Application Note

Food, Beverage,

Life Sciences

The finest HPAEC-PAD applications for carbohydrate analysis

Food and Beverage
Mono– and disaccharides
Sugars in meat & fish
Carbohydrates in food according to AOAC
Carbohydrates in instant coffee
Carbohydrates in Dutch candy
Carbohydrates in honey
Oligo– and Polysaccharides in honey

Prebiotics Food Additives
Analysis of Maltodextrin in Syrups
Fructans in infant formula
TGOS in food products
Profiling of FOS

Lactose Free Products
Lactose in dairy & meat
Lactose in lactose-free products

Artificial Sweeteners
Sugar alcohols
Sucralose

Carbohydrates in food according to AOAC 2018.16

- **Mono-, di-, tri- and tetrasaccharides in food matrices**
- **ALEXYS Carbohydrate Analyzer**
- **SweetSep™ AEX20 anion-exchange column**
- **AOAC 2018.16**
- **Improved and 'green' analytical method**

Summary

AOAC Method 2018.16, is the official analytical procedure for the quantification of nutritionally relevant sugar compounds in food, dietary supplements, pet food, and animal feeds [1]. AOAC method 2018.16 employs High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) to separate and detect mono-, di-, tri- and oligosaccharides without derivatization. This method allows precise identification and quantification of sugars such as galactose, glucose, fructose, sucrose, lactose, isomaltulose (palatinose), maltose, maltotriose, and maltotetraose. Measuring sugars in food is essential for regulatory compliance, accurate food product labeling, quality control, and detecting potential product adulteration.

This study presents an analysis method, based on the AOAC method 2018.16, using a novel polymeric anion-exchange column SweetSep™ AEX20, offering fast separation of 9 carbohydrates in under 38 minutes with baseline resolution. In addition, the use of narrow-bore (2.1 mm ID) columns reduces mobile phase consumption and waste, aligning with green chemistry principles. A commercial infant cereal product was analyzed using the presented method, demonstrating its versatility for carbohydrate analysis for carbohydrate analysis without the need for derivatization and post-column addition.

Introduction

Carbohydrates are the most abundant biomolecules found in living organisms. These carbohydrates, also known as saccharides, are produced by photosynthesis and play important roles in metabolism, storage of energy and nutrition [2]. Carbohydrates can be classified in different groups based on their structures. Monosaccharides, like glucose and fructose, can be described as aldehyde– or ketone-alcohols containing three to six carbon atoms and are the subunits for more complex carbohydrates such as disaccharides and polysaccharides. Maltose is an example of a disaccharide which is mainly found in grains and cereals. Carbohydrate profiling in food is performed for several reasons: to ensure compliance with regulatory requirements and to inform consumers about the nutritional content of food products. Various techniques are routinely applied to analyze sugar profiles in food [3-6].

Enzymatic techniques provide fast but indirect analysis by converting saccharides to easily measurable compounds. However, these methods often lack specificity and are unable to simultaneously quantify all sugars. Gas chromatography is also applied for profiling of sugars in food but requires derivatization [1,7]. High performance liquid chromatography is commonly used in combination with universal detectors such as refractive index (RID), since carbohydrates are aliphatic molecules they lack an UV chromophore. RI detectors lack sensitivity and cannot be used in combination with gradient elution since a change in mobile phase composition will result in a change in refractive index.

Figure 1. ALEXYS™ Carbohydrate Analyzer consisting of the ET210 eluent tray (for N_2 blanketing), a P6.1L quaternary LPG pump, AS6.1L autosampler, CT2.1 column thermostat, and the DECADE Elite electrochemical detector.

High Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) is the method of choice because it combines high selectivity with high sensitivity without the need for derivatization.

The AOAC method 2018.16 describes the analysis of common mono– and disaccharides in human food, pet food, and animal food using HPAEC-PAD [1,7]. In this application note, an improved HPAEC-PAD method derived from the AOAC 2018.16, is presented for the compositional analysis of sugars in food products, using the ALEXYS™ Carbohydrate Analyzer in combination with a novel polymeric anion-exchange SweetSep AEX20 anion-exchange column. In contrast to the AOAC method, the presented method is based on direct PAD detection without post-column addition of sodium hydroxide. This approach resulted in a more user-friendly workflow, reduction in instrumentation cost and improvement in sensitivity. To demonstrate the method's performance and applicability, a commercial infant cereal is analyzed.

Method

Carbohydrate analysis of the food samples was conducted using the Antec Scientific ALEXYS™ Carbohydrate Analyzer (Figure 1), equipped with the new SweetSep AEX20 column and operated under the conditions listed in Table 1. This dedicated high-pressure ion chromatography system with metal-free flow path consists of the ET210 eluent tray, P6.1L quaternary LPG pump, CT2.1 column thermostat, and DECADE Elite electrochemical detector. The ET210 eluent tray has an integrated gas distribution system which enables blanketing of the eluent bottles with inert gas (Nitrogen or Helium), to avoid diffusion of CO_2 into the eluents and the build up of carbonate ions (CO_3^{2-}), ensuring reproducible analysis. Carbonate ions act as a strong modifier in HPAEC-PAD, negatively affecting the separation, due to faster elution of the components of interest over time.

Separation

Carbohydrates are weak acids with pKa values ranging between 12 and 14. Therefore, at high pH carbohydrates they will become partially ionized and can be separated by means of HPAEC using alkaline mobile phases. Due to the extreme alkaline conditions, only polymeric anion-exchange columns are suitable for carbohydrate separation. The retention time of carbohydrates is inversely correlated with pKa value and increases significantly with molecular weight.

