
Glucose	Sigma Aldrich, pn G8270
Sucrose	Sigma Aldrich, pn S9378
Lactose	CarboSynth, pn OL04771
Maltose	Sigma Aldrich, pn M5885
Nitrogen 5.0 (purity 99.999%)	Messer Netherlands, pn 100542102

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.

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