Table 1

HPAEC-ECD Conditions

HPLC system	ALEXYS™ Carbohydrate Analyzer
Detector	DECADE™ Elite electrochemical detector
Columns	SweetSep™AEX20, 2.1 x 200 mm analytical column, 5 µm SweetSep™AEX20, 2.1 x 50 mm precolumn, 5 µm Borate ion trap, 2.1 x 50 mm column, 10 µm (Antec Scientific)
Mobile phase (MP)	A: DI Water (resistivity > 18 MOhm.cm and TOC < 5ppb) B: 100 mM NaOH C: 200 mM NaOH D: 600 mM NaOH Eluents blanketed with Nitrogen 5.0
Flow rate	0.18 mL/min
Back pressure	About 150 bar (at start of the run)
Injection	6 µL (full loop)
Temperature	30°C for separation (CT2.1) 35°C for detection (DECADE Elite)
Flow cell	SenCell with Au WE and HyREF Palladium RE, AST 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
I-cell	about 0.02 - 0.40 µA
ADF	0.1 Hz
Range	20 µA/V

Table 2

Gradient program

Time (min)	*Mobile phase*	*A %*	*B %*	*C %*	*D %*	*Description*
0.00	3 mM NaOH	97	3	0	0	Isocratic elution & detection
7.00	3 mM NaOH	97	3	0	0	Gradient elution & detection
13.00	50 mM NaOH	75	0	25	0	
13.02	10 mM NaOH	90	10	0	0	Isocratic elution & detection
17.00	10 mM NaOH	90	10	0	0	Gradient elution & detection
25.00	200 mM NaOH	0	0	100	0	
25.02	504 mM NaOH	16	0	0	84	Isocratic elution, detection & column clean-up/regeneration
30.00	504 mM NaOH	16	0	0	84	
30.02	3 mM NaOH	97	3	0	0	Equilibration to the starting condition
38.00	3 mM NaOH	97	3	0	0	

In HPAEC-PAD carbohydrate analysis, low µg/L borate eluent contaminations can harm chromatographic efficiency for mannose, sugar alcohols, and fructose due to complexation reactions with borate. To prevent this, a borate ion trap column was installed between the pump and injector as a precaution. Glassware can potentially release borate ions, particularly at high pH. Thus, all solutions were prepared in polypropylene bottles and samples stored in polypropylene vials.

The separations were performed at 30°C on a SweetSep AEX20 analytical column (2.1 x 200 mm ID). An AEX20 precolumn (2.1 x 50 mm ID) was installed in series upfront the analytical column as a guard to protection it against contaminants and particulate matter. The gradient program used for the separation, shown in Table 2, was slightly modified from AOAC Method 2018.16 to optimize the resolution between target sugars and potential interfering sugars which might be present in food samples. Modifications to the gradient program are permitted under AOAC Method 2018.16, as long as the end time of the 10 to 200 mM NaOH linear gradient step remains 25 minutes [7].

Arabinose, glucose and galactose were separated using isocratic elution with 3 mM NaOH at a flow rate of 0.18 mL/min for 7 minutes. Followed by a linear gradient step to 50 mM NaOH from t = 7 to 13 minutes to separate sucrose and fructose. Subsequently, an isocratic elution step from t = 13 to 17 minutes with 10 mM NaOH was executed to elute lactose, followed by a linear ramp to 200 mM NaOH for the separation of isomaltulose and maltose. The more strongly retained oligosaccharides, maltotriose and maltotetraose elute during the wash/regeneration step. The final step of the gradient program involves an 8-minute re-equilibration to the initial conditions, resulting in a total run time of 38 minutes. The gradient program in the AOAC method has a slightly shorter run time of 36 minutes, but does not take into account the analysis of the maltooligosaccharides. As noted in the Introduction, the adapted method does not make use of post-column addition of NaOH but is based on direct PAD detection. See the paragraph Post-column addition for more details.

To minimize the introduction of carbonate ions in the mobile phase the eluents were carefully prepared manually using a carbonate-free 50% w/w NaOH solution (commercially available). The diluent was ultrapure type 1 water (resistivity >18 MΩ.cm, TOC <5 ppb), which was sparged with Nitrogen 5.0 (purity 99.999%). During analysis the mobile phase headspace is blanketed with Nitrogen gas (0.2—0.5 bar N_2 gauge pressure) using the ET210 eluent tray to ensure reproducible analysis.

Detection

The ALEXYS Carbohydrate Analyzer was equipped with a DECADE Elite electrochemical detector in combination with a SenCell™ electrochemical flow cell for the pulsed amperometric detection [8]. The SenCell has a confined wall-jet flow cell design and consists of a gold working electrode (WE), HyREF palladium reference electrode (RE), and stainless-steel auxiliary electrode (AE). The flow cell has an adjustable cell volume and was set to position 1, which corresponds to approximately 25 µm spacing and a 80 nL working volume. A 4-step potential waveform was applied: E_1, E_2, E_3, and E_4 were +0.10, –2.0, +0.6, and –0.1 V, respectively, with pulse duration of t_1 = 0.40 s, t_2 = 0.02 s, t_3 = 0.01 s, and t_4 = 0.07 s, resulting in a total pulse time of 0.5 s (corresponding to a data rate of 2 Hz). The signal (cell current) is acquired for 200 ms at t_1 between 0.20 - 0.40 s (ts). This particular 4 -step waveform has several benefits: (1) long-term reproducible response factor for all analytes of interest and (2) minimal electrode wear [9]. The detection temperature was set to 35°C. Under the conditions specified, the typical cell current was in a range of 0.02-0.40 µA during the gradient elution. The filter setting for the detection was set to 0.1 Hz. Preparation of standards, reagents and samples

Standards: 1 mg/mL stock standards of the individual sugars were prepared in 95/5 (v/v%) water/acetonitrile to suppress bacterial and fungal growth and to prevent fast degradation. Stock standards under these conditions are approximately stable for more than a month in the fridge at 4°C. Working standards in the concentration range of 0.5 – 15 µg/mL were prepared by serial dilution of the stock standards with DI water. AOAC method 2018.16 specifies arabinose as the internal standard (ISTD) for carbohydrate analysis. 10 µL of 1 mg/mL arabinose is added to every standard and sample resulting in a total concentration of 10 µg/mL ISTD.

Sample: An infant cereal sample was kindly provided by Nestlé. This sample was prepared as follows: 0.5 g of infant cereal sample was transferred to a 50 mL centrifuge tube and diluted in 30 mL hot deionized water. This is mixed using a vortex mixer and placed in a hot water bath at 70°C. Subsequently, the sample solution is cooled down to room temperature, vortexed and transferred to a 50 mL volumetric flask and brought to volume using deionized water. After mixing, aliquots of the sample solution were transferred to 2 mL Eppendorf tubes and centrifuged for 20 minutes. The supernatant is filtered through a 0.22 µm polyethersulfone (PES) filter (25 mm Ø FFL/MLS) and diluted (1:100) with water and 6 µL injected into the LC system.

Results

In Figure 2 a chromatogram obtained from a 6 µL injection of a 10 µg/mL standard mix of 9 carbohydrates (including ISTD) in water is shown, using the specified conditions in Table 1 and 2. All compounds of interest eluted within 33 minutes. The total run time is 38 minutes due to the wash/regeneration step and re-equilibration of the column to starting conditions. The wash step is required to remove late eluting compounds which might be present in real food samples. The analyzed standard mix represents a group of mono-, di- and maltooligosaccharides (DP3 and 4) commonly found in food products. All carbohydrates in the standard mix were baseline separated

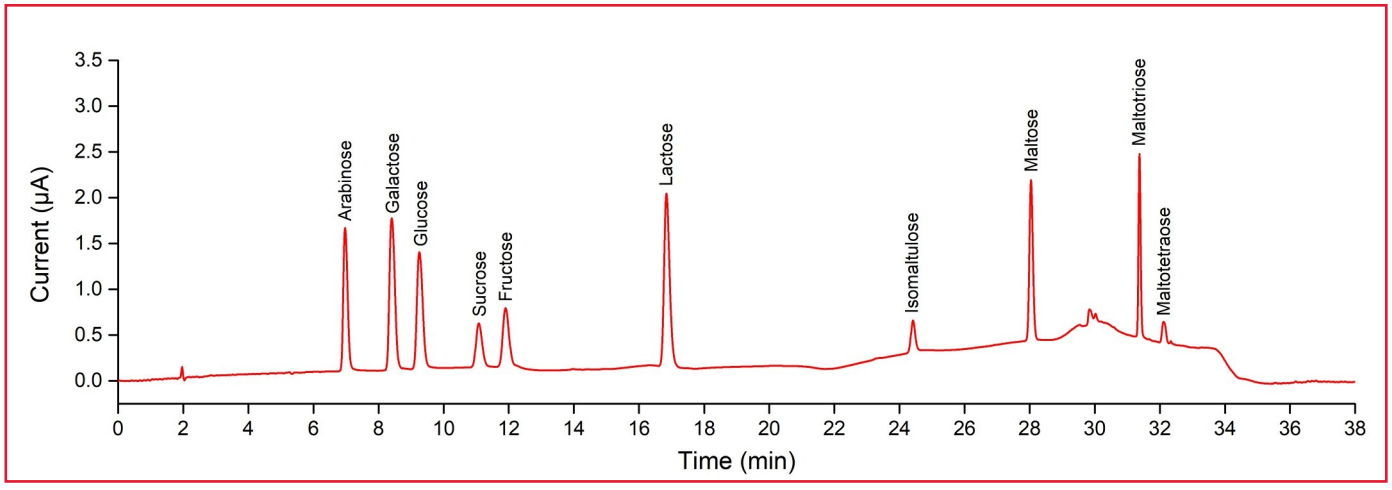

Figure 2. Chromatogram obtained from a 6 µL injection of 10 µg/mL carbohydrate standard mix containing arabinose (ISTD), galactose, glucose, sucrose, fructose, lactose, isomaltulose, maltose, maltotriose, maltotetraos in ultrapure water.

(>1.5). The peak asymmetry values for all carbohydrate peaks ranged from 1.1 to 1.3.

Potential interferences

The AOAC Standard Method Performance Requirements (SMPR) 2018.001 for sugars in animal feed, pet food and human food also requires that the method takes into account potential interferences which might be present in these sample matrices. Interferences such as non-targeted mono-, di– and trisaccharides, sugar alcohols and amino sugars. The interferants which were assessed for the 2018.06 AOAC method as described in the 'first action' publication [1] were: fucose, maltitol, rhamnose, glucosamine, xylose, mannose, ribose, isomaltose, lactulose, raffinose and stachyose [7]. In this study the same potential interferences were evaluated by injecting reference solutions of the interferants and comparing the retention times with the standard mix. The chromatogram of the standard mix including the interferants is shown in Figure 3. All peaks from the standard mix, including the internal standard (ISTD) and maltooligosaccharides, are baseline-resolved from interfering compounds (resolution, r > 1.5), highlighting the exceptional resolving power of the SweetSep AEX20 column. It is evident that the method using the AEX20 column enables accurate quantification of the 9 target carbohydrates in food samples.

Repeatability

The relative standard deviation (RSD) for the retention time and peak area were determined for 10 repetitive injections of a 1 µg/mL, as well as a 10 µg/mL standard mix in DI water. The results are listed in Table 3. Low RSD values were obtained for both the retention time and peak areas. The retention time RSD values for all compounds in both the 1 µg/mL and 10 µg/mL standard were < 0.28%. The RSD values of the peak areas for the 10 µg/mL standard was slightly lower than that of the 1 µg/mL standard, attributed to the higher signal response at the higher concentration. Nevertheless, the peak area RSD values remained < 1.32 %, which are significantly lower than the RSD limit (7%) specified in AOAC method 2018.16 [7,23]. These low RSD values for peak area demonstrate the good repeatability of the analysis method.

Calibration

The method's dynamic range was investigated over a concentration range of 0.5 – 15 µg/mL for all carbohydrates ((6 calibration levels). The calibration curves of all 9 sugars are shown in Figure 4. The response factor (RF) is calculated using formula (1) on the next page.

Table 3

Repeatability of 6 µL injections of a 10 and 1 µg/mL carbohydrate standard mix in DI water (n=10)

	RSD (%)		*RSD (%)*	
	10 µg/mL		*1 µg/mL*	
Compound	t_R	*Area*	t_R	*Area*
Galactose	0.20	0.80	0.15	0.85
Glucose	0.20	0.19	0.19	0.56
Sucrose	0.07	0.47	0.16	0.99
Fructose	0.19	0.51	0.22	0.67
Lactose	0.19	0.40	0.28	1.32
Isomaltulose	0.10	0.31	0.14	1.12
Maltose	0.03	0.49	0.06	0.62
Maltotriose	0.11	0.35	0.10	0.47
Maltotetraose	0.11	0.33	0.10	1.26

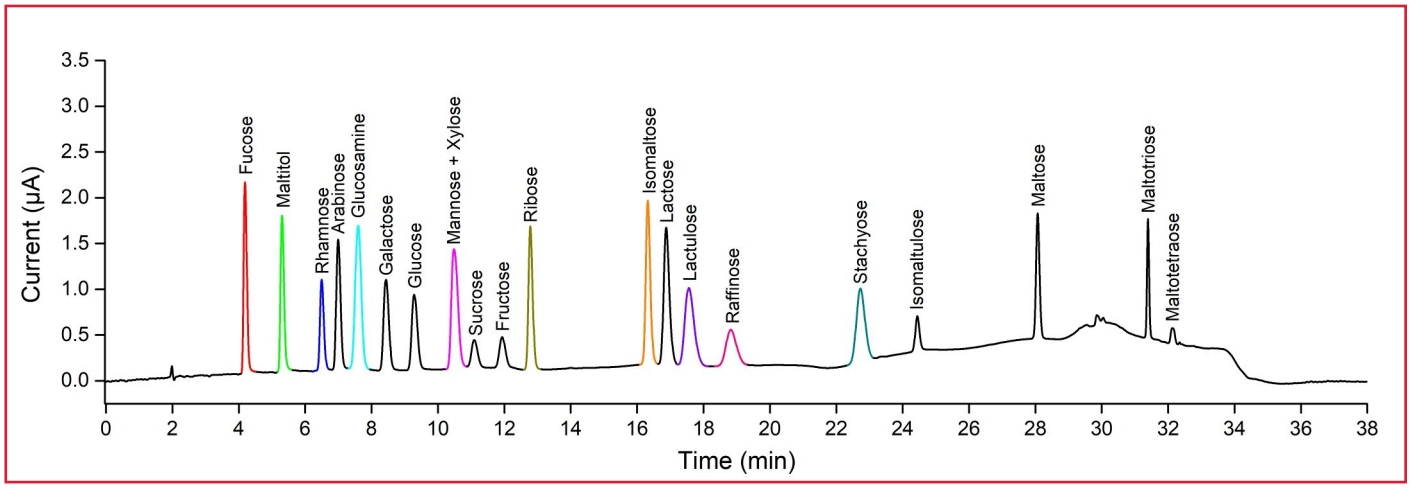

Figure 3. Chromatogram obtained from a 6 µL injection of 10 µg/mL carbohydrate standard mix including the ISTD and potential interferences in ultrapure water. Retention times (minutes) for identifying potential interference peaks: fucose = 4.2, maltitol = 5.3, rhamnose = 6.5, glucosamine = 7.6, mannose = 10.5, xylose = 10.5, ribose = 12.8, isomaltose = 16.3, lactulose = 17.6, raffinose = 18.8, and stachyose = 22.7.

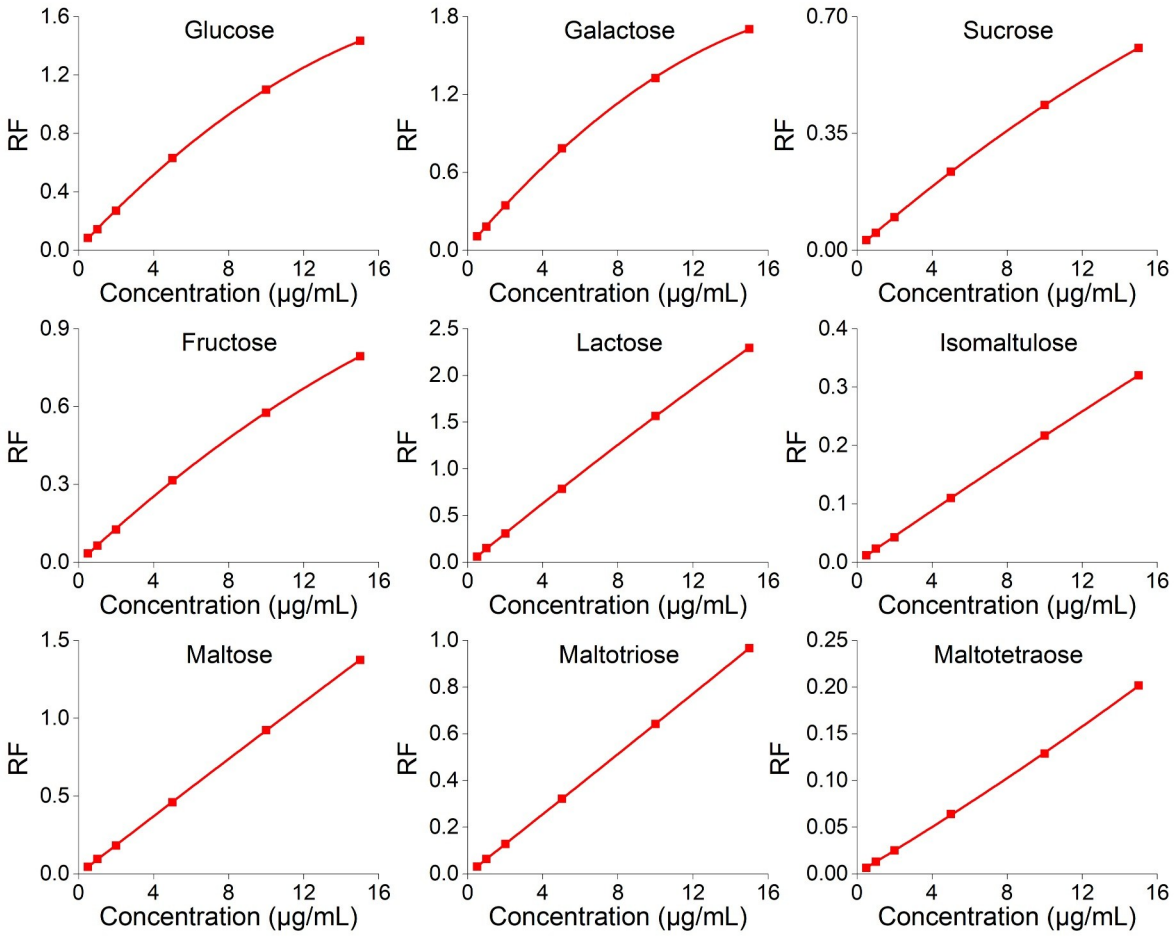

Figure 4. Calibration curves of the 9 sugars in the range of 0.5 - 15 µg/mL for all sugars. A quadratic fit was applied for all sugars, were the origin was ignored and a weight factor of 1/concentration^2 was used.

$$\text{Response factor (RF)} = \frac{\text{Analyte peak area}}{\text{Internal standard peak area}} \quad (1)$$

The AOAC 2018.16 describes the use of 1/x weighted linear regression (not forced through zero) for calibration using the response factors (RF) as function of concentration. Although, the requirements with respect to linearity ($r^2 \geq 0.998$) are met using this approach*, a weighted quadratic model was used instead. This quadratic model, using a $1/x^2$ weighting factor (x = concentration) and excluding the origin, outperforms others calibration models for all analytes. The relative standard error was calculated to assess the goodness-of-fit of the calibration curves, see Table 4. The predicted concentration closely matched the actual concentration of the standards. A relative standard error of ≤ 15 – 20% is typically advised, and in this case the relative standard error (RSE) for all compounds are less than 3.4 %, demonstrating the high calibration accuracy of the method [10,11]. AOAC method 2018.16 requires that the relative error for the lowest calibration level should be ≤ 10% and ≤ 5% for the other levels [1,7]. Table 4 shows the relative error for the lowest 0.5 µg/mL calibration level and the highest relative error for the 1 - 15 µg/mL levels. The relative error for all calibration levels fall within the specified limits of the AOAC. The calibration curves shown in Figure 4 were used for the actual quantification of the carbohydrates in the infant cereal sample.

Table 4

Relative standard error (RSE) and relative error

Compound	*RSE (%)*	*Relative error (%) 0.5 µg/mL std*	*Relative error (%) 1 - 15 µg/mL std*
Galactose	1.9	1.8	-3.7
Glucose	3.2	7.2	-2.5
Sucrose	2.9	6.7	-1.9
Fructose	3.4	7.6	-2.8
Lactose	2.3	-3.9	4.0
Isomaltulose	3.0	4.4	-4.1
Maltose	1.4	-0.3	3.2
Maltotriose	0.3	0.3	-0.6
Maltotetraose	3.2	-7.7	1.4

*) Note: the coefficient of determination r^2 was ≥ 0.998 for all components using SenCell AST setting 2 corresponding to a spacing of approximately 50 µm and a cell volume of 180 nL.

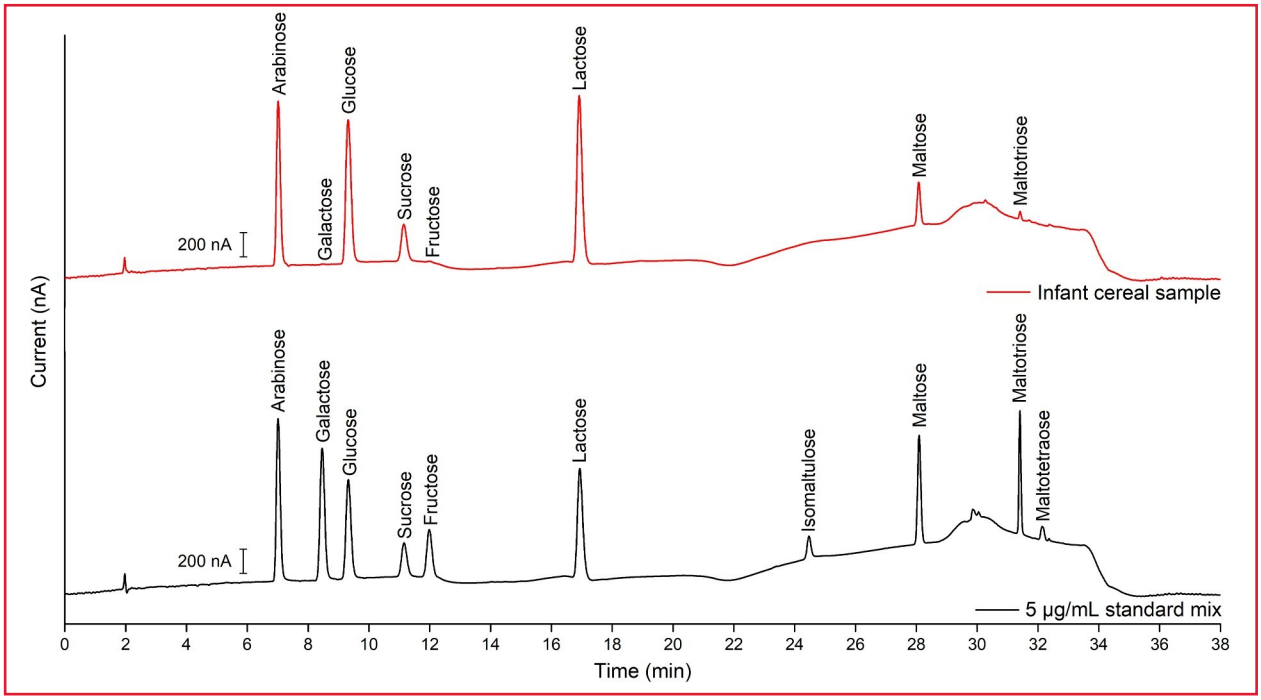

Figure 5. Chromatogram obtained from a 6 µL injection of the infant cereal sample (red) and a 5 µg/mL standard mix including ISTD in DI water (black).

Detection limit

The limit of detections (LOD) and limit of quantifications (LOQ) for all carbohydrates are summarized in Table 5. The LOD values were calculated as the analyte response corresponding to 3× average peak-to-peak baseline noise (ASTM noise).

The ASTM noise was calculated based on 0.5-minute segments between t = 18.5 min and t = 20.5 min in a blank injection (total of 4 segments). The average analyte responses of 10 replicate injections of the 1 µg/mL standard mix were used to calculate the LOD. The detection limit ranged from 2 – 25 ng/mL for all compounds. The LOQ was calculated in a similar way to the LOD, using a 10× signal-to-noise (S/N) ratio. The LOQ for all carbohydrates ranges from 7 - 83 ng/mL, indicating the high sensitivity of this method for quantification of carbohydrates.

Sample analysis

To evaluate the method's applicability a commercially available infant cereal product was analyzed. The infant cereal sample was diluted 100x before injection. For peak identification, the sample was spiked with a known amount of carbohydrate standards. The chromatograms obtained from the infant cereal

Table 5

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compound	*LOD*		*LOQ*
	nmol/L	*ng/mL*	*ng/mL*
Galactose	11	2	7
Glucose	15	3	9
Sucrose	25	9	29
Fructose	34	6	21
Lactose	18	3	11
Isomaltulose	42	15	48
Maltose	8	3	10
Maltotriose	5	3	9
Maltotetraose	37	25	83

Table 6

Carbohydrate content of infant cereal sample

Compound	*Quantified amount in g/100g*
Galactose	0.1
Glucose	10.7
Sucrose	7.1
Fructose	0.2
Lactose	8.9
Isomaltulose	-
Maltose	2.3
Maltotriose	0.4
Maltotetraose	-
Total	29.6

* - = not detected

sample is shown in Figure 5. The carbohydrate content was determined based on the calibration curves (figure 4) and are reported in g/100g (%w/w) in table 6.

Cereals, also called grains, refer to crops that are harvested for dry grain only. They include, for example, wheat, rice, oats, and maize [12]. Cereal grains are among the most important sources of the world's food supply. Infant cereals are defined as "processed cereal-based foods" that are divided into "simple cereals which are or have to be reconstituted with milk or other appropriate nutritious liquids"; or "cereals with an added high protein food which are or have to be reconstituted with water or another protein-free liquid" [13].

Infant cereals are nowadays processed on an industrial scale by manufacturers of dietetic products. These infant cereal products undergo toasting, boiling, hydrolysis, and drying to enhance their sensory qualities, digestibility, safety, and shelf life. Hydrolysis has a significant impact on the sugar profile in infant cereals. Enzymatic hydrolysis is commonly used to improve dispersibility in liquids and starch digestibility, increase sweetness and reduce syneresis effect [14,15]. Enzymatic hydrolysis typically involves α-amylase and glucoamylase, which break down starch into simple sugars (16). Starch is a polysaccharides composed by two molecules: amylose and amylopectin. Amylose is composed of long, linear chain of D-glucose units linked through α -(1,4)-glycosidic bonds and amylopectin is made up of highly branched chains of D-glucose units linked by both α -(1,4)- and α -(1,6)-glycosidic bonds [17]. By breaking these α -(1,4)- and α -(1,6)-glycosidic bonds, glucose, maltose, maltotriose, and maltotetraose can be formed [18]. Table 6 shows that glucose has the highest concentration among these four compounds, followed by maltose and maltotriose. Maltotetraose was not detected in the sample. When α-amylase is used during enzymatic hydrolysis it produces glucose, maltose, maltotriose and maltotetraose. In contrast, glucoamylase primarily forms glucose and only low concentrations of maltose, maltotriose, and maltotetraose [16]. The high glucose concentration observed in this infant cereal sample might be an indication that an enzyme such as glucoamylase was used.

During the hydrolysis stage, other ingredients may be added, including sucrose, fructose, sweeteners, powdered fruit, powdered milk, minerals, vitamins and flavorings. Powdered milk is often included to enhance the nutritional value of the infant cereal and to allow it to be prepared simply by mixing with water before consumption [19,20]. The addition of powdered milk may explain the high concentration lactose detected in this infant cereal sample (see Table 6). A low concentration galactose was detected. Galactose is not typically added as ingredient to cereal products, but can be naturally present in small amounts [21]. Sucrose was also detected at a high concentration, whereas fructose was present at a low concentration (see Table 6). Isomaltulose is a sweetener that can be added as substitute for sucrose to cereal food products. However, it was not detected in this infant cereal sample [22].

The use of carbohydrates in processed cereal-based foods and baby foods for infants and young children is regulated under Directive 2006/125/EC. This directive specifies the maximum allowable amount of added carbohydrates and added fructose in cereal-based foods. 'Added carbohydrates' refer to sugars introduced through ingredients such as sucrose, dextrose, high-fructose corn syrup, glucose syrup or honey. According to the directive, the total amount of added carbohydrates must not exceed 1.80 g/100kJ, and the added fructose content must not exceed 0.90 g/100kJ [13]. To calculate the content in g/100kJ, the following formula is used:

$$\text{Content (g/100kJ)} = \frac{\text{Carbohydrate content in g/100g}}{\text{Energy content in kJ/100g}} \times 100$$

Due to the lack of specific energy content data (kJ/100 g) for the sample, nutritional information from similar commercially

Table 7

Added carbohydrates and fructose

Compound	*Content g/100kJ*	*Maximum permitted amount g/100kJ (2006/125/EC)*
Glucose, sucrose and Fructose	1.07	1.80
Fructose	0.01	0.90

Table 8

Recovery values

Compound	*Recovery (%)*
Galactose	104.2
Glucose	92.2
Sucrose	94.7
Fructose	105.5
Lactose	108.8
Isomaltulose	106.5
Maltose	108.4
Maltotriose	106.7
Maltotetraose	106.0

available infant cereals was used. The average of these energy content values was calculated and used as a representative estimate (1672 kJ/100g). It is evident from table 7 that the amount of added carbohydrates and added fructose are within the maximum permitted amount.

Recovery

In order to assess the accuracy of the sample analysis, the recoveries were calculated based on the response factor of the analytes in the sample, the spiked sample and 5 a µg/mL standard (the final spike concentration in the sample was 5 µg/mL). The recovery is calculated using the equation below:

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

Whereby RF is the response factor of the analyte to the internal standard. The calculated recoveries are listed in table 8. The recovery values for all samples ranged between 92 - 109%. These values are in accordance with the specifications drawn in the standard method performance requirements of the AOAC 2018.001 [7,23].

Post-column addition

AOAC Method 2018.16 employs post-column addition of NaOH claiming that it will enhance baseline stability and to reduce overall run time by minimizing the equilibration time required when transitioning from the high NaOH concentration used for column cleanup and conditioning. Therefore, the method was also shortly evaluated in combination with post-column addition using 200 mM NaOH at a flowrate of 0.2 mL/min. Comparing the chromatograms recorded without post-column addition (figure 2) and with post-column addition (Figure 6) shows a slight improvement of the baseline stability with post-column addition. However, the baseline noise increased and peak heights were slightly lower when using post-column addition. The limit of detections (LOD) and limit of quantifications (LOQ) for all carbohydrates are summarized in Table 9. It is evident that the sensitivity is a factor 3.5 - 4.5 lower when using post-column addition compared to direct PAD detection (for LOD's see table 5). In addition to the observed loss in sensitivity and marginal improvement in baseline stability, post-column addition requires a more complex and thus costly HPAEC-PAD system. For these reasons it was decided to evaluate the improved method based on the SweetSep AEX20 column without the use of post-column addition.

Table 9

Limit of Detection (LOD) and Limit of Quantification (LOQ)

Compound	*LOD*		*LOQ*
	nmol/L	*ng/mL*	*ng/mL*
Galactose	38	7	23
Glucose	46	8	27
Sucrose	65	22	74
Fructose	105	19	63
Lactose	59	11	35
Isomaltulose	190	65	217
Maltose	38	13	43
Maltotriose	26	13	44
Maltotetraose	157	105	348

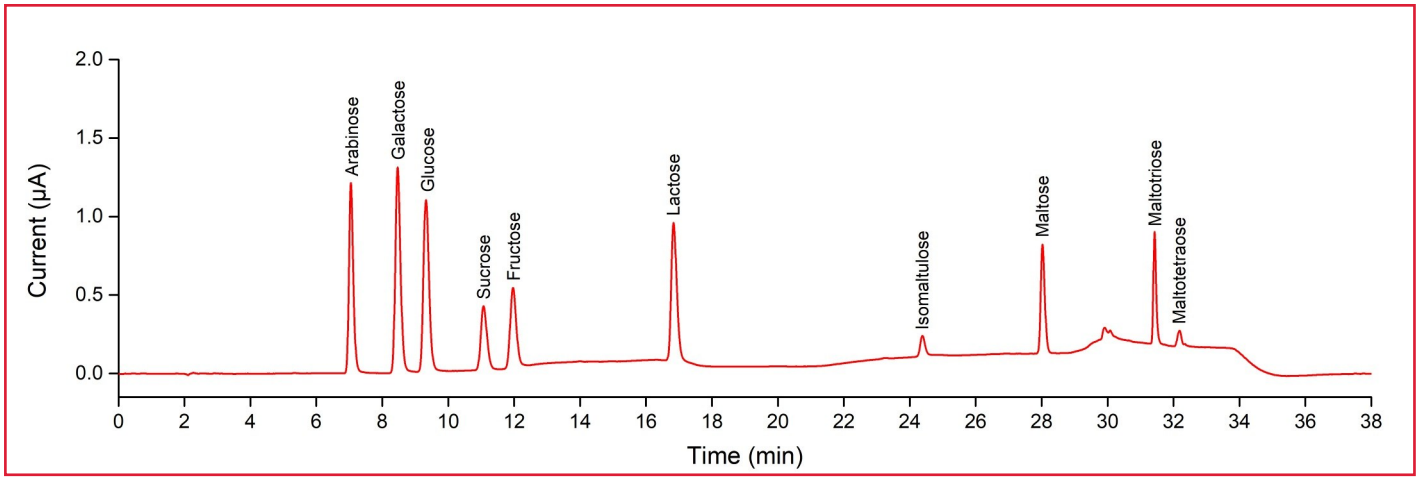

Figure 6. Chromatogram obtained from a 6 µL injection of 10 µg/mL carbohydrate standard mix containing arabinose (ISTD), galactose, glucose, sucrose, fructose, lactose, isomaltulose, maltose, maltotriose, maltotetraos in ultrapure water using post-column addition.

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Conclusion

An improved HPAEC-PAD method for the analysis of carbohydrates in food has been developed based on AOAC2018.16. The workflow—eluent delivery, automated injection, separation, and detection—is managed with a dedicated ALEXYS carbohydrate analyzer. The amperometric detector's high sensitivity eliminates the need for post-column addition of sodium hydroxide prior to detection, making it a more user-friendly and cost effective analysis solution.

The nine target carbohydrates are separated in 33 minutes with high chromatographic resolution (R > 1.5) using a polymer-based SweetSep™ AEX20 column (2.1 mm ID). Moreover, it was demonstrated that none of the target sugars coeluted with any of the 11 potential interfering sugars that may be present in food products, ensuring accurate and interference-free quantification . The applicability of the method was confirmed through the successful analysis of a commercially available infant cereal product.

The use of a narrow-bore column minimizes solvent consumption and waste, thus reducing environmental impact.

Carbohydrates in food according to AOAC

Ordering information

ALEXYS analyzer	
180.0057W	ALEXYS Carbohydrates Analyzer - gradient (quaternary LPG)
116.4321	SenCell 2 mm Au HyREF
186.ATC00	CT2.1 Column Thermostat
Optional equipment*	
180.0605	Post Column Kit Carbohydrates
Columns	
260.0021	SweetSep™ AEX20, 2.1 x 200 mm column, 5 µm
260.0026	SweetSep™ AEX20, 2.1 x 50 mm precolumn, 5 µm
260.0031	Borate ion trap, 2.1 x 50 mm column, 10 µm
260.0100**	Pre-column filter PEEK, 0.5 µm
Software#	
195.0035	Clarity CDS single instr. incl LC, AS module

*) In the case post-column addition of NaOH is required, the post-column kit for carbohydrates can be ordered as an option. The kit consist of an isocratic P6.1L pump with dual channel degasser and solvent selection valve, all LC connections and mixer.

**) In case samples might contain particulate matter it is advised to use a pre-column filter.

#) The ALEXYS Carbohydrates Analyzer (full system) can also be controlled under Thermo Fisher Scientific Chromeleon™ CDS. Please contact Antec for more details.

Reagents, standards and sample prep accessories

NaOH 50%, carbonate –free	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
Arabinose	Sigma Aldrich, pn A3131
Lactose	BioSynth, pn OL04771
Maltose	Sigma Aldrich, pn M5885
Isomaltulose	Sigma Aldrich, pn p2007
Maltotriose	BioSynth, pn OM06486
Maltotetraose	BioSynth, pn OM02796
Syringe filter	0.22 µm PES (Polyethersulfone) 25 mm Ø FFL/MLS
Centrifuge tubes	Fisherbrand™ Polypropylene Centrifuge Tubes (50 mL), Fisher Scientific, pn 05-539-9
Eppendorf tubes	Eppendorf™ Safe-lock tubes 2.0 mL, Fisher Scientific, pn 15635367

For research purpose only. The information shown in this communication 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. Specifications mentioned in this application note are subject to change without further notice.

